2008 Proceedings of the 6th MID-ATLANTIC NUTRITION CONFERENCE

March 26-27, 2008
Timonium, Maryland

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Pennsylvania State University
University of Pennsylvania Veterinary School
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Conference Note: The Mid-Atlantic Nutrition Conference is a regional meeting that evolved from the Maryland Nutrition Conference for Feed Manufactures. Program content and format remain the same.
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* These papers were peer reviewed by program committee members. Manuscripts received in a timely manner were reviewed and edited. We thank the program participants for their cooperation in providing the material in this document.

Mark your Calendar

March 25-26, 2009

For the 7th Mid-Atlantic Nutrition Conference
General Session
EFFECT OF FEED-BORNE MYCOTOXINS ON PERFORMANCE AND REPRODUCTION OF LIVESTOCK AND POULTRY

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Summary

A series of experiments were conducted with gestating and lactating sows, broiler breeder chickens and lactating dairy cows to determine the effect of feed-borne Fusarium mycotoxins on performance, health status and reproduction. Blends of naturally-contaminated wheat and corn were found to contain deoxynivalenol (DON, vomitoxin) as the major contaminant with 15-acetyl DON, zearalenone and fusaric acid as lesser contaminants. Mid-lactation Holstein cows were fed: (1) control diet (2) contaminated grains (3) contaminated grains + 0.2% polymeric glucomannan mycotoxin adsorbent (GMA, Mycosorb, Alltech, Inc., Nicholasville, KY, U.S.A) for 56 days. Gestating and lactating sows were fed similar diets for 21 days pre-farrowing and 21 days following parturition. Broiler breeder chickens were fed similar diets for 84 days. In the dairy cow studies, there was no effect of diet on milk production, feed intake, somatic cell counts or milk composition. The feeding of contaminated grains to cows did, however, significantly reduce serum concentrations of IgA and also resulted in a significant increase in serum urea concentrations. The feeding of GMA prevented these effects. There was no effect of diet on milk production, feed intake, somatic cell counts or milk composition. The feeding of contaminated grains to cows did, however, significantly reduce serum concentrations of IgA and also resulted in a significant increase in serum urea concentrations. The feeding of GMA prevented these effects. There was no effect of diet on feed intake of gestating sows. The feeding of contaminated grains, however, significantly reduced sow weight gain during gestation and this was partially prevented by the feeding of GMA. Weight of piglets born alive, however, was not affected by diet. The feeding of contaminated grains significantly increased the incidence of stillborn piglets, however, and this was prevented by the feeding of GMA. The feeding of diets containing contaminated grains in the lactation period resulted in significantly reduced feed intake and weight loss compared to controls. The nutrient content of milk was not affected by diet. The feeding of contaminated diets tended to reduce egg production in broiler breeder hens. No effect was seen on fertility of roosters. The feeding of contaminated grains significantly reduced eggshell thickness and this resulted in a significant increase in early embryonic mortality. The feeding of GMA prevented these effects. It was concluded that reproducing pigs, broiler chickens and dairy cows are all adversely affected by the feed-borne Fusarium mycotoxins and that contaminated feedstuffs should be fed only with caution.

Introduction

Mycotoxins are fungal metabolites which can reduce performance and alter metabolism of livestock and poultry (Wannemacher et al., 1991). The pathological states arising from the consumption of feeds contaminated with mycotoxins are mycotoxicoses. Mycotoxins can be formed in the field preharvest and may continue to be formed under suboptimal storage conditions postharvest. High moisture content often predisposes feedstuffs to fungal growth and mycotoxin formation. Temperature is another key factor. Some fungi, such as Aspergillus flavus, are usually found in tropical and semi-tropical climates. This mold produces the carcinogenic hepatotoxin aflatoxin. Fusarium fungi, however, are more common in temperate climates and Fusarium mycotoxins are likely the most common mycotoxins on a global basis (Wood, 1992).
There are many reports of the effects of *Fusarium* mycotoxins on growth rates and metabolism in livestock and poultry but less research has been devoted to the effects of *Fusarium* mycotoxins on reproduction. This is no doubt a reflection of the complexity of such experiments. Ruminant animals have been considered to be more resistant to the effects of feed-borne mycotoxins because of the detoxifying potential of rumen microorganisms. Charmley et al. (1993); Ingalls (1996); Trenholm et al. (1985) reported few adverse effects of deoxynivalenol (DON, vomitoxin) contaminated feed on performance of lactating and non-lactating dairy cows. Friend et al. (1983) fed 3.45 mg DON / kg feed to gestating sows and noted a significant reduction in feed intake, body weight gain, fetal length and fetal weight at 20-54 days of gestation. Chavez (1984) fed sows naturally-contaminated wheat to provide up to 3.29 mg DON / kg feed for the last 90 days of gestation. No effect was seen on litter size or piglet weight at birth but reduced feed intake and weight gain were observed. There have been even fewer reports of the effect of feed-borne *Fusarium* mycotoxins on reproduction in broiler breeder chickens. Brake et al. (1999) fed up to 20 mg diacetoxyscirpenol (DAS)/kg feed to breeder hens and roosters and observed decreased fertility in broiler breeder males.

Experiments were conducted, therefore, to determine the effect of feed-borne *Fusarium* mycotoxins on reproduction and metabolism in sows, broiler breeder hens and roosters and lactating dairy cows.

**Materials and Methods**

**Feeding Trial With Dairy Cattle**

A study was conducted to determine the effect of feeding lactating dairy cows TMR containing wheat, corn and hay naturally-contaminated with *Fusarium* mycotoxins. A total of 18 mid-lactation Holstein cows (6 cows per diet) with an average milk production of 30 – 35 kg / day were fed for 56 days. Treatments included: (1) control (2) contaminated grains (3) contaminated grains + 0.2% GMA (Mycosorb, Alltech Inc., Nicholasville, KY), a polymeric glucomannan mycotoxin adsorbent. DON was the major contaminant and was found at up to 3.6 mg/kg in TMR dry matter. Zearalenone and 15-acetyl DON were found in lesser concentrations. Body weight, body condition score, milk production, milk composition, somatic cell count (SCC), blood serum chemistry, hematology, total immunoglobulin (Ig) count and coagulation profile were measured.

**Feeding Trial with Swine**

A study was conducted to determine the effects of feeding a blend of corn and wheat naturally-contaminated with *Fusarium* mycotoxins to gestating and lactating sows. A total of 36 first parity Yorkshire gilts (12 per treatment) were housed in individual stalls for 21 days before farrowing and 21 days after farrowing. During gestation, feed was restricted to 2.4 kg/pig/day. Treatments included (1) control (2) contaminated grains (3) contaminated grains + 0.2% GMA. DON was the major contaminant and was present at 5.7 mg/kg in contaminated diets and 0.2 mg/kg in the control diet. Zearalenone and 15-acetyl DON were found in lesser concentrations. Parameters measured included body weight change, feed consumption, numbers and weights of piglets born, numbers of stillborn and mummified piglets, milk composition, viability of piglets until weaning, blood chemistry and weaning to estrus interval.

**Feeding Trial with Poultry**

A study was conducted to determine the effects of feeding a blend of corn and wheat naturally-contaminated with *Fusarium* mycotoxins to broiler breeder hens and roosters. Forty-two 26-wk-old broiler breeder hens and nine roosters (Ross 308) were weighed and randomly assigned to individual wire cages serving as 14 and 3 replicates respectively for each of the three treatment groups. Feed consumption
of hens was restricted to 133 g/bird/d increasing to 155 g/bird/d at the end of the experiment. Diets included (1) control (2) contaminated grains (3) contaminated grains + 0.2% GMA. The major contaminant was DON which was found at about 13 mg/kg in contaminated diets and 0.2 mg/kg in the control diet. Zearalenone and 15-acetyl DON were again found in lesser concentrations. Contaminated rooster diets contained an average of 7.8 mg/kg DON with the control diet containing 0.9 mg/kg. Hens were individually inseminated three times during the week before egg collection with 50 ul of fresh pooled semen from roosters fed corresponding diets. Experimental parameters measured included feed consumption, body weight change, egg production, egg weight, shell deformity, albumin height, yolk weight, shell weight, shell thickness, weights of liver, spleen and kidney, biochemistry, hematology, serology, hatchability, progeny performance and rooster fertility.

**Determination of Dietary Mycotoxin Concentrations**

Dietary contents of 19 mycotoxins including DON, 3-acetyl DON, 15-acetyl DON, nivalenol, T-2 toxin, iso T-2 toxin, acetyl T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, fusarenone-X, diacetoxyscirpenol (DAS), scirpentriol, 15-acetoxyscirpentriol, neosolaniol, zearalenone, zearalenol, aflatoxin and fumonisin were analyzed by gas chromatography and mass spectrometry (Raymond et al., 2003). The detection limits were 0.2 mg/kg with exception of aflatoxin and fumonisin which were detected at 0.02 and 2 mg/kg respectively. Mycotoxin concentrations reported are for the complete experimental diets. The major sources of contamination were wheat and corn. Dairy rations also included contaminated hay and silage. Diets were formulated by replacing control wheat and corn with equivalent amounts of contaminated wheat and corn.

**Results and Discussion**

**Feeding Trial with Dairy Cattle**

There was no effect of diet on feed consumption, body weight change, body condition score, milk production, milk composition or milk somatic cell count. Total serum protein and globulin concentrations were increased after 42 days of feeding in cows fed contaminated TMR while albumin:globulin ratio decreased compared to controls (Table 1). Cows fed contaminated TMR + GMA were not significantly different from controls. These changes might reflect the beginning of liver damage due to mycotoxin exposure. The changes in serum proteins do not appear to be a sign of acute inflammation as there was no elevation in other markers of inflammation.

The feeding of contaminated TMR resulted in a continuous elevation in serum urea concentrations throughout the experiment and this effect was prevented by dietary supplementation with GMA. It is not clear whether the elevated blood urea concentrations are due to the effect of DON and other trichothecene mycotoxins in inhibiting protein synthesis in rumen microbes or in inhibiting hepatic protein synthesis.

The feeding of contaminated TMR also significantly reduced serum IgA concentrations after 36 days of feeding and this was prevented by dietary supplementation with GMA. This likely reflects the immunosuppressive effects of *Fusarium* mycotoxins as has been described in monogastric species.

It was concluded that feed naturally contaminated with *Fusarium* mycotoxins, even in low concentrations, can affect metabolic parameters and immunity of dairy cows and the feeding of GMA can prevent many of these effects.
Table 1. Effect of feeding TMR naturally-contaminated with *Fusarium* mycotoxins on production and metabolism of dairy cows\(^1\).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Feed Intake (kg/cow/day)</th>
<th>Milk Production (kg/cow/day)</th>
<th>SCC(^2) (sc/ml x 10(^3))</th>
<th>Serum IgA (g/L serum)</th>
<th>Serum Urea (mmol/L)</th>
</tr>
</thead>
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<tr>
<td>Control</td>
<td>48.5</td>
<td>30.0</td>
<td>64.56</td>
<td>0.35</td>
<td>5.3</td>
</tr>
<tr>
<td>Contaminated</td>
<td>49.5</td>
<td>34.0</td>
<td>57.25</td>
<td>0.16</td>
<td>6.3</td>
</tr>
<tr>
<td>Contaminated + 0.2% GMA</td>
<td>44.4</td>
<td>28.9</td>
<td>40.88</td>
<td>0.27</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Control vs Contaminated NS\(^3\) NS NS NS NS

Control vs Contaminated + GMA NS NS NS NS NS

\(^1\)From Korosteleva *et al.*, 2007.

\(^2\)Somatic cell count in milk.

\(^3\)Not significant (\(P>0.05\)).

**Feeding Trials with Swine**

There was no effect of diet on average daily feed intake of gilts in gestation (Table 2). Weight gain and gain:feed ratios, however, were reduced by the feeding of contaminated grains and this was prevented by the feeding of GMA. Serum chemistry was unaffected by diet. The percentage of stillbirths was higher and the total piglets born was lower for gilts fed contaminated grains compared to those fed contaminated grains + GMA. There was no effect of diet on frequency of mummies at birth, total piglets born or body weight of piglets at birth. In the lactation period, feed intake and weight gain were reduced by diets containing contaminated grains (Table 3). Blood chemistry, milk composition and piglet weights at weaning were not affected by diet. There was a strong trend, however, to increased weaning to estrus interval when sows were fed contaminated grains.

Table 2. Effect of feeding blends of grains naturally-contaminated with *Fusarium* mycotoxins on performance of gestating gilts.\(^1\)

<table>
<thead>
<tr>
<th>Diet</th>
<th>ADFI (kg/d)</th>
<th>ADG (kg/d)</th>
<th>G:F (%)</th>
<th>Stillbirths (%)</th>
<th>Born Alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.41(^{a})</td>
<td>1.14(^{a})</td>
<td>0.37(^{a})</td>
<td>6.27(^{ab})</td>
<td>90.5(^{ab})</td>
</tr>
<tr>
<td>Contaminated grains</td>
<td>2.12(^{a})</td>
<td>0.62(^{b})</td>
<td>0.19(^{b})</td>
<td>15.52(^{b})</td>
<td>80.8(^{b})</td>
</tr>
<tr>
<td>Contaminated grains + 0.2% GMA</td>
<td>2.15(^{a})</td>
<td>0.80(^{ab})</td>
<td>0.37(^{ab})</td>
<td>4.6(^{a})</td>
<td>95.4(^{a})</td>
</tr>
</tbody>
</table>

\(^1\)From Diaz-Llano and Smith, 2006.

\(^{a,b}\) Means within a row with different superscripts are different (\(P<0.05\)).
Table 3. Effect of feeding blends of grains naturally-contaminated with *Fusarium* mycotoxins on performance of lactating sows.1

<table>
<thead>
<tr>
<th>Diet</th>
<th>ADFI (kg/d)</th>
<th>ADG (kg/d)</th>
<th>Weaning To Estrus Interval (d)</th>
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<tbody>
<tr>
<td>Control</td>
<td>4.87a</td>
<td>0.050a</td>
<td>6.33a</td>
</tr>
<tr>
<td>Contaminated grains</td>
<td>3.56b</td>
<td>-0.592b</td>
<td>15.00b</td>
</tr>
<tr>
<td>Contaminated grains + 0.2% GMA</td>
<td>3.43b</td>
<td>-0.465b</td>
<td>15.33a</td>
</tr>
</tbody>
</table>

1From Diaz-Llano and Smith, 2007.  
abc Means within a row with different superscripts are different (P<0.05).

It was concluded that the feeding of grains naturally contaminated with *Fusarium* mycotoxins to gestating and lactating sows results in increased numbers of stillborn piglets but piglets that are born alive are viable and thrive throughout the lactation period. This is achieved, however, by a marked depletion of body reserves resulting in trend towards increased weaning to estrus intervals.

*Feeding Trials with Poultry*

There was no effect of diet on feed consumption or feed efficiency (feed consumed / egg produced) and body weights were also not affected (Table 4). There was a trend towards reduced egg production in birds fed the contaminated grains and this was significant in week 6. The feeding of contaminated grains did, however, reduce eggshell thickness after 4 weeks and this was accompanied by an increase in early (1-7 d) embryonic mortality. These effects were prevented by the feeding of GMA. It has been demonstrated that shell thickness affects moisture loss during incubation prompting early embryonic mortality. There was no effect of diet on other egg parameters including weight, yolk weight, albumen height, eggshell deformity or eggshell weight. Weights of liver, spleen and kidney were also not affected by diet. There was no effect of diet on weight or viability of newly hatched chicks.

The feeding of contaminated grains decreased serum antibody titers against infectious bronchitis virus after 12 weeks and this was prevented by the feeding of GMA. There was no effect of diet, however, on serum antibody titers against Newcastle disease virus. The absence of the effect of diet on titers against Newcastle disease virus is likely due to the fact that Newcastle disease is not endemic in Canada. Reduced antibody titers against infectious bronchitis is a reflection of the immunosuppressive properties of the trichothecene mycotoxins.

Rooster semen volume and sperm concentration, viability, motility and relative weights of testes were not significantly affected by diet.
Table 4. Effect of feeding blends of grains naturally-contaminated with *Fusarium* mycotoxins on performance of broiler breeder hens.¹

<table>
<thead>
<tr>
<th>Diet</th>
<th>Egg Production (%)</th>
<th>Eggshell Thickness (μm)</th>
<th>Early Embryonic Mortality (%)</th>
<th>IBV titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>84.3</td>
<td>32.1</td>
<td>5.4</td>
<td>12,653</td>
</tr>
<tr>
<td>Contaminated grains</td>
<td>78.8</td>
<td>30.1</td>
<td>21.5</td>
<td>8,012</td>
</tr>
<tr>
<td>Contaminated grains + 0.2% GMA</td>
<td>86.7</td>
<td>31.5</td>
<td>2.3</td>
<td>9,340</td>
</tr>
</tbody>
</table>

| Control vs Contaminated     | NS²                | 0.04                    | 0.03                         | 0.02      |
| Control vs Contaminated + GMA | NS                 | NS                      | NS                           | NS        |

¹From Yegani *et al.*, 2006.
²Not significant (*P*>0.05).

**Conclusions**

It can be concluded that there are adverse effects of feed-borne *Fusarium* mycotoxins on reproduction in swine, poultry and dairy cows with the severity declining in that order. These effects can largely be prevented by the feeding of GMA. This has important economic consequences when widespread contamination of the feed supply forces the feeding of contaminated grains or when favorable pricing prompts the intentional feeding of contaminated materials.

**References**


IMPACT OF FEED-BORNE *Fusarium* MYCOTOXINS ON PERFORMANCE, BLOOD PARAMETERS AND IMMUNOCOMPETENCE OF TURKEYS

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Summary

An experiment was conducted to investigate the effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on performance, hematology, plasma chemistry and immunological parameters of turkeys. The efficacy of polymeric glucomannan mycotoxin adsorbent (GMA, Mycosorb®, Alltech, Inc., Nicholasville, KY) in preventing these adverse effects was also evaluated. Three hundred 1-d-old male turkey poults were fed wheat, corn and soybean meal-based starter (0-3 wk), grower (4-6 wk), developer (7-9 wk), and finisher (10-12 wk) diets formulated with control grains, contaminated grains and contaminated grains + 0.2 % GMA. Feeding contaminated grains significantly decreased body weight gains during the grower and developer phases and GMA supplementation prevented these effects. There was no effect of diet, however, on feed intake or feed efficiency. The feeding of contaminated grains reduced total lymphocyte counts at wk 3 (P<0.05). Dietary supplementation with GMA increased plasma total protein concentrations compared to controls and birds fed the contaminated diet. Plasma uric acid concentrations in birds fed contaminated grains were increased at the end of the experiment compared to controls and the feeding of GMA prevented this effect. Feeding contaminated grains significantly increased percent CD4+ lymphocyte populations during wk 6, however there was no change in the percent CD8+ and B-lymphocyte populations. Contact hypersensitivity to dinitrochlorobenzene, which is a CD8+ T-cell-mediated delayed type hypersensitivity response, was significantly decreased after 24 hr and 72 hr by feed-borne mycotoxins compared to controls. Supplementation of the contaminated diet with GMA prevented the decrease in response after 24 hr. Secondary antibody (IgG titer) response against sheep red blood cell antigens (CD4+ T-cell dependent) was significantly decreased after feeding contaminated grains compared to controls. It was concluded that turkey performance and some blood and immunological parameters were adversely affected by feed borne *Fusarium* mycotoxins, and GMA prevented many of these effects.
FATE AND CONSEQUENCES OF WASTE-ASSOCIATED CONTAMINANTS ENTERING THE WATERWAYS

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Summary

In a broad sense, this paper is about the fate and consequences of animal waste-associated contaminants. This paper argues that although they differ by the animals they serve, human waste water treatment plants (WWTP), concentrated animal feeding operations (CAFO), and aquaculture facilities face common environmental challenges that are the result of housing large numbers of animals under high density. Human WWTPs and CAFOs are considered point sources of contaminants and certain aquaculture facilities seem likely to be regulated similarly. Until recently, the regulatory focus has been on nutrient contributions by these entities, but there is growing interest in other contaminants, including dust, odors, antimicrobials, antibiotic resistant microbes, pharmaceuticals, and hormones. Environmental contaminants that can mimic, antagonize, or through other mechanisms, alter normal endocrine system function are called endocrine disrupting chemicals. The goal of this paper is to discuss CAFOs as sources of natural and synthetic hormones which have the ability to alter reproduction and development of exposed fish and potentially other organisms.

The Issue

We have long recognized the importance of clean water for drinking, recreation, and commerce. In response to this recognition, Congress enacted environmental laws such as the Clean Water Act, Safe Drinking Water Act, and Solid Waste Disposal Act among others (USEPA 2006a). In the United States, it is estimated that human waste production is about 150 million tons compared to agricultural animal waste at 500 million tons per year (USEPA 2003). Much of human waste is concentrated and processed in sewage treatment plants before effluent is introduced into the environment. In the past, the comparatively larger amounts of livestock waste were diluted by deposition or spreading on large areas of land, but this is not as feasible on a growing number of CAFOs. We have also come to realize that the concentration of waste, whether from human or agricultural animal origins, requires that we control the introduction of nutrients (e.g., nitrogen and phosphorus) into surface and shallow ground water.

A combination of economic pressure to control the costs of meat production in developed nations and a rapidly growing demand for meat in developing nations is changing the traditional agricultural practices of raising livestock (Naylor et al., 2005). In the past, beef cattle, dairy cows, pigs, poultry, and other livestock were fed mainly by grazing in the fields and supplemented with farm-raised grain. Animal waste, in turn, was used to fertilize the fields and provide needed nutrients and humus to the crops. Today, there is a growing trend to centralize and concentrate the husbandry of livestock in animal feeding operations, where livestock are housed in buildings or fenced enclosures and food is brought to them (Naylor et al., 2005; USEPA 2003). Often some of the waste must be trucked to offsite disposal areas, as ability of the farm to process its own waste has been exceeded. In the United States, there are approximately 1.3 million farms that have livestock and about 238,000 of these farms are considered...
animal feeding operations. Animal feeding operations of a certain size (livestock specific), with known connections to surface waters, or with the potential to discharge waste are called CAFOs (USEPA 2003). While not currently regulated as CAFOs, aquaculture using large pens containing tens of thousands of salmon in nearshore marine facilities or freshwater catfish ponds, has raised concerns for their local environmental effects (Pew Ocean Commission 2003; Kolodziej et al., 2004) There is growing concern about the potential environmental and human health effects of CAFOs, including ammonia, dust, and odor as air pollutants and ammonia/ammonium, nutrients, pathogens, antibiotics, antibiotic-resistant microbes, pharmaceuticals, and natural and synthetic hormones as water pollutants emanating from animal waste. See papers by (Daughton and Ternes, 1999; Hamscher et al., 2003; Heederik et al., 2007; Khetan and Collins, 2007; Kolpin et al., 2002; Lee et al., 2007) and (Sapkota et al., 2007) for more information on these areas of concern.

Fate and Consequences of Natural and Synthetic Hormones Emanating from CAFOs

Naturally occurring sex steroid hormones such as androstenedione, testosterone (T), 17β-estradiol (E2), estrone (E1), and progesterone are known to be present in dairy cow wash water, downstream of aquaculture raceways, and in spawning runs of large schools of salmon (Kolodziej et al., 2004). Water samples collected downstream of farms where poultry litter was applied contained T and E2 (Shore 2004). These hormones have been demonstrated to occur in the environment in sufficient concentration to disrupt the reproductive biology of fishes, e.g. low ng/L for E2. In another study, estrogenic activity was detected in painted turtles, Chrysemys picta, living in a pond below a feedlot that was housing dairy cows, which were not receiving any implants (Irwin et al., 2001). Thus far, the focus of researchers has been on synthetic estrogens, e.g., 17α-ethynylestradiol, as these are not only more potent than the natural estrogens, but have a longer half-life compared to natural estrogens in the environment. The data, however, on transport and fate of natural sex steroid hormones in the environment are equivocal, are mostly on estrogens, and depend greatly on temperature, presence of oxygen, exposure to sunlight, etc., and decreases in soil concentrations do not usually account for losses due to surface runoff (Khanal et al., 2006). At least one research group concluded that although laboratory studies suggest rapid degradation of E2 and E1 in soils, field studies show that they are both mobile and persistent enough to reach ground water (Hanselman et al., 2003). The synthetic androgenic growth promoter, 17β-trenbolone, has been measured in drainage water that percolated through soil underlying a cattle barn in the Midwest USA (Durhan et al., 2006). Furthermore, estrogens, especially E1 in human WWTP effluent, have been shown to migrate into sediment underlying rivers receiving the effluent (Labadie et al., 2007).

A wide array of pharmaceutical agents, including endocrine disrupting chemicals, has been reported in sewage and open waters in various countries (Daughton and Ternes, 1999; Kolpin et al., 2002; Stumpf et al., 1999; Ternes et al., 1999). Environmental contaminants that can mimic, antagonize, or through other mechanisms, alter normal endocrine system function are called endocrine disrupting chemicals or EDCs (Crews and McLachlan, 2006; Damstra et al., 2002; Guillette and Crain, 2000; McLachlan, 2001). Pharmaceutical agents include drugs commonly prescribed for the treatment of heart disease, stress, inflammation, bacterial infections (antibiotics) and birth control (Daughton, 2007; Khetan and Collins, 2007). Further, although veterinary drugs, such as growth promoters and antibiotics, are used extensively in agriculture, few studies have examined their presence in the environment, although some studies have recently reported the presence of these compounds in ground water near farms (Lee et al., 2007; Peterson et al., 2000).

In the United States, hormone supplements are used in the production of approximately 90% of the beef cattle (Balter, 1999). These supplements promote rapid growth and increase the conversion of feed to muscle mass. The androgenic trenbolone acetate, estrogenic zeranol, and progestogenic melengestrol acetate commonly used singly or combined with natural steroid hormones including T, E2, or progesterone, are approved by the FDA for use in beef cattle only (FDA et al., 2006; Velle 2002). In
general, these supplements increase body protein, metabolism of fat stores, and mineral uptake across the gut and decrease amino acid metabolism (Meyer, 2001).

Recent studies have indicated that there is a basis for concern about the ecological effects of these synthetic growth supplements. Trenbolone acetate is a potent androgen and has antiglucocorticogenic activity as well (Meyer, 2001). In cattle, following dermal implants in the ear, it is metabolized into trenbolone-β, the biologically active molecule, and excreted mostly as trenbolone-α and some trenbolone-β (Schiffer et al., 2001). 17β-Trenbolone and 17α-trenbolone have half-lives in liquid manure of approximately 260 days, suggesting that they could have ecological impacts if released into the environment from CAFOs (Schiffer et al., 2001). In laboratory exposures, both 17β-trenbolone and 17α-trenbolone have been shown to masculinize the development of the mosquitofish, Gambusia affinis (Sone et al., 2005) and negatively affect the reproductive biology of the fathead minnow, Pimephales promelas (FHM, (Ankley et al., 2003; Jensen et al., 2006)). In female FHMs exposed to 17β-trenbolone, fecundity decreased, male-like secondary sex characteristics developed (nuptial tubercles) and plasma concentrations of T, E₂, and vitellogenin were all significantly decreased. In male FHMs, plasma concentrations of 11-ketotestosterone were decreased and E₂ and vitellogenin were increased (Ankley et al., 2003). Similar results were observed in the 17α-trenbolone study and these results were curious as 17β-trenbolone is known to have a greater affinity for the human androgen receptor by about one order of magnitude (Bauer et al., 2000). The authors demonstrated that the fish were likely converting the 17α-isof orm, as the tissue concentration of 17β-trenbolone were similar to or greater than 17α-trenbolone and it was not used in the experiment (Jensen et al., 2006).

Zeranol is a synthetic, estrogenic, anabolic growth supplement for beef cattle (FDA et al., 2006). Zearalenone is one of a number of mycoestrogenic substances naturally produced by species of the fungus Fusarium and is readily found in cereal grains that are stored damp (Le Guével and Pakdel, 2001). Overall the potency of zeranol is about equal to E₂ as it has been shown to be more potent than E₂ in stimulating growth of the Ishikawa cell and have slightly less affinity for the human and rainbow trout, Oncorhynchus mykiss, estrogen receptors (Arukwe et al., 1999; Le Guével and Pakdel, 2001). A metabolite of zeranol, 17α-zearalenol induced zona radiata and vitellogenin at the mRNA and protein levels in rainbow trout livers (Celius et al., 2000). While we have confirmed the estrogenicity of zeranol and its metabolites in fishes, that is all that is known about its potential effects on captive fishes. There is no information currently available on the effects of zeranol or its metabolites on the reproductive biology of wild fishes.

Melengestrol acetate (MGA) is a synthetic progestogenic growth supplement that is administered orally to immature female beef cattle or heifers (Velle, 2002). It is also used as an estrus suppresser, which both increases available energy for growth and well as improves management of facilities containing maturing steer and bulls. MGA is thought to act through the stimulation of endogenous synthesis of E₂ in the ovaries and its subsequent anabolic effects on muscle growth (Meyer, 2001). Used at the FDA allowable dose, MGA suppresses ovulation and induces continuous follicle development, which increases plasma E₂ concentration sufficiently for promotion of growth. It is thought that estrogens, which are normally low in heifers, have greater anabolic activity than androgens because estrogen receptors outnumber androgen receptors in the muscle tissue (Meyer, 2001). MGA is a potent progestin, with greater than five times the binding affinity for the bovine PR compared to endogenous progesterone (Bauer et al., 2000). Furthermore, MGA in manure applied to soil remained detectable 195 days following application to soil (Schiffer et al., 2001). No information is available about the potential effects of MGA on the reproductive biology of fishes.

Potential impacts of CAFOs to aquatic ecosystems depend upon how efficiently CAFO-derived hormones are transported to the receiving water. While many natural and synthetic hormones break down
relatively rapidly in stored manure and soils (half-lives on the order of hours to days; (Das et al., 2004; Hanselman et al., 2003; Johnson et al., 2006; Lee et al., 2003), trenbolone and MGA persist for months (Schiffer et al., 2001) in manure-amended soils. Persistence in soils increases the potential for these potent synthetic hormones to reach surface waters.

While there is much to learn about the transport and fate of natural and synthetic hormones in waste from CAFOs, there is far less known about the potential reproductive and developmental effects of these hormones singly or as mixtures on aquatic or terrestrial organisms. Currently, there is only one published field study that reports the effects of CAFO runoff on fishes (Orlando et al., 2004) and none on amphibians or terrestrial animals.

**Effects of CAFO Runoff on Fish**

The FHM was chosen because it is a well-characterized toxicological model, and the species has high ecological relevance as it is widely distributed in the USA, with that distribution often coinciding with regions having the greatest concentration of CAFOs. FHMs are becoming an important sentinel species for exposure to endocrine disrupting chemicals. Much of what we know about the effects on fish reproduction from exposure to CAFO growth supplements has been gained from laboratory exposures of FHMs (Ankley et al., 2003; Jensen et al., 2006), and, furthermore, a 21 day reproduction toxicity protocol based on FHMs is being considered as part of a screening and testing battery of assays for endocrine disrupting chemicals (USEPA 2006b).

We know that FHMs are sensitive to exposure to environmental androgens, estrogens, and their antagonists. Various reproductive endpoints, e.g. endogenous sex steroids and vitellogenin concentrations, gonadosomatic index, histopathology, secondary sex characteristics, and fecundity are altered following exposure to these compounds (Ankley and Villeneuve, 2006; Filby et al., 2007; Filby et al., 2006; Panter et al., 2004; Van Aerle et al., 2002). Certain endpoints, such as fecundity, can be used to model ecological effects. For example, data from adult female FHM exposed to 17β-trenbolone in the laboratory were used to predict that FHM populations exposed continuously to 27 ng/L of the growth promoter would have average equilibrium population sizes approaching zero (Miller and Ankley, 2004).

In this study, feral adult FHMs living upstream and downstream of cattle feedlots in Nebraska (USA) were collected and reproductive and developmental endpoints were examined. Fathead minnows collected below a feedlot exhibited altered reproductive biology including decreased T synthesis, altered head morphometrics, and smaller testis size in males and decreased E2/T ratio in female fish. We did not observe overt characteristics in either male or female fish suggesting environmental exposure to estrogens. Using an *in vitro* CV-1 assay having cells transfected with the human androgen receptor, we detected potent androgenic responses from the CAFO effluent. Taken together, our morphological, endocrinological, and *in vitro* gene activation assay data suggest two hypotheses: (1) there is an androgenic substance(s) in the CAFO effluent and/or (2) there is a mixture of endocrine active substances that alter the hypothalamic-pituitary-gonadal axis (Orlando et al., 2004). Work performed in collaboration with the laboratory of Professor Ana Soto (Tufts University) adds further support to the hypothesis that androgens are present in the CAFO effluent, as her lab observed androgenic activity. However, her lab also reported estrogenic activity in the effluent using the MCF-7 cell *in vitro* E-Screen assay, suggesting that there could be a complex mixture of natural and pharmaceutical compounds in the effluent (Soto et al., 2004).

In our research, we demonstrated that endocrine activity could be detected in natural stream/river systems below CAFOs by studying the reproductive endocrinology and secondary sex characteristics of wild fish populations. However, we were unable to do the analytical chemistry to identify the hormones in the water and so were not able to conclude whether the observed effects were due to natural or synthetic
hormones. In a recent study by the EPA, Dr. Elizabeth Durhan and colleagues collected water samples multiple times over more than a year from sites upstream, downstream, and directly from a discharge drain of a CAFO in Ohio (Durhan et al., 2006). The CAFO operator told the researchers that trenbolone acetate was administered to cattle at the facility. Using the same CV-1 assay as in our study, they reported in vitro androgenic activity in water from the discharge drain and using GC/MS demonstrated the presence of 17α- and 17β-trenbolone at concentrations high enough to negative impact FHM populations (Durhan et al., 2006; Miller and Ankley, 2004). Given the quantitative limits of the CV-1 assay and the varying detection limits in the GC/MS data, the authors say that the androgenic activity could very well be due to a combination of natural and synthetic androgens.

Furthermore, while much is known about the effects of exposure to natural and synthetic estrogens, comparatively less is known about the effects of exposure to androgens on the reproductive biology of fishes. Moreover, our understanding of environmental progestogens or their antagonists is even more limited. In teleosts fishes, the final stages of egg and sperm maturation as well as the early events in spermatogenesis are controlled by the progestogens 17α, 20β–dihydroxy-4-pregnenone (Miura et al., 2006; Senthilkumaran et al., 2004) or 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S, (Thomas et al., 2002). Some prostaglandins and these progestogens, when excreted in their free or glucuronidated and sulfated conjugate forms, are known to also function as pheromones that help coordinate reproductive behavior in many fishes (Stacey et al., 2003). Considering the role of excreted natural progestogens as pheromonal cues to courtship behavior, MGA may also alter reproductive behavior in exposed populations. Acting as an environmental progestin, MGA could potentially induce final maturation; alternatively, MGA could inhibit final maturation of gametes by antagonizing the action of the endogenous progestogen. The insecticides fenvalerate and permethrin have been shown to significantly antagonize the action of progesterone in human breast cancer (IT47D) cells (Kim et al., 2005). Both the progesterone receptor (PR) mediated induction of alkaline phosphatase gene expression and the concentration of PR protein were decreased in fenvalerate and permethrin in vitro exposures. In fishes, a number or organochlorine pesticides, e.g. o,p’-DDD and kepone, competitively displace 20β-S and have been shown to inhibit final maturation of eggs and sperm in the Atlantic croaker (Micropogonias undulatus) and the spotted seatrout (Cynoscion nebulosus), respectively (Das and Thomas, 1999; Ghosh and Thomas, 1995). There are no fish PR binding assays with MGA. Given the potency of MGA and several of its metabolites for the bovine PR, its use as a growth promoter in cattle, and its persistence in the environment, it is reasonable to expect that MGA and its metabolites could be present in aquatic environments. Exposure to MGA could interfere with the timing of final maturation of egg and sperm and the coordination of courtship behavior and thus affect the reproductive health of exposed fishes. Work by our lab is currently underway examining these hypotheses, but at this time, there are no published studies available on either captive or wild fishes.

Conclusions

Among vertebrates and some invertebrates, there is impressive evolutionary conservation of steroid hormones and hormone receptors in their molecular structure. Generally, this conservation in structure extends to their function as well, for together they regulate similar processes such as reproduction. EDCs, contaminants that affect endocrine system function, have been shown to alter development and reproductive biology of animals, including humans (Crews and McLachlan, 2006; Damstra et al., 2002; Guillette and Crain, 2000; McLachlan, 2001). WWTPs and CAFOs are some of the sources for EDCs, and thus, they have become a concern to government regulatory agencies throughout the world. It is clear that there is insufficient information regarding the transport, fate, or effect of hormones and other EDCs emanating from WWTPs and CAFOs and that more research is necessary. The USEPA and USDA are currently conducting in-house research and funding grants and contracts for research that are in support of furthering our understanding of this issue.
While there is reason for concern, there is no consensus that natural or synthetic hormones from either source are threatening the health of aquatic ecosystems or the nation’s drinking water quality. Having said that, it is also important to highlight several related issues that up to this time have garnered comparatively little attention from either research labs or regulatory agencies. First is the potential contribution of hormones and other EDCs from domestic residential septic systems. Approximately 25% of residences in the US have domestic septic systems and many of these are poorly maintained and leaking. To date there is only one study examining this issue and they measured E1, E2, and other EDCs in the groundwater surrounding the septic fields in Cape Cod, Massachusetts USA (Swartz et al., 2006).

Second, an even more important issue is that of the fate and potential effect of hormones and other EDCs in the biosolids from human WWTPs. WWTPs were not designed to remove hormones and EDCs from the waste they receive. However, during processing, WWTP’s remove approximately 85 – 99% of E2 and 25 – 80% of E1 from the effluent before it is delivered to the environment (Khanal et al., 2006; Khetan and Collins, 2007). What remains in the effluent, though, is biologically active. Many EDCs and other contaminants are effectively removed from the effluent, but settle out and become part of the biosolids. Biosolids are trucked to landfills, burned as fuel, spread in forests and fields, and some – following sterilization – are packaged and sold to consumers as soil supplements for their gardens. There is a dearth of information about the hormone and other EDC content of biosolids but research on two antimicrobial compounds recently identified as EDCs gives pause for concern. Triclosan and triclocarban are ubiquitous components of antimicrobial soaps, detergents, cutting boards, and toothpaste that are known to partition to biosolids and to have long half-lives in soil (Chu and Metcalfe, 2007; Halden and Paull, 2005; Heidler et al., 2006). Triclosan treated frog tadpoles have accelerated thyroid hormone mediated metamorphosis (Veldhoen et al., 2006; Veldhoen et al., 2007). Triclocarban enhances the action of testosterone in male laboratory rats resulting in greatly increased prostate and other reproductive tract accessory organs (Chen et al., 2007). It is evident that more research and a reassessment of regulations may be required of (all) waste-associated contaminants entering our waterways either directly as WWTP effluent or CAFO runoff, or indirectly from WWTP biosolids or CAFO waste spread onto the land.

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CURRENT TECHNOLOGIES FOR IDENTIFICATION AND MEASUREMENT OF FECAL POLLUTION IN WATER

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Summary

Fecal pollution of worldwide water resources is an increasingly critical problem. Specific water quality standards for recreational waters, based on measurement of nonpathogenic fecal indicator bacteria, have been employed in the US since 1986. Since these limits are not indicative of source, various methods for identifying the hosts-of-origin (human or animal) of fecal pollution have subsequently been developed. Techniques for profiling particular host-associated microbial strains have been based on analysis of their functional (phenotypic) or genetic (genotypic) characteristics. These microbial source tracking methods differ with respect to accuracy of discrimination, sensitivity, ease of performance, time required and cost. Many have proven useful for field application and public health risk analysis while others are promising but still experimental. Results of these assays are useful for identification, characterization and mitigation of water contamination.

Introduction

US Environmental Protection Agency requirements for recreational waters are based on a study done to correlate numbers of indicator bacteria with incidence of bather illness. Associated regulations indicate that water used for this purpose should contain no more than 200 fecal coliforms per 100 ml fresh water or no more that 126 \textit{E. coli} per 100 ml. Since host-of-origin of these indicator organisms is an important issue, a variety of methods for host-association of specific microbial strains have subsequently been developed.

Practical application of these methods for monitoring water quality and pollution sources generally occurs in the following sequence. Basic water sampling procedures are performed routinely by various governmental agencies. In the event that unacceptable levels of sentinel organisms are found, microbial source tracking (MST) procedures may be chosen to provide evidence of host source(s) of pollution. Examples of potential sources of fecal pollution are: human sewage treatment systems (private and collective), concentrated animal feeding operations (CAFOs), pastured animals, pet animals, migratory birds and wild animals.

Methods

MST methods vary widely with respect to technologic basis. Many relate to determination of differences in bacterial genotype or phenotype. Some require culture of bacterial isolates and development of a database or reference library of profiles of environmental strains. Others directly target specific host-associated microbial genes and, therefore, do not require a library.

Antibiotic resistance analysis (ARA) and carbon source utilization are examples of phenotypic methods. These techniques require extensive libraries for reference comparison. Genotypic methods, such
as ribotyping (based on differences in the 16S ribosomal RNA gene); rep-PCR (based on the presence and location of multiple repeat elements within the bacterial chromosome); and pulsed-field-gel electrophoresis (PFGE) are based on genetic variation between bacterial strains and also require reference libraries. Detection of F+ phages (viruses that infect certain bacteria) is indicative of the presence of human feces as is the detection of human-associated polyomavirus, adenoviruses or enteroviruses. Chemical methods, particularly focused on human-related compounds such as caffeine or laundry detergents, have also been successfully applied for source tracking. Direct detection of specific pathogens is theoretically useful but not generally practical due to relative scarcity of these targets in the environment.

Relative qualities of the most commonly applied technologies employed for MST are shown in Table 1. High levels of associated specificity and sensitivity are ultimately the most important characteristics. Accuracy in predicting host source(s) of pollution is essential. Concomitant low levels of false negative and positive results are also of extreme importance.

Table 1. Comparison of selected MST methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
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</tr>
</thead>
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<td>inexpensive</td>
<td>Library required</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inexpensive</td>
<td></td>
<td>Harwood (2003)</td>
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<td>Ribotyping</td>
<td>High specificity</td>
<td>Library required</td>
<td>Parveen (1999)</td>
</tr>
<tr>
<td>Rep-PCR</td>
<td>High specificity</td>
<td>Library required</td>
<td>Dombek (2000)</td>
</tr>
<tr>
<td>PFGE</td>
<td>Very high specificity</td>
<td>Lengthy procedure</td>
<td>Myoda (2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Library required</td>
<td></td>
</tr>
<tr>
<td>Host-specific PCR</td>
<td>Good to high accuracy; may be universal</td>
<td>Few, if any</td>
<td>Bernhard (2000)</td>
</tr>
<tr>
<td>F+ phage typing</td>
<td>Good accuracy</td>
<td>Often not detected</td>
<td>Gerba (1987)</td>
</tr>
<tr>
<td>Enterovirus/</td>
<td>Good accuracy</td>
<td>Sophisticated equipment needed</td>
<td>Noble (2001)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td></td>
<td></td>
<td>Jiang (2001)</td>
</tr>
<tr>
<td>Pathogen ID</td>
<td>Excellent accuracy</td>
<td>Scarce targets</td>
<td>Griffin (2001)</td>
</tr>
<tr>
<td>Chemical methods</td>
<td>Indicative of human impact</td>
<td>Often not detected</td>
<td>McDonald (2006)</td>
</tr>
</tbody>
</table>

To more clearly describe the mechanics used for discrimination between host-associated bacterial strains the following two examples are presented in detail. Example 1, DNA fingerprinting via rep-PCR, is based on the presence of repeated elements in the *E. coli* chromosome. These elements vary in number of copies and location (Figure 1).
The “fingerprint pattern”, resembling a bar-code, becomes the identification signature of *E. coli* strains in the human or animal gut. Short pieces (primers) of DNA are designed to match and bind to specific DNA sequences between these repeats. Primer attachment initiates the polymerase chain reaction (PCR) which multiplies these “intergenic” regions millions of times so that they can be visualized in an agar gel after electrophoresis. These patterns are captured in digital form and subjected to computer-assisted analysis. “Unknown” patterns of *E. coli*, isolated from environmental water samples (Figure 2), are compared for “closest-match” to known-host patterns in the computer database.

**Figure 1. rep-PCR test based on location of target gene in *E. coli*.**

**Figure 2. Fecal *E. coli* isolates from Little Sac River water samples.**
Example 2, host-specific gene targeting, is an increasingly popular MST approach due to its high level of accuracy and its library-independence. These PCR methods (Table 2) using particular host-specific primer sets have been reported as being indicative of feces from human (Bernhard and Field, 2000; Carson et al., 2005; Shanks et al., 2007); dog (Dick et al., 2005); cattle (Bernhard and Field, 2000; Reischer, 2006); horse (Dick et al., 2005); pig (Dick et al., 2005; Ufnar et al., 2007); chicken (Lu et al., 2007); turkey (Lu et al., 2007); duck (Hamilton et al., 2006); and goose (Hamilton et al., 2006).

**Table 2. Microbial gene-specific MST targets indicative of host source of fecal pollution.**

<table>
<thead>
<tr>
<th>Host source</th>
<th>Microbial targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Bacteroides, Enterococcus, Methanobrevibacter, unknown*, viruses</td>
</tr>
<tr>
<td>Dog</td>
<td>Bacteroides</td>
</tr>
<tr>
<td>Cattle</td>
<td>Bacteroides, Methanobrevibacter</td>
</tr>
<tr>
<td>Horse</td>
<td>Bacteroides</td>
</tr>
<tr>
<td>Pig</td>
<td>Bacteroides, Methanobrevibacter</td>
</tr>
<tr>
<td>Chicken</td>
<td>Unknown*</td>
</tr>
<tr>
<td>Turkey</td>
<td>Unknown*</td>
</tr>
<tr>
<td>Duck</td>
<td>E. coli</td>
</tr>
<tr>
<td>Goose</td>
<td>E. coli</td>
</tr>
</tbody>
</table>

* Unknown = based on metagenomics

*Enterococcus, Bacteroides, Methanobrevibacter* and viruses are among those target microbes reportedly used in this approach. Genomic targets, related to unknown and yet uncultured bacteria, have also been derived by use of metagenomics (Sadowsky and Santo Domingo, 2007). *Bacteroides*, for example, are anaerobic bacteria which occur in dominant numbers in human and nonhuman feces and exist as different host-associated strains. Particular target gene sequences are chosen for their specific presence in the genomes of *Bacteroides* strains which inhabit the gut of specific hosts (human or animal). Matching primers are designed for strict attachment only to those targets and the PCR amplifies millions of DNA molecules indicative of a specific bacterial strain. The PCR products, as previously described, form a “band” of specific size (Figure 2); indicative of the presence of the host-associated bacterial strain in an environmental water sample.

We have applied several MST methods for environmental water quality monitoring throughout Missouri. One example is an analysis done on the Upper Shoal Creek watershed. This region encompasses a three county (Newton, McDonald and Barry) area in extreme Southwestern Missouri. It is one of the most agriculturally productive areas in the state, with 91,000 acres in the watershed. Ninety percent is pasture land grazed by over 300,000 head of cattle and fertilized by spreading poultry litter. There are 50-80 million poultry produced in the area yearly. Thirteen miles of Shoal Creek are designated as impaired, by regulatory agencies, due to high fecal coliform levels. Table 3 shows the results of MST in the Shoal Creek watershed using the rep-PCR with a five-class reference library. Water sample collection dates are presented with total fecal coliform counts (related to each water sample) and numbers of DNA fingerprint patterns assigned to each library host class. Study conclusions were as follows: cattle (particularly in streams) contribute substantially to water pollution; waste from pastured animals and spread poultry litter also contribute via runoff to streams; and there are multiple host sources of feces that combine for the total contribution.
Table 3. Shoal Creek BST data.

<table>
<thead>
<tr>
<th>Date</th>
<th>FC/100ml</th>
<th># Patterns</th>
<th>Cattle</th>
<th>Dogs</th>
<th>Human</th>
<th>Poultry</th>
<th>Wildlife</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/29/02</td>
<td>1,470</td>
<td>14</td>
<td>6</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>7/23/02</td>
<td>637</td>
<td>18</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>8/2/02</td>
<td>870</td>
<td>19</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>5/7/03</td>
<td>300</td>
<td>16</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>6/12/03</td>
<td>125</td>
<td>9</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion

MST methods are powerful tools to resolve questions of host sources of fecal pollution and associated high bacterial counts in water. Current consensus is to simultaneously use a combination of methods with different targets. Results must be interpreted carefully, combined with local observations and based on multiple samples collected over a period of time.

MST methods can be applied to saltwater and freshwater. In some cases the information required relates simply to determination of the presence of human vs. nonhuman pollution. Further discrimination between animal hosts, with respect to presence and relative contribution, can also be accomplished. Geographic and temporal variation of host-associated bacterial strains is known to exist and must be considered. Therefore, watershed specific analysis is often recommended and the method of choice may require a location-specific target. Water pollution is an issue that is highly visible, often emotionally charged and even politically and legally sensitive. The technology is now available for producing factual information (as opposed to supposition) to support conclusions. In our experience there is rarely a single host-source of pollution, but rather a combination of sources. Therefore, use of MST has the potential to encourage meaningful dialogue and facilitate solutions to problem situations. Only through development of science-based remediation efforts involving all stakeholders, can equitable plans be designed to protect our water resources.

References


Technical Session
Efficacy of Essential Oils as Dietary Supplements for Dairy Cows

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Summary

Effects of essential oils (EO) on rumen microbial fermentation in vitro are well established in the literature, but the impact of dietary EO supplementation on ruminant animal performance has been equivocal (Calsamiglia et al., 2007). Seven reports on the effects of EO supplemented in diets fed to lactating dairy cows were reviewed herein. Averaged across all treatment comparisons, EO increased DMI and yields of milk, FCM, fat and protein by 0.4, 0.9, 1.4, 0.07, and 0.03 kg/cow/d over control; these responses to EO increased milk income minus feed cost $0.27 to $0.42/cow/d depending on the milk component and feed prices evaluated. Milk fat and protein percentage and feed efficiency responses to EO were positive on average. Other reported (P < 0.10) in vivo responses were increased ruminal OM and N digestibility (Yang et al., 2007), increased ruminal pH and reduced total VFA (Benchaar et al., 2007), and increased total tract ADF digestibility and ruminal pH (Benchaar et al., 2006). Unpublished results of a recent UW-Madison trial to evaluate transition cow and 15-wk postpartum lactation performance responses to dietary EO supplementation were reported herein. Treatments were a control diet and an EO diet supplemented with 1.2 g/cow/d EO mixture (CRINA Ruminants) fed to 20 multiparous Holstein cows per treatment from 4 wk prepartum through 15 wk of lactation. Transition cow measurements were unaffected by EO. Lactation DMI was 1.8 kg/cow/d lower for EO (P < 0.04). Milk yield was numerically lower for EO during lactation wk 1-5 (-2.4 kg/cow/d), similar during wk 6-10, and numerically higher (+2.1 kg/cow/d) for EO during wk 11-15. Average feed efficiencies (Milk/DMI and FCM/DMI) tended to be greater for EO (P < 0.08 and P < 0.07, respectively). Feed efficiency was unaffected by treatment during lactation wk 1-5, but was greater for EO during wk 6-10 and wk 11-15 (P < 0.04 and P < 0.02, respectively). In a meta analysis performed on combined data from the literature review and the UW-Madison trial, milk, fat and protein yields were 1.2 (P < 0.04), 0.06 (P < 0.03) and 0.05 (P < 0.06) kg/d, respectively, higher for EO. More dairy cattle research regarding potential interactions between basal diet, stage of lactation and dietary EO supplementation is warranted.

Introduction

Newbold et al. (2006) and Calsamiglia et al. (2007) described EO as follows: volatile aromatic compounds with an oily appearance extracted from plant materials typically by steam distillation; alcohol, ester or aldehyde derivatives of phenylproponoids and terpenoids. Some of the more common EO compounds available include thymol (thyme and oregano), eugenol (clove), pinene (Juniper), limonene (dill), cinnamaldehyde (cinnamon), capsaicin (hot peppers), terpinene (tea tree), allicin (garlic), anethol (anise), etc. They have antimicrobial activity and have been shown to modify rumen microbial fermentation. With regard to EO as modifiers of rumen microbial fermentation, Calsamiglia et al. (2007) from an extensive review of the literature (primarily in vitro, in situ or continuous culture based) concluded the following: inhibition of deamination and methanogenesis, which results in lower ammonia-N, methane and acetate and higher propionate and butyrate concentrations; effects may vary depending on the specific EO or combination of EO supplemented; effects of some EO are pH and diet dependent.
Readers are referred to Calsamiglia et al. (2007) for an in depth review of EO and effects on rumen microbial fermentation. The purpose of this paper is to review and summarize the available reports involving EO as dietary supplements for dairy cows and effects on lactation performance.

**Literature Review**

Seven reports on the effects of EO supplemented in diets fed to lactating dairy cows were reviewed. Six of these reports involved the CRINA ruminants (CRINA S.A., Gland, Switzerland) mixture of natural and synthesized EO including thymol, eugenol, vanillin, guaiacol, and limonene. The other report involved EO (Axiss France SAS, Bellegarde-sur-Valserine, France) from garlic (allicin) and juniper berry (pinene) fed separately. The seven experiments are described in Tables 1 (EO tested, experimental design, and cows), 2 (Diet ingredient and nutrient composition and control DMI and milk yield), and 3 (Experimental measurements). There were 9 and 10 treatment comparisons, respectively, for intake and production related measurements across the seven experiments.

**Table 1. Literature review: Essential oils tested, experimental design, and cows.**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Essential Oils Product</th>
<th>Experimental Design</th>
<th>Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al., 2007</td>
<td>Garlic^1 5 g/cow/d  Juniper Berry^1 2 g/cow/d</td>
<td>4x4 LS^4 21-d periods</td>
<td>n=4 &gt;113 DIM^4 Parity&gt;1</td>
</tr>
<tr>
<td>Benchaar et al., 2007</td>
<td>Crina^2 0.75 g/cow/d  Juniper Berry^1 2 g/cow/d</td>
<td>4x4 LS 28-d periods</td>
<td>n=4 &gt;61 DIM Parity&gt;1</td>
</tr>
<tr>
<td>Benchaar et al., 2006</td>
<td>Crina 2 g/cow/d</td>
<td>4x4 LS 28-d periods</td>
<td>n=4 &gt;98 DIM Parity&gt;1</td>
</tr>
<tr>
<td>Offer et al., 2005</td>
<td>Crina 0.5, 1, and 2 g/cow/d</td>
<td>4x4 LS 28-d periods</td>
<td>n=16 &gt;50 DIM Parity&gt;1</td>
</tr>
<tr>
<td>Schmidt et al., 2004</td>
<td>Crina 1.2 g/cow/d</td>
<td>RCB^5 56-d period</td>
<td>Parity=1 n=4 Parity&gt;1 n=26 &gt;50 DIM</td>
</tr>
<tr>
<td>Varga et al., 2004</td>
<td>Crina 1.2 g/cow/d</td>
<td>Unreplicated pens 120-d period</td>
<td>n=170 High group Parity 1 &amp; &gt;</td>
</tr>
<tr>
<td>LaCount, 1997</td>
<td>Crina 1.5 g/cow/d</td>
<td>CRD^6 70-d period</td>
<td>n=33 &gt;42 DIM Parity&gt;1</td>
</tr>
</tbody>
</table>

^1 Axiss France SAS, Bellegarde-sur-Valserine, France; Garlic standardized at 1.5% of allicin; Juniper Berry standardized at 35% of pinene.
^2 CRINA Ruminants, CRINA S.A., Gland, Switzerland; Mixture of natural and synthesized essential oils including thymol, eugenol, vanillin, guaiacol, and limonene.
^3 Latin square design. ^4 Days in milk. ^5 Randomized complete-block design. ^6 Completely randomized design.
Table 2. Literature review: Diet ingredient and nutrient composition and control DMI and milk yield.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Diet Ingredient Composition (DM basis)</th>
<th>Diet Nutrient Composition (DM basis)</th>
<th>Control DMI kg/d</th>
<th>Control Milk kg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al., 2007</td>
<td>40:60 F:C&lt;sup&gt;1&lt;/sup&gt;</td>
<td>16% CP, 32% NDF, &amp; 33% Starch</td>
<td>20.7</td>
<td>29.0</td>
</tr>
<tr>
<td></td>
<td>Barley silage&amp; grain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benchaar et al., 2007</td>
<td>50:50 F:C&lt;sup&gt;2&lt;/sup&gt; or CS&lt;sup&gt;3&lt;/sup&gt;</td>
<td>16% CP, 38% NDF, &amp; 21% Starch</td>
<td>17.5</td>
<td>28.9</td>
</tr>
<tr>
<td></td>
<td>Corn &amp; barley grain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benchaar et al., 2006</td>
<td>48:52 F:C&lt;sup&gt;4&lt;/sup&gt;</td>
<td>19% CP, 36% NDF, &amp; 20% Starch</td>
<td>22.6</td>
<td>34.3</td>
</tr>
<tr>
<td></td>
<td>75:25 Grass silage:CS Corn grain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-/+ 350 mg/d monensin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offer et al., 2005</td>
<td>Grass silage ad lib 12 kg/d (as fed) DC&lt;sup&gt;5&lt;/sup&gt;</td>
<td>19% CP &amp; 35% NDF</td>
<td>20.8</td>
<td>31.1</td>
</tr>
<tr>
<td>Schmidt et al., 2004</td>
<td>50:50 F:C&lt;sup&gt;6&lt;/sup&gt;</td>
<td>16% CP, 35% NDF, &amp; 19% Starch</td>
<td>26.4</td>
<td>39.8</td>
</tr>
<tr>
<td></td>
<td>50:30:20 CS:AS:AH&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corn grain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varga et al., 2004</td>
<td>42:58 F:C&lt;sup&gt;8&lt;/sup&gt;</td>
<td>18% CP, 31% NDF, &amp; 27% Starch</td>
<td>NA&lt;sup&gt;9&lt;/sup&gt;</td>
<td>40.1</td>
</tr>
<tr>
<td></td>
<td>70:30 CS:AS High in byproducts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LaCount, 1997</td>
<td>51:49 F:C&lt;sup&gt;10&lt;/sup&gt;</td>
<td>18% CP &amp; 35% NDF</td>
<td>22.5</td>
<td>44.0</td>
</tr>
<tr>
<td></td>
<td>50:50 CS:AS Pelleted complete feed</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Forage:Concentrate Ratio. <sup>2</sup> Alfalfa silage. <sup>3</sup> Corn silage. <sup>4</sup> 18% CP (as-fed basis) Dairy concentrate. <sup>5</sup> Alfalfa hay. <sup>6</sup> Not available.

Table 3. Literature review: Experimental measurements.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al., 2007</td>
<td>Ruminal fermentation parameters; Ruminal &amp; total tract nutrient digestibility; Duodenal nutrient flows; intake &amp; production</td>
</tr>
<tr>
<td>Benchaar et al., 2007</td>
<td>Ruminal microbial counts &amp; fermentation parameters; Total tract nutrient digestibility; N balance; intake &amp; production; milk fatty acid profiles</td>
</tr>
<tr>
<td>Benchaar et al., 2006</td>
<td>Ruminal fermentation parameters &amp; protozoa counts; Ruminal in situ nutrient degradation; Total tract nutrient digestibility; N balance; intake &amp; production; milk fatty acid profiles</td>
</tr>
<tr>
<td>Offer et al., 2005</td>
<td>Intake &amp; production</td>
</tr>
<tr>
<td>Schmidt et al., 2004</td>
<td>Intake &amp; production</td>
</tr>
<tr>
<td>Varga et al., 2004</td>
<td>Production field trial; Continuous culture fermenters</td>
</tr>
<tr>
<td>LaCount, 1997</td>
<td>Intake &amp; production</td>
</tr>
</tbody>
</table>
DMI, milk yield, composition and component yield, and feed efficiency responses to EO relative to control are presented in Table 4. Averaged across all treatment comparisons, EO increased DMI and yields of milk, FCM, fat and protein by 0.4, 0.9, 1.4, 0.07, and 0.03 kg/cow/d over control. Milk fat and protein percentage and feed efficiency responses to EO were positive on average.

Table 4. Literature review: DMI, milk yield, composition and component yield, and feed efficiency responses relative to control.

<table>
<thead>
<tr>
<th>Trial</th>
<th>DMI</th>
<th>Milk</th>
<th>FCM</th>
<th>Fat</th>
<th>Protein</th>
<th>Milk/ DMI</th>
<th>FCM/ DMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al., 2007</td>
<td>-0.3</td>
<td>+0.9</td>
<td>+2.5</td>
<td>+0.32</td>
<td>+0.14</td>
<td>-0.08</td>
<td>0</td>
</tr>
<tr>
<td>Garlic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juniper Berry</td>
<td>-0.2</td>
<td>+0.4</td>
<td>+1.8</td>
<td>+0.26</td>
<td>+0.11</td>
<td>-0.03</td>
<td>0</td>
</tr>
<tr>
<td>Benchaar et al., 2007</td>
<td>-0.1</td>
<td>-0.9</td>
<td>-0.7</td>
<td>-0.05</td>
<td>+0.01</td>
<td>-0.04</td>
<td>-0.03</td>
</tr>
<tr>
<td>Benchaar et al., 2006</td>
<td>+0.1</td>
<td>-1.3</td>
<td>-1.3</td>
<td>+0.04</td>
<td>-0.04</td>
<td>-0.01</td>
<td>-0.05</td>
</tr>
<tr>
<td>Offer et al., 2005</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 g/cow/d Crina</td>
<td>+0.3</td>
<td>+1.4</td>
<td>+1.2</td>
<td>-0.03</td>
<td>+0.04</td>
<td>+0.03</td>
<td>+0.06</td>
</tr>
<tr>
<td>1.0 g/cow/d Crina</td>
<td>+0.2</td>
<td>+1.7</td>
<td>+1.6</td>
<td>-0.01</td>
<td>+0.07</td>
<td>+0.02</td>
<td>+0.07</td>
</tr>
<tr>
<td>2.0 g/cow/d Crina</td>
<td>+0.3</td>
<td>+2.0</td>
<td>+1.8</td>
<td>-0.03</td>
<td>+0.06</td>
<td>+0.03</td>
<td>+0.08</td>
</tr>
<tr>
<td>Schmidt et al., 2004</td>
<td>+1.9</td>
<td>+1.9</td>
<td>+2.7</td>
<td>+0.10</td>
<td>+0.11</td>
<td>-0.04</td>
<td>+0.04</td>
</tr>
<tr>
<td>Varga et al., 2004</td>
<td>NA</td>
<td>+1.6</td>
<td>+1.6</td>
<td>+0.02</td>
<td>+0.06</td>
<td>+0.05</td>
<td>+0.07</td>
</tr>
<tr>
<td>LaCount, 1997</td>
<td>+1.0</td>
<td>+1.6</td>
<td>+2.6</td>
<td>+0.15</td>
<td>+0.13</td>
<td>+0.11</td>
<td>+0.10</td>
</tr>
<tr>
<td>Average</td>
<td>+0.4</td>
<td>+0.9</td>
<td>+1.4</td>
<td>+0.08</td>
<td>+0.07</td>
<td>+0.02</td>
<td>+0.03</td>
</tr>
</tbody>
</table>

P < 0.10. *Not available.

To calculate the economic value derived from EO at the average response, the following milk and feed prices were used: $3.10/kg fat, $9.18/kg protein, $0.51/kg other solids, and an add-on premium of $0.036/kg milk (based on pay period ending 10/31/07 for a Wisconsin dairy), $0.18/kg TMR DM, and $0.06/cow/d cost for 1.2 g/cow/d supplemental EO (Will Seymour, DSM, personal communication). At the average response and under this milk and feed price scenario, dietary supplementation with EO increased milk income minus feed cost $0.42/cow/d. To calculate the average economic value derived from EO under a lower milk and feed price scenario, the following milk and feed prices were used: $2.91/kg fat, $4.69/kg protein, $0.42/kg other solids, and an add-on premium of $0.030/kg milk (based on 2006 average pay prices for a Wisconsin dairy), $0.15/kg TMR DM, and $0.06/cow/d cost for supplemental EO. At the average response and under this milk and feed price scenario, dietary supplementation with EO increased milk income minus feed cost $0.27/cow/d. Responses to EO were average or above average for 7/10, 5/10 and 6/10 of milk, fat and protein yield treatment comparisons, respectively.

Other significant (P < 0.10) in vivo responses found in these seven reports are summarized in Table 5. These responses include increased ruminal OM and N digestibility (Yang et al., 2007), increased ruminal pH and reduced total VFA (Benchaar et al., 2007), and increased total tract ADF digestibility and ruminal pH (Benchaar et al., 2006).
Table 5. Literature review: Other significant (P < 0.10) responses reported.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Other P &lt; 0.10 Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al., 2007</td>
<td>ROMD¹ +5.8%; RND² +6.5%</td>
</tr>
<tr>
<td>Garlic, Juniper Berry</td>
<td>ROMD +7.1%; RND +5.7%</td>
</tr>
<tr>
<td>Benchaar et al., 2007</td>
<td>Ruminal pH +0.10; Total VFA -9.2 mM for CS³</td>
</tr>
<tr>
<td>Benchaar et al., 2006</td>
<td>TTADFD +2.9%; Ruminal pH +0.12;</td>
</tr>
<tr>
<td>Offer et al., 2005</td>
<td>NR²</td>
</tr>
<tr>
<td>Schmidt et al., 2004</td>
<td>NR</td>
</tr>
<tr>
<td>Varga et al., 2004</td>
<td>Continuous culture fermenter data</td>
</tr>
<tr>
<td>LaCount, 1997</td>
<td>NR</td>
</tr>
</tbody>
</table>

¹Ruminal organic matter digestibility (truly) as % of intake. ²Ruminal nitrogen digestibility (truly) as % of intake. ³Corn silage based diet. ⁴Total tract acid detergent fiber digestibility. ⁵None reported.

UW-Madison Trial

Our objective was to evaluate transition cow and 15-wk postpartum lactation performance responses to dietary EO supplementation. Forty multiparous Holstein cows were used in a completely randomized design. Treatments were a control diet supplemented with a placebo premix (57 g/cow/d) and an EO diet supplemented with 1.2 g/cow/d CRINA Ruminants (CRINA S.A., Gland, Switzerland; mixture of natural and synthesized EO including thymol, eugenol, vanillin, guaiacol, and limonene) provided through a premix (57 g/cow/d). Treatment diets were fed from 4 wk prepartum through 15 wk of lactation. Prepartum and lactation TMR ingredient and nutrient composition are presented in Table 6. Cows were fed individually a TMR once daily in tie-stalls and the amounts fed and refused were recorded daily. Body weights and condition scores were recorded weekly throughout the trial. Blood samples from each cow obtained prior to feeding on d -21, -7, -1, 1, 8, 15, 22, and 29 were analyzed for glucose, BHBA, NEFA, and urea-N. Milk yield was recorded daily on individual cows from throughout the lactation trial. Milk samples obtained from all cows weekly on two consecutive days of the week from am and pm harvests throughout the lactation trial were analyzed for fat, true protein, lactose and MUN concentrations.

Results are presented in Table 7 and Figures 1-3. There was no affect of EO on prepartum DMI. Lactation DMI was 1.8 kg/cow/d lower for EO (P < 0.04). Milk and component yields were unaffected by treatment. Milk true protein was 0.15%-units lower for EO (P <0.03). Milk yield was numerically lower for EO during lactation wk 1-5 (-2.4 kg/cow/d), similar during wk 6-10, and numerically higher (+2.1 kg/cow/d) for EO during wk 11-15 (Figure 1). Unfortunately, the feeding trial was not continued any further into the lactation. Average feed efficiencies (Milk/DMI and FCM/DMI) tended to be greater for EO (P < 0.08 and P < 0.07, respectively). Feed efficiency (Milk/DMI) was unaffected by treatment during lactation wk 1-5, but was greater for EO during wk 6-10 and wk 11-15 (P < 0.04 and P < 0.02, respectively; Figure 2). Average lactation energy balance tended to be lower for EO (P < 0.06). Energy balance was unaffected by treatment during lactation wk 1-5, but was lower for EO during wk 6-10 and wk 11-15 (P < 0.04 and P < 0.03, respectively; Figure 3). Control cows returned to positive energy balance during lactation wk 6-10 (+1.5 Mcal/d), while EO cows remained in slightly negative energy balance even during wk 11-15 (-0.4 Mcal/d; Figure 3). Prepartum and lactation body weight, body condition score, and blood sample measurements were unaffected by treatment.
Table 6. UW-Madison trial diet ingredient and nutrient composition (Tassoul and Shaver unpublished).

<table>
<thead>
<tr>
<th>Ingredients, % DM</th>
<th>Prefresh TMR</th>
<th>Lactation TMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa silage</td>
<td>11.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Corn silage</td>
<td>48.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Mixed Alfalfa/Grass Hay</td>
<td>--</td>
<td>3.7</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>11.0</td>
<td>--</td>
</tr>
<tr>
<td>Ground shelled corn</td>
<td>18.2</td>
<td>22.0</td>
</tr>
<tr>
<td>Soybean meal-48%</td>
<td>9.2</td>
<td>9.2</td>
</tr>
<tr>
<td>Distillers dried grains</td>
<td>--</td>
<td>9.2</td>
</tr>
<tr>
<td>Whole cottonseed-linted</td>
<td>--</td>
<td>5.6</td>
</tr>
<tr>
<td>Tallow</td>
<td>--</td>
<td>0.9</td>
</tr>
<tr>
<td>Minerals &amp; Vitamins</td>
<td>2.6</td>
<td>2.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrients†</th>
<th>Prefresh TMR</th>
<th>Lactation TMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, % as fed</td>
<td>46.1 ± 2.9</td>
<td>53.6 ± 3.0</td>
</tr>
<tr>
<td>CP %</td>
<td>12.5 ± 0.7</td>
<td>17.1 ± 0.8</td>
</tr>
<tr>
<td>NDF %</td>
<td>38.1 ± 4.6</td>
<td>35.3 ± 1.9</td>
</tr>
<tr>
<td>Starch %</td>
<td>29.9 ± 4.6</td>
<td>24.7 ± 2.1</td>
</tr>
<tr>
<td>Fat %</td>
<td>3.5 ± 0.4</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>TDN 1x %</td>
<td>68.9 ± 1.9</td>
<td>--</td>
</tr>
<tr>
<td>NEL 3x, Mcal/kg</td>
<td>--</td>
<td>1.71 ± 0.03</td>
</tr>
</tbody>
</table>

†TMR sampled weekly, composited by month, and analyzed using wet chemistry by Dairy One (Ithaca, NY).

Table 7. UW-Madison trial results (Tassoul and Shaver unpublished).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Crina</th>
<th>SEM</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepartum DMI, kg/d</td>
<td>13.8</td>
<td>13.1</td>
<td>0.4</td>
<td>NS†</td>
</tr>
<tr>
<td>Lactation DMI, kg/d</td>
<td>24.5</td>
<td>22.7</td>
<td>0.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Milk Yield, kg/d</td>
<td>48.2</td>
<td>48.1</td>
<td>1.1</td>
<td>NS</td>
</tr>
<tr>
<td>4% FCM, kg/d</td>
<td>43.9</td>
<td>44.0</td>
<td>1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Fat %</td>
<td>3.48</td>
<td>3.46</td>
<td>0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Fat kg/d</td>
<td>1.65</td>
<td>1.64</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>True Protein %</td>
<td>3.10</td>
<td>2.95</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>True Protein kg/d</td>
<td>1.46</td>
<td>1.41</td>
<td>0.06</td>
<td>NS</td>
</tr>
<tr>
<td>MUN, mg%</td>
<td>12.9</td>
<td>13.4</td>
<td>0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Milk/DMI</td>
<td>1.99</td>
<td>2.15</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>FCM/DMI</td>
<td>1.83</td>
<td>1.98</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>Lactation EB 2, Mcal/d</td>
<td>-1.1</td>
<td>-3.6</td>
<td>0.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Body Condition Score</td>
<td>Prepartum</td>
<td>3.9</td>
<td>3.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Body Weight, kg</td>
<td>Prepartum</td>
<td>734.2</td>
<td>745.3</td>
<td>16.0</td>
</tr>
<tr>
<td>Blood Data†</td>
<td>NEFA, mEq/L</td>
<td>524.1</td>
<td>530.9</td>
<td>34.5</td>
</tr>
<tr>
<td></td>
<td>BHBA, mg/dL</td>
<td>6.9</td>
<td>7.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Urea-N, mg/dL</td>
<td>53.8</td>
<td>55.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**Not significant (P > 0.10).  †Energy balance = ((DMI*NEL\_Diet) – ((0.08*BW^{0.75})+(NEL\_Milk*Milk))).  ‡Averaged across -21, -7, -1, 1, 8, 15, 22, and 29 d samples.**
Figure 1. Milk yield (kg/d) summarized by 5-wk slices from wk 1-15 of lactation (P >0.10 differences and SEM = 1.3 kg/d for each slice).

Figure 2. Feed efficiency (kg Milk/ kg DMI) summarized by 5-wk slices from wk 1-15 of lactation (Slice 1 - P > 0.10; Slice 2 - P < 0.04; Slice 3 - P < 0.02; SEM = 0.07 by slice).
Figure 3. Energy balance (Mcal/d) summarized by 5-wk slices from wk 1-15 of lactation (Slice 1 - P > 0.10; Slice 2 - P < 0.04; Slice 3 - P < 0.03; SEM = 1.1Mcal/d by slice).

Meta Analysis

Combined data from the literature review and the UW-Madison trial were analyzed using the MIXED procedure of SAS to evaluate animal response to dietary EO supplementation for DMI and milk, fat and protein yields. The model included the fixed effect of EO supplementation and the random effect of trial (St. Pierre, 2001). Each response was weighted according to the number of animals used to test for it using the WEIGHT statement. DMI was unaffected by treatment (P > 0.10). Milk, fat and protein yields were 1.2 (P < 0.04), 0.06 (P < 0.03) and 0.05 (P < 0.06) kg/d, respectively, higher for EO.

Conclusions

Averaged across all treatment comparisons from the reports reviewed, EO increased DMI and yields of milk, FCM, fat and protein; these responses to EO increased milk income minus feed cost $0.27 to $0.42/cow/d depending on the milk component and feed prices evaluated. Milk fat and protein percentage and feed efficiency responses to EO were positive on average. In a recent UW-Madison trial: transition cow measurements were unaffected by EO; lactation DMI was lower for EO (P < 0.04); milk yield was numerically higher (+2.1 kg/cow/d) for EO during lactation wk 11-15; average feed efficiencies tended to be greater for EO; feed efficiency was greater for EO during lactation wk 6-10 and wk 11-15 (P < 0.04 and P < 0.02, respectively). In a meta analysis performed on combined data from the literature review and the UW-Madison trial, milk, fat and protein yields were 1.2 (P < 0.04), 0.06 (P < 0.03) and 0.05 (P < 0.06) kg/d, respectively, higher for EO. More dairy cattle research regarding potential interactions between basal diet, stage of lactation and dietary EO supplementation is warranted.
References


VITAMIN E DEFICIENCY IS A RISK FACTOR FOR EQUINE MOTOR NEURON DISEASE

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Summary

Epidemiological, pathological, laboratory, and experimental studies all support the hypothesis that equine motor neuron disease is an oxidative disorder associated with prolonged vitamin E deficiency. The role that pro-oxidants play in the disease has not been determined. All horses without access to green forage and/or with low plasma vitamin E levels should be supplemented with vitamin E.

Introduction

Natural vitamin E is comprised of eight different forms $\alpha$, $\beta$, $\gamma$, and $\delta$ tocopherols and $\alpha$, $\beta$, $\gamma$ and $\delta$ tocotrienols (Feki et al., 2001; Brigelius-Flohe and Traber, 1999; Zingg 2007). Vitamin E occurs naturally in plants (highest in the leaf and seeds) with all isomers having similar antioxidant potential. In mammals, $\alpha$-tocopherol is the predominant form of vitamin E being concentrated in the mammalian body by the $\alpha$-tocopherol transfer protein. Vegetable oils often have high concentrations of tocopherols and tocotrienols with soybean oil reportedly particularly high in $\delta$-tocopherol and olive and sunflower oils particularly high in $\alpha$-tocopherol. The overall antioxidant properties of the different forms of vitamin E are similar, although $\alpha$, presumably due to its selective retention in liver and high concentration storage in adipose tissue has the greatest effect in mammals (Zingg, 2007; Chandan et al., 2007; Hargreaves, 2002).

Most natural $\alpha$-tocopherol sold for a supplementation comes from vegetable seed oil and marketed as d-$\alpha$-tocopherol or d-$\alpha$-tocopherol acetate. The biologic activity of d-$\alpha$-tocopherol acetate is 1.36 IU/mg and dl-$\alpha$-tocopherol 1.10 IU/mg and dl-$\alpha$-tocopherol acetate the lowest (Acuff et al., 1994). Papas suggested that in the equine, d-$\alpha$-tocopherol acetate had nearly 3x the bioavailability/effectiveness as the same amount of dl-$\alpha$-tocopherol acetate (Papas et al., 1990).

Absorption and Metabolism of Vitamin E

Vitamin E molecules are absorbed in esterified forms which are hydrolyzed to a free form. Bile emulsifies the tocopherols and tocotrienols to micells which are absorbed across the gut as chylomicrons. Tocotrienols may be directly deposited along with triglycerides into adipose tissue, the main storage site for all vitamin E. The tocopherols circulate in the lymph and associated with lipoprotein lipase, are transferred to lipoproteins (apolipoprotein E) or into tissue. Apolipoprotein E enters the liver and then is secreted with VLDL where it is further hydrolyzed by lipase to LDL which, in most species, contains most of the circulating tocopherols. The liver $\alpha$-tocopherol transfer protein is important in the incorporation of $\alpha$ tocopherol into VLDL. This protein is responsible for recirculating and concentrating $\alpha$-tocopherol in plasma. Additionally, $\alpha$-tocopherol binding protein (TBP) enhances the transport of $\alpha$-tocopherol to mitochondria (Zingg, 2007; Hargreaves, 2002). The turnover rate of $\alpha$-tocopherol is rapid in
rat lung and liver, higher in spinal cord (76 days), likely reflecting the importance of vitamin E as a neuroprotectant and 4 months in adipose tissue, the primary storage site (Ingold et al., 1987).

**Action of Vitamin E**

Vitamin E action is mostly, but not solely, as a chain-breaking antioxidant. Vitamin E is the major lipid soluble antioxidant in cell membranes. It is a radical scavenging antioxidant that inhibits chain initiation and propagation of lipid peroxidase in cell membranes (Zingg, 2007; Hargreaves, 2002).

Briefly, reactive oxygen species (ROS, e.g., OH and NO\(_2\)) are normally formed with oxygen consumption. The ROS attack lipids (which is a component of all cell membranes) to further form peroxyl radicals which keep the “chain” going. As a ROS scavenging molecule, vitamin E works in conjunction with vitamin C (which has a sparing and recycling promotion effect of the oxidized vitamin E (\(\alpha\)-tocopherol quinone) and glutathione peroxidase, both water soluble cytosol antioxidants. Undoubtedly, the activity of the vitamin E are more diverse than as a chain breaking antioxidant, likely having an effect on platelet aggregation and signal transduction and gene expression in a number of cells. Although most vitamin E research has focused on biologic benefits of \(\alpha\)-tocopherol, the other tocopherols and the tocotrienols are now known to possess neuroprotective and anticancer properties not exhibited by \(\alpha\)-tocopherol, \(\gamma\)-tocopherol, and \(\delta\)-tocotrienol are vitamin E molecules that provide functions distinct from \(\alpha\)-tocopherol (Chandan et al., 2007). Vitamin E may also be crucial for maintenance of immune competence.

In the late gestating mare, vitamin E supplementation has also been shown to increase IgG and IgM in colostrums and nursing foal serum (Hoffman et al., 1999). Vitamin E administered to horses maintained on an otherwise low vitamin E diet caused an increased humoral immune response to routine vaccines (Bualsrud and Overnes, 1986).

**Vitamin E in Horse Feeds**

As previously mentioned, the greatest source would be in leafy green pastures and some cereal grains. Therefore, it is no surprise that seasonal variation in plasma vitamin E has been reported in horses with highest levels during grazing periods and lowest at the end of winter as reserves are depleted (Blakley and Bell, 1994; Mäenpää et al., 1988). Storage of hays, prolonged exposure to sunlight, exercise, moisture, and high temperatures will decrease vitamin E concentration. Most stored hays have < 50 IU/kg DM, although concentration may vary.

Natural vitamin E in stored concentrate could provide antioxidant protection for both the feed and the horse. Unfortunately, much of the natural vitamin E in processed and stored grain concentrates are destroyed by heat processing, storage, minerals, etc. Synthetic vitamin E, which are esters of \(\alpha\) tocopherol do not have all the biologic properties of natural vitamin E and are less potent on a mg to mg basis, may not protect the feed against oxidative damage, but are more stable and provide antioxidant benefits to the horse (Hargreaves, 2002).

**Vitamin E Deficient Disease in the Horse**

Equine motor neuron disease (EMND) is a naturally occurring neurodegenerative disease of the somatic lower motor neuron system in adult horses. The disease was first described in 11 horses in 1990 and bore clinical resemblance to a progressive muscular atrophy form of ALS, a motor neuron disease of humans (Cummings et al., 1990). EMND exists in many parts of the world.
Clinical signs of EMND include weight loss from muscle wasting, trembling, muscle fasciculations, and prolonged periods of recumbency (Divers et al., 1994). Accumulation of lipopigment in spinal cord capillaries and retinal pigment epithelium (RPE) and the predilection for denervation of the highly oxidative type I muscle fibers suggest that EMND is an oxidative disorder (Cummings et al., 1995). Epidemiologic studies (Mohammed et al., 1994; de la Rua-Domenech et al., 1995a; de la Rua-Domenech et al., 1995b; de la Rua-Domenech et al., 1997a) reveal that absence of green forage for at least 18 months, high-grain diets, and coprophagia or geophagia were risk factors for EMND. Low plasma concentrations of the antioxidant vitamin E has been a consistent laboratory finding in clinical cases (de la Rua-Domenech et al., 1997b). Analysis of the mineral content of spinal cords from affected horses and age-matched controls reveals increased amounts of copper (a potential pro-oxidant) in the spinal cord of horses with EMND. The finding of hepatic iron (another potential pro-oxidant) content has also been reported. These findings suggest that a deficiency in antioxidant elements (vitamin E) and an excess of pro-oxidant elements (copper and iron) may be responsible for causing EMND.

We have conducted a study to determine if EMND could be induced in adult horses fed a diet low in vitamin E and high in copper and iron. Eight horses were fed a low vitamin E, high copper and iron diet for up to 30 months. Four control horses, fed the same concentrate but with normal vitamin E, copper and the iron and the same source of hay but without the 1 year storage which occurred for the experimental horse hay, were used to compare hepatic concentration of measured variables. Plasma vitamin E concentrations in all 8 experimental horses fed a low vitamin E diet decreased significantly over time, and values (0.09 to 0.51 µg/mL) at euthanasia or study end were less than reference range (Divers et al., 2006). Four horses developed clinical signs and pathologic lesions of EMND. The mean ± SD of the regression coefficient for change in vitamin E over time was not different between experimental horses that developed EMND (-0.043 ± 0.009) and experimental horses that did not develop EMND (-0.053 ± 0.009). There was no significant change in the concentrations of vitamin A, selenium, copper and ferritin over time. Although plasma selenium concentrations decreased some during the study period in all 8 experimental horses, changes were not significant and values at euthanasia or study end were only slightly less than laboratory reference values. There was no significant change in vitamin A concentration in any of the experimental horses, with all 8 horses beginning and ending the trial with values in or slightly less than the reference range. Plasma copper concentrations did not change over time in the experimental horses, and all horses had plasma copper concentrations within reference range throughout the study. Values for serum ferritin concentration were slightly greater than reference range in 7 treatment horses (281 to 528 ng/mL) and were within reference range in 1 horse upon entry into the study. Serum ferritin values were variable during the study, and no horse had a significant increase in serum ferritin concentration over time. One horse had a significant decrease in serum ferritin over time. There were no differences in mean serum ferritin or plasma vitamin E, vitamin A, copper, or selenium concentrations at the time of euthanasia or study end between experimental horses that developed clinical signs of EMND and experimental horses that did not.

At the end of the 30-mo treatment period, the 4 experimental horses that did not have clinical signs of EMND appeared clinically normal, except that 1 horse had lost weight (77 kg). That horse was the only surviving horse in the treatment group that had obvious lesions (i.e. 1 to 2 degenerative fibers/fascicle and bands of Schwann cells and endoneurium resulting from complete nerve fiber degeneration) in the spinal accessory nerve at the time biopsy specimens were collected at the end of 30 mo. The other 3 surviving horses had either no definitive lesions of EMND (1 horse) in the spinal accessory nerve or minimal lesions (i.e. demyelinated fibers in scattered nerve fascicles; 2 horses).

Mean ± SE dry-weight hepatic vitamin E concentration was low (2.57 ± 1.25 µg/g) and significantly different in the 4 experimental horses with signs of EMND, compared with reference
values and values in the 4 control horses (21.1 ± 1.3µg/g). Mean ± SE wet-weight hepatic copper concentration (503.5 ± 65.59 ppm) in horses with EMND was more than 50 x the upper limit of the reference range and more than 100 x the mean value for liver copper concentration in the 4 control horses (4.06 ± 0.44 ppm), differences that were significant. Only one of the horses with EMND had wet-weight hepatic iron concentrations greater than the reference range for the testing laboratory, and there was no significant difference in mean values between horses with EMND (462.3 ± 113.71 ppm) and the 4 control horses (251.0 ± 27.54 ppm). Hepatic wet-weight selenium concentration was within reference range in all 4 horses with EMND (1.59 ± 0.16 ppm) and in 3 of 4 control horses (1.27 ± 0.19 ppm). Although dry-weight hepatic vitamin A concentrations were slightly less than reference range in 3 of the experimental horses that developed EMND, the mean value (317.8 ± 55.8 µg/g) was not significantly different from that in the control horses (476.3 ± 26.3 µg/g). No control horses developed EMND and each had liver concentrations of vitamin E, copper, iron, selenium and vitamin A that were within normal range.

Our results yielded further evidence that lack of access to pasture and deficiency of vitamin E are important risk factors for EMND. The 21-mo interval between the beginning of the study and development of clinical signs in the experimental horses correlates with our previous findings that, in naturally occurring EMND, affected horses had been on the property for at least 18 mo prior to developing clinical signs. Tissue stores of vitamin E are likely abundant in most horses that have seasonal access to green forage, and several months of feeding a vitamin E-deficient diet may be required to develop a deficiency severe enough for oxidative injury and EMND to occur. The gradual depletion of vitamin E in the experimental horses was apparent in the decline of plasma vitamin E concentrations over time. The experimental horses were fed a diet similar to that consumed by horses with naturally occurring EMND (eg, absence of pasture and green hay). Although most horses with naturally occurring EMND were reportedly fed a variety of commercial concentrates containing variable amounts of added synthetic vitamin E, the amounts of vitamin E added to equine concentrate feeds have been low until recently and may not prevent vitamin E deficiency or EMND if access to green forage is minimal. The synthetic vitamin E added to concentrates is usually dl-α-tocopherol acetate (recently renamed all-me-tocopherol acetate), which has both lower bioavailability and lower potency than natural vitamin E (recently renamed RRR-α-tocopherol).

We believe that our results, together with results of previous studies, indicate that vitamin E deficiency has a major causative role in EMND. Although all 8 experimental horses developed severe deficiencies in plasma vitamin E, only 4 horses developed classic clinical signs of EMND during the 30-mo study. Vitamin E was not measured in spinal cord tissue of the horses, but in humans, tocopherol concentrations in CSF are highly correlated with serum concentrations (Vatassery et al., 2004). It is uncertain why some horses develop EMND but others fed the identical feed and that have similar plasma vitamin E concentrations do not. This finding was also observed in our field studies of naturally occurring disease in which only 1 or 2 horses in an at-risk stable developed clinical signs of EMND, although nutritional, managemental, and environmental conditions were similar for all horses in the stable. This suggests there could be individual susceptibility to a disturbed antioxidant-pro-oxidant balance (oxidative stress). The fact that there were obvious histologic findings of EMND in 1 experimental horse with no overt clinical signs, mild histologic lesions in 2 horses with no clinical signs of disease, and an absence of lesions or signs in 1 horse suggests that in stables where 1 horse develops EMND, other horses fed the same diets may be mildly or subclinically affected.

The role of copper and iron as potential pro-oxidants is less obvious than the role of vitamin E deficiency in the pathogenesis of EMND. Both copper and iron have the potential to act
as strong pro-oxidants via the Fenton reaction and generation of free hydroxyl ions with or without peroxynitrite production (Urbanski and Beresewica, 2000). Hepatic copper concentrations were remarkably high (nearly 125 x those in control horses) in the 4 horses with clinical signs of EMND. Copper content is also high, compared with that in control horses, in the spinal cord of horses with naturally occurring EMND (Polack et al., 2000). The role of copper in the development of EMND remains questionable, however. In a separate study (Mohammed et al., 2007) that we performed concurrently, horses fed a diet similarly low in vitamin E content but with NRC-recommended copper concentrations (100 ppm in the concentrate feed) developed signs of EMND at a similar rate (i.e. 4 of 10 horses) and time of onset (19 to 27 mo after beginning a vitamin E-deficient diet). Despite substantially high hepatic copper concentrations in the 4 horses with EMND, there was no evidence of liver necrosis associated with these concentrations and plasma copper concentration did not change over time in any of the treated horses. This finding suggests that horses are relatively resistant to copper-induced hepatopathy, and as has been reported if liver copper stores are adequate, increasing copper intake does not alter blood copper concentrations but will increase the accumulation of copper in the liver.

High hepatic concentrations of iron and high serum concentrations of ferritin have been detected in horses with naturally occurring EMND, but the role that iron plays in the development of EMND is questionable because iron concentrations are not high in the spinal cord of those horses, and in our experiment, horses fed a diet that was similarly low in vitamin E content but that contained iron closer to reference range concentrations (ie, 140 ppm in concentrate feed) also developed EMND (Mohammed et al., 2007). The 4 horses in the initial study with clinical signs of EMND had either unremarkable or only mildly high hepatic iron concentrations at euthanasia. This finding was surprising because the amount of iron in the concentrate the horses consumed was > 10 times the amount recommended or contained in most commercial concentrate feeds. The failure of this diet to cause an increase in serum ferritin concentrations or significantly higher hepatic iron concentrations than in control horses supports the concept that the percentage of iron absorbed in the intestine decreases when body stores are sufficient; this protective mechanism may prevent iron overload when dietary excess of the mineral is mild to moderate. Copper and iron cannot be ruled out as candidates for pro-oxidant activity in horses with EMND, however, because even moderate increases of either cation may be harmful if body stores of antioxidants such as vitamin E are severely depleted.

Abnormal selenium content in plasma and spinal cord tissue has not been detected in horses with naturally occurring EMND, but we measured selenium in the experimental horses because of the close physiologic relationship between vitamin E and selenium. Although the changes were not significant, plasma selenium concentrations decreased over time in all 8 vitamin E-deficient horses, despite the fact that the diet met NRC requirements for idle horses. This decrease in plasma selenium concentration in the vitamin E-deficient horses may have been caused by increased use of selenium as a result of progressive vitamin E deficiency.

Results of our studies of experimental EMND (in which horses were fed a diet low in vitamin E but with copper and iron content in the range recommended by NRC), and published clinical investigations suggest that a green forage deficiency, likely leading to vitamin E deficiency, is the major risk factor for EMND. Although control horses received only marginally sufficient vitamin E in the concentrate and hay, they had access to grass for several months of the year. Each of the control horses that were euthanatized had liver concentrations of vitamin E that were > 10 times the hepatic vitamin E in experimental horses that developed EMND. Previous studies have revealed that consumption of grass increases plasma vitamin E concentration in horses, and after grazing is suspended, several weeks are required for the plasma vitamin E concentration to decrease.
The decrease in plasma vitamin E concentration is presumed to be a result of tissue storage (Mäenpää et al., 1988).

Vitamin A deficiency may be expected under feeding and housing conditions similar to those that cause vitamin E deficiency (i.e. lack of green forage). We do not believe vitamin A deficiency plays an important role in the development of EMND because the amount of vitamin A added to commercial feeds is sufficient to meet minimum daily NRC maintenance requirements (40 IU/kg of body weight) when 0.5% to 1% of concentrate/kg of body weight is fed, even without the additional vitamin A contained in roughage. Hepatic vitamin A concentration in the 4 experimental horses with EMND was within reference range or marginally low and was not significantly different from hepatic vitamin A content in control horses. Plasma vitamin A concentrations remained within or close to the reference range throughout the study and did not change over time.

Equine motor neuron disease is not the only neurologic disorder in horses that is believed to be associated with vitamin E deficiency. In young growing equids, vitamin E deficiency and a suspected heritability may cause equine degenerative myeloencephalopathy (EDM) a diffuse neurodegenerative disorder of white-matter neurons (Blythe et al., 1991). Lipopigment accumulation is a prominent feature of EDM and EMND, suggesting that both diseases may be oxidative disorders. In horses with EDM, lipopigment is detected in ascending and descending white-matter pathways, whereas in horses with EMND, lipopigment accumulation is pronounced in the capillary endothelium in the ventral gray column of the spinal cord, within macrophages at the site of dead neurons, and in RPE. Although EMND and EDM are distinct entities and differ in certain clinical and pathologic features, we have examined 3 yearlings with clinical signs and histologic changes associated with classic EDM that also had histologic lesions associated with EMND, further supporting a relationship between vitamin E deficiency and both EDM and EMND. It is possible that vitamin E has protective effects against oxidative damage in the spinal cord at neuroanatomic sites that vary on the basis of age. Our results support previous recommendations (Craig and Blythe, 1992; Nutrient Requirements of Horses, 2007) that all horses without access to green forage for prolonged periods should receive dietary supplementation with vitamin E to ensure a daily intake of at least 1 to 2 IU/kg of body weight per day, irrespective of age. Vitamin E concentration in feed should total at least 50-80 IU/kg DM (NRC, 2007).

Additional Comments on Equine Requirements

Horses maintained on marginal or low vitamin E diets, horses with intestinal malabsorption and/or chronic liver disease and equines with hyperlypemic and/or those inflammatory and degenerative diseases of the nervous system should be supplemented with vitamin E. Pregnant mares should also be supplemented in parts of the world where pasture grazing does not occur during late pregnancy. Growing foals and weanlings should also be supplemented when they have limited or no access to green pasture. I have seen three grazing foals that developed EDM when they were confined to a stall for at least two months because of orthopedic problems. Also, horses asked to perform prolonged aerobic activities should also be supplemented. Adding the vitamin E to grain (top dressing) would likely increase its absorption.

Vitamin E deplete horses will have rapid increases in plasma vitamin E after supplementation (assuming normal intestinal function), even with dl-α-tocopherol acetate. Prolonged supplementation may be required to obtain plasma levels similar to the normal pasture fed range (4-10 ug/mL).

A concern regarding supplementation with synthetic vitamin E is (1) does this form of vitamin E actually mimic the natural vitamin E (found in green forage or cereal grain oils), and their many biologic functions, (2) could supplementation of α-tocopherol actually decrease tissue levels of the other forms of
vitamin E? Other than this possibility, there is little data to suggest that even high level supplementation is harmful.

**Testing for Vitamin E Deficiency in the Horse**

The normal plasma vitamin E concentration is suggested to be >1.5 ug/mL. In my opinion, this would be adequate for the sedentary, non-pregnant horse in the winter. Horses on green grass should have much higher concentrations as the concentration will decrease during the winter months or if there is excessive demand. Horses with plasma values >1.5 ug/mL can have significant fluctuations throughout the day which is an indication that organ stores are likely high. Horses that are deficient in vitamin E usually have levels <1.0 ug/mL and do not have the daily/hourly fluctuations in plasma concentration seen in vitamin E replete horses. Vitamin E deficiency can be further confirmed by liver and/or preferably adipose analysis (Steiss et al., 1994).

**References**


NON-STARCH POLYSACCHARIDE ENZYMES FOR POULTRY

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Abstract

Endogenous enzymes in poultry do not sufficiently digest the non-starch polysaccharide (NSP) portion of plant ingredients. The complex nature of the NSP indicates that an array of enzymes would most effectively degrade this otherwise inert component. Thus, fine-tuning of corn/SBM (soybean meal) diets to combat unprecedented ingredient costs can include feed enzymes. Historically, the growth response has not been particularly evident when adding carbohydrases to nutritionally fit corn/SBM diets, with performance improvements potentially concealed by an array of factors in commercial production. Yet, under controlled study conditions and with similar corn/SBM diets, these enzymes demonstrate sufficient efficacy to be considered as a means to provide meaningful reductions in commercial feed costs.

Introduction

U.S. feed costs have risen by 50% or more within the past 12-18 months. While being driven by higher energy costs and by the diversion of massive quantities of corn toward DDGS (distillers dried grains with solubles), subsequent domino effects on peripheral ingredient prices are also accountable. Being that feed costs represent 70-75% of total production expenditures, prices have been particularly painful in poultry production, especially when that ingredient accounts for 60-70% of the diet.

Carbohydrate enzymes in corn/SBM (soybean meal) diets have been tested for several decades. Technology continues to improve the selection and development for more effective enzymes, and when coupled with efficient costs of enzyme production, opportunities for U.S. poultry feeds look exceptionally bright. Ultimately, the aim for these enzymes is to reduce feed costs through an improvement in the utilization of the feed. Indeed, a $3-5/ton savings is often experienced even with the cost of enzymes.

The NSP (non-starch polysaccharide) portion in corn and SBM serves as the target substrate (Table 1). The relatively high-energy availability for corn (~85%), as compared to other cereals, has generally limited corn-based diets from being as successful as viscous grains for enzyme inclusion. But with today’s costs, the potential gain of 10-15% in energy improvement becomes quite significant.

Table 1. Major dietary carbohydrates.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Monosaccharides</th>
<th>Glucose, galactose, fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disaccharides</td>
<td>Sucrose, lactose, trehalose</td>
<td></td>
</tr>
<tr>
<td>Polyols</td>
<td>Sorbitol, mannitol</td>
<td></td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>Malt-oligosaccharides</td>
<td>Maltodextrins</td>
</tr>
<tr>
<td>Other oligosaccharides</td>
<td>Raffinose, stachyose, fructo-oligosaccharides</td>
<td></td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>Starch</td>
<td>Amylose, amylopectin, modified starches</td>
</tr>
<tr>
<td>Non-starch Polysaccharides</td>
<td>Cellulose, hemicellulose, pectins, hydrocolloids</td>
<td></td>
</tr>
</tbody>
</table>
Soluble NSP

The soluble NSPs present in ingredients such as wheat, barley, and rye, have been well documented. Increased intestinal viscosity, lowered feed intake, subdued growth rates and F/G, problems associated with wet litter, and a proliferation of intestinal microorganisms related to soluble NSP grains are factual (Choct, 2001). Based on the NSP substrates in these feeds, endo-ß-xylanases, ß-glucanases and α-amylases are commonly added to commercial diets to counter the effects of soluble NSP.

Insoluble NSP

Water insoluble NSP are probably more pertinent for ingredients commonly used in the US. For corn grain, NSP exist primarily as arabinoxylans (ie, pentosans) and cellulose. In corn, these are essentially water insoluble and lack the viscous nature of those found in wheat, barley and rye. Hence, viscosity is seldom an issue with corn and insoluble NSP, albeit this carbohydrate fraction can imbibe small amounts of water and influence intestinal dynamics.

The NSP in corn are within the cell walls (Figure 1), and encapsulate starch, protein, oil, and other nutrients, inside the plant cell. This impermeable cell wall is a physical barrier between the intestinal enzymes and the cell components, and prevents full use of the potential nutritional value (Hesselman and Aman, 1986). Referred to as “the cage effect”, nutrients entrapped within the structure are inaccessible to endogenous enzymes. The energy gained from the complete NSP digestion of these cell walls is insignificant when compared to that from the release of the cell contents. Hence, with insoluble NSP, the goal is to attain “nutrient release” via enzymatic degradation. In addition, high levels of NSP stimulate mucin secretion, and increase the number of goblet cells (Satchithanandan et al., 1990). Therefore, the presence of NSP can disrupt the movement of fat micelles and hinder the normal digestive process. The chemical-physical complexity of the NSP fiber matrix can alter the digestibility of all nutrients. A multitude of factors, such as animal species, solubility, chemical structure and quantity in the feed, ultimately influence the digestibility of NSP (Choct, 2001).

- NSP are a major component of dietary fiber, originating from plant cell walls

Figure 1. Cell wall nonstarch polysaccharide organization in corn.

Corn Grain NSP. We recently analyzed two groups of U.S. corn grain (2006 and 2007 crop years) for NSP composition, and across 23 samples, the arabinoxylan content averaged close to 4% with a
CV of 13.1% (Figure 2). The cellulose in corn is not high, and generally falls within a 2-4% range. Along with about 1% pectins, the arabinoxylan and cellulose make up about 90% of the total NSP in corn grain (Malathi and Devegowda, 2001), or constitute about 9-10% of the dry matter.

![Figure 2. Arabinoxylan Content of U.S. Corn Samples from Various Sources (DSM Internal, 2008).](image)

Corn DDGS NSP. We also assessed the levels of NSP in corn DDGS, as well as the variability of these levels (Ward et al., 2008; Table 2). Samples were randomly collected from DDGS sources throughout the US, and NSP analysis was completed at the University of Alberta. Ether extract, crude protein and acid detergent fiber were also determined.

Table 2. Nutritional and Total NSP Components of Corn DDGS (Ward et al., 2008).

<table>
<thead>
<tr>
<th>Component</th>
<th>Average, %</th>
<th>Std Dev, %</th>
<th>Range, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Deter Fiber</td>
<td>13.65</td>
<td>1.98</td>
<td>9.09 to 17.33</td>
</tr>
<tr>
<td>Ether Extract</td>
<td>11.17</td>
<td>2.09</td>
<td>3.18 to 13.50</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>32.56</td>
<td>3.34</td>
<td>27.80 to 46.76</td>
</tr>
<tr>
<td>Arabinose</td>
<td>4.98</td>
<td>0.49</td>
<td>4.09 to 6.08</td>
</tr>
<tr>
<td>Xylose</td>
<td>6.42</td>
<td>0.72</td>
<td>4.81 to 7.78</td>
</tr>
<tr>
<td>Glucose</td>
<td>7.86</td>
<td>0.86</td>
<td>6.72 to 9.68</td>
</tr>
<tr>
<td>Mannose</td>
<td>1.62</td>
<td>0.43</td>
<td>1.16 to 2.44</td>
</tr>
<tr>
<td>Galactose</td>
<td>1.61</td>
<td>0.19</td>
<td>1.19 to 2.08</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>0.08</td>
<td>0.01</td>
<td>0.05 to 0.09</td>
</tr>
<tr>
<td>Ribose</td>
<td>0.11</td>
<td>0.04</td>
<td>0.06 to 0.20</td>
</tr>
<tr>
<td>Fucose</td>
<td>0.06</td>
<td>0.04</td>
<td>0.01 to 0.18</td>
</tr>
</tbody>
</table>

The total NSP content comprised 23.1% of the dry matter, and the water-insoluble portion made up about 88% of this total. The high level of insoluble NSP is not unexpected since much of the small amount of the soluble fraction is fermented during ethanol production. The arabinose + xylose portion provides an estimate of the arabinoxylan fraction which was 11.7%. The glucose portion (as estimate of
cellulose) was about 7.9% of the dry matter for this group. Values for other corn DDGS generally put cellulose in the range of 9-12%. The fractions recognized as arabinoxylan and cellulose accounted for about 85% of the NSP in corn DDGS.

One could surmise that xylanases and cellulases have the best potential to improve diets with corn DDGS simply because these enzymes degrade those substrates. For corn grain, this would also hold true, with the exception that the inclusion that \( \alpha \)-amylase may also increase starch digestibility. Thus, while we generally view xylanases as being “wheat enzymes”, \( \beta \)-glucanases as “barley enzymes”, and \( \alpha \)-amylases as needed for wheats, barleys, ryes, etc., these same enzymes have application for corn grain and corn DDGS by virtue of the substrates present.

**SBM and NSP.** SBM contains a relatively large amount of soluble indigestible carbohydrates, both oligosaccharides and polysaccharides. The oligosaccharides, mainly \( \alpha \)-galactosides (raffinose and stachyose), cannot be digested by endogenous enzymes in poultry, yet comprise about 6% of SBM dry matter, and have been associated with increased wet litter. In total, SBM contains approximately 18-21% NSP, of which 2.5 to 3% is soluble (Bach Knudsen, 1997).

The ratio of ME (metabolizable energy) to GE (gross energy) in SBM is about 0.51 for poultry (NRC, 1982). In other words, only about 51% of the gross energy in SBM is used for metabolic functions. TME\(_{n}\) and improved NSP digestibility in SBM can occur through the removal of the oligosaccharides with ethanol extraction (Coon et al., 1990) by 10-15% or more, clearly implicating NSP as a detriment to SBM nutritional value.

Bacterial degradation of SBM \( \alpha \)-galactosides in the lower intestinal tract can set the stage for diarrhea. Pouls fed higher levels of SBM produced wet feces, resulting in foot pad dermatitis (Jensen et al., 1970). Indeed, wet litter issues occur with commercial all veggie diets, even after attempting to adjust for the higher K in SBM.

Significantly higher litter moisture and footpad dermatitis occurred when broilers were fed corn/SBM diets with 46 and 43% SBM in the starter and grower (Eichner et al., 2007). Enzymes were not fed, and differences in litter moisture might have been a reflection of the 20-22% higher level of K in the all-veggie diet versus the control (10% poultry by-product meal). Even so, the presence of galactosides in SBM can create conditions for wet litter and footpad problems, and this could be crucial when high petroleum costs often lead to reduced ventilation during cold weather.

### Supplemental Carbohydrases

**Amylase.** The potential need for supplemental \( \alpha \)-amylase in corn/SBM diets exists, being that starch digestion is not necessarily quick and efficient (Tester et al., 2003; Noy and Sklan, 1985). Poultry can be sensitive to “starch overloading,” and starch digestion probably has some dependency on the kinetics of feed intake (Carre, 2003; Svihus and Hetland, 2001). Genetic progress in today’s birds places emphasis on high feed consumption, and possibly encumbered with the use of pelleted feeds to promote maximal nutrient intake, starch digestibility certainly could suffer.

In addition, one could easily assume that young birds benefit from added amylase. In controlled studies (Uni et al., 1995; Siddons, 1969), pancreatic \( \alpha \)-amylase and intestinal maltase increased from day 4 to day 21 of age, suggesting that starch digestion improves with age. In fact, University of Illinois chick studies with corn/SBM diets (Batal and Parsons, 2001) and turkey studies at The Ohio State University (Persia et al., 2002) noted that more energy was garnered from the diet as birds matured. Perdue University investigators (Olukosi et al., 2007) also noted that total tract nutrient retention and ME increased as chicks grew to 21 days when fed a basic corn/SBM diet.

In a 42-day trial, broiler corn/SBM diets were supplemented with \( \alpha \)-amylase to ascertain effects on live performance and digestive traits (Gracia et al., 2003). Within 7 days into the trial, the \( \alpha \)-amylase group experienced a 9.4% increase in daily gain, and 4.2% improvement in F/G. Through the grower-
finisher stages, the group supplemented with α-amylase continued superior growth and F/G. Although performance improvements were greatest early in life, the advantage attributed to α-amylase supplementation certainly was present well past 21 days of age.

Dr. S.L. Vieira’s group (Universidade Federal De Lavras) recorded strong responses by broilers fed a corn/SBM diet supplemented with a combination of α-amylase and β-glucanase (Bertechini et al., 2006). Enzymes were added to diets with 3% less energy than the positive control. Enzyme addition over a 42-day period gave significant improvements in body weights and F/G in the starter period, as well as the grower-finisher phase (Tables 3 and 4).

Table 3. Body Weight Gain (g) of Broilers Fed Corn/SBM to 42 days of Age (Bertechini et al., 2006).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 - 7</th>
<th>8 - 21</th>
<th>1 - 21</th>
<th>21 - 42</th>
<th>1 – 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>133</td>
<td>700</td>
<td>833&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,711&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2,544&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control (NC)</td>
<td>134</td>
<td>695</td>
<td>829&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,630&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2,460&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>NC + Ronozyme A 300 g/t</td>
<td>139</td>
<td>704</td>
<td>843&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,803&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,646&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NC + Ronozyme A 400 g/t</td>
<td>137</td>
<td>717</td>
<td>854&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,795&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,649&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>P Value</td>
<td>NS</td>
<td>NS</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>CV, %</td>
<td>3.97</td>
<td>1.99</td>
<td>1.75</td>
<td>1.85</td>
<td>1.36</td>
</tr>
</tbody>
</table>

Negative control contained 3% less ME than the positive control

Table 4. Feed Conversion (F/G) of Broilers Fed Corn/SBM to 42 days of Age (Bertechini et al., 2006).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 - 7</th>
<th>8 - 21</th>
<th>1 - 21</th>
<th>21 - 42</th>
<th>1 – 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>1.133</td>
<td>1.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.382&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.947&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.761&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control (NC)</td>
<td>1.168</td>
<td>1.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.423&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.129&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.891&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NC + Ronozyme A 0.3 kg/Ton</td>
<td>1.147</td>
<td>1.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.390&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.955&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.773&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NC + Ronozyme A 0.4 kg/Ton</td>
<td>1.147</td>
<td>1.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.372&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.991&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.789&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Prob.</td>
<td>NS</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>CV, %</td>
<td>3.33</td>
<td>2.75</td>
<td>2.51</td>
<td>2.27</td>
<td>1.43</td>
</tr>
</tbody>
</table>

Negative control contained 3% less ME than the positive control

1 Ronozyme® A
That there seems a deficit of enzymes in a developing intestinal system at an early age clearly portrays a potential need for exogenous enzymes. Yet, studies find that performance improvements can also exist through the grower-finisher age. And while starch digestion is generally considered to be high for corn/SBM diets, evidence is available to challenge this assumption.

**Pectinase.** Marsman *et al.* (1997) reported a combination of carbohydrases\(^2\) (high in pectinase) to effectively increase the digestibility of the NSP fraction of a corn/SBM diet. The exogenous enzymes reduced the concentration of soluble carbohydrates in the chime. Crude protein digestibility was significantly improved, presumably through an increase in protein solubility. However, live performance was unaffected over a 25-day period, leading the authors to suggest that an abundance of dietary protein may have masked any potential improvement.

The same mix of enzymes\(^2\) (pectinase, β-glucanase and other enzymic activities) improved (P<0.05) AME\(_n\) and ileal protein digestibility (Kocher *et al.*, 2002) for broilers consuming diets with 36.5% SBM. Significant reductions were found in fecal rhamnose and galactose, and a trend for a decrease in litter moisture was evident. In another 35-day broiler feeding trial with corn/SBM, a significant linear increase in weight gain and feed intake was dose-dependent for this product (Vahjen *et al.*, 2005).

*In vitro* SBM digestibility was improved with this combination of enzymes, and was attributed to the presence of pectinase since SBM is relatively high in pectins (Malathi and Devegowda, 2001). Compared to other enzyme mixtures, the group containing pectinase released more total sugars from SBM, and produced the lowest viscosity. On the other hand, combinations that included xylanase and cellulase were especially effective in a typical corn/SBM broiler starter diet.

**Multiple Enzyme Mixes**

Carbohydrates within ingredients are a complex mixture of polymers, and the NSP fraction can constitute 70-90% of the total cell wall (Bach Knudsen, 2001). The building blocks of the cell wall include the pentosans arabinose and xylose, the hexoses glucose, galactose and mannose, the 6 deoxyhexoses rhamnose and fucose, and the uronic acids glucuronic and galacturonic acids (Bach Knudsen, 2001). Chief polysaccharides include cellulose, arabinoxylans, and mixed linked polymers. Lignin is interspersed and cements various components.

**Amylase, glucanase, xylanase.** Over the years, a number of enzyme combinations have been investigated. These combinations are often aligned with the complexity in the overall fiber fraction associated with the ingredients formulated in diets. Not one enzyme is designed to degrade various substrates, thus a combination of enzymes would theoretically offer the best opportunity for meaningful improvements.

The combination\(^3\) of α-amylase, β-glucanase and endo-xylanase has shown efficacy for birds fed basic corn/SBM diets. In two experiments that tested three combinations of this group of three enzymes, broilers were raised during hot and cool growing conditions in floor pens and fed corn/SBM diets (with 4.6% fish meal) over a 38-day period (Yu and Chung, 2004). Added to a negative control with 3% less ME, this group of enzymes fully restored live performance to that of the birds fed a positive control diet.

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\(^2\) Energex, marketed as Ronozyme® VP

\(^3\) Ronozyme® AX
The combination of enzymes from this study (Yu and Chung, 2004) that showed the best response was further tested with broilers (Rutherfurd et al., 2007). In this second evaluation, improvements (P<0.05) occurred in the utilization of corn/SBM diets that included a 2.3% increase in AME (Table 5).

Table 5. Improvement in apparent metabolizable energy (AME) of broilers fed a corn/SBM diet with α-amylase, β-glucanase and endo-xylanase (Rutherfurd et al., 2007).

<table>
<thead>
<tr>
<th>Component</th>
<th>Corn-soy diet</th>
<th>Corn-soy diet + enzymes</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEM(^1), %</td>
<td>67.5</td>
<td>69.0</td>
<td>0.0952</td>
</tr>
<tr>
<td>AME(^2), kcal/kg</td>
<td>2,766</td>
<td>2,829</td>
<td>0.0499</td>
</tr>
</tbody>
</table>

\(^1\)AEM = apparent energy metabolizability  
\(^2\)AME = apparent metabolizable energy  
\(^3\)Ronozyme® AX

Furthermore, the apparent ileal digestibility of all amino acids was significantly higher in the corn/SBM diets supplemented with the carbohydrases, as compared to the diet without (Rutherfurd et al., 2007). Overall, amino acid digestibility was improved by more than 5%, excluding cystine. In the absence of a supplemental protease, this increase could be due to the degradation of the fiber matrix surrounding the protein, thus allowing intestinal proteases greater access. Enzyme supplementation did not influence ileal endogenous amino acid loss, evidence that the improved amino acid digestibility likely occurred by the actual breakdown of protein by proteases already present.

Improvements in amino acid digestibility for birds receiving carbohydrases have been reported elsewhere (Zanella et al., 1999; Saleh et al., 2005; Meng and Slominski, 2005).

The findings of Rutherfurd et al. (2007) are consistent with work from the University of Illinois, in which chicks fed a corn/SBM diet experienced an increase 46 kcal/lb feed in AME with this combination of enzymes. Similarly, when tested with the TME (true metabolizable energy) method at the University of Georgia, the addition of this blend of enzymes to a corn/SBM diet increased TME by 26 kcal/lb feed. The relative difference between the AME and TME methods to determine the energy contribution is unknown.

**Cellulase, glucanase, xylanase.** Another unique blend of enzymes has been shown to improve the feeding value of corn/SBM diets. In one study with pigs, ileal digestibility of various fiber fractions increased (P<0.05) in diets when this blend of cellulose, β-glucanases, endo-xylanase and other enzymes was included (Table 6). Without enzyme supplementation, total NSP digestibility was 11.3%, but with the highest supplementation rate, the digestibility was increased to 25.8%. In corn/SBM diets, this group of carbohydrases increased TME\(_a\) by 39 kcal/lb feed. This corresponds well with a replicated study with a 4.9% and 6.2% improvement 35-day body weight and F/G for broilers fed corn/SBM based diets with this enzyme combination (Unreported, 2005).

\(^4\)Ronozyme® AX  
\(^5\)Roxazyme® G2
Table 6. Enzyme increase in ileal digestibility in pigs of various fiber fractions (DSM Internal, 2005).

<table>
<thead>
<tr>
<th>Enzyme Combination¹, mg/kg</th>
<th>N</th>
<th>Arabinoxylans</th>
<th>Total NSP</th>
<th>Dry Matter</th>
<th>NDF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ileal Digestibility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11</td>
<td>2.6a</td>
<td>11.3a</td>
<td>62.7a</td>
<td>57.8a</td>
</tr>
<tr>
<td>50</td>
<td>11</td>
<td>7.9ab</td>
<td>18.2ab</td>
<td>64.7ab</td>
<td>60.6ab</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>18.8b</td>
<td>27.6b</td>
<td>65.9b</td>
<td>64.5bc</td>
</tr>
<tr>
<td>200</td>
<td>11</td>
<td>15.0b</td>
<td>25.8b</td>
<td>66.3b</td>
<td>64.8c</td>
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</tbody>
</table>

abcP<0.05 within columns
¹Enzyme blend of cellulase, beta-glucanase, endo-xylanase, and others; Roxazyme® G2

Conclusion

Endogenous enzymes in poultry do not sufficiently digest the NSP portion of plant ingredients. The complex nature of the NSP indicates that an array of enzymes would most effectively degrade this otherwise inert component. Thus, fine-tuning of corn/SBM diets to combat unprecedented ingredient costs can include feed enzymes. Historically, the growth response has not been particularly evident when adding carbohydrases to nutritionally fit corn/SBM diets, with performance improvements potentially concealed by an array of factors in commercial production. Yet, under controlled study conditions and with similar corn/SBM diets, these enzymes demonstrate sufficient efficacy to be considered as a means to provide meaningful reductions in commercial feed costs.

References


BACTERIAL DIRECT-FED MICROBIALS FOR DAIRY COWS: THEORY AND PRACTICE

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Summary

Direct-fed microbials, formerly called probiotics, are live, naturally occurring microorganisms. Bacterial direct-fed microbials (DFM) have been promoted for a number of years for their benefits in the intestinal tract, especially in calves. Bacterial DFM include species of *Lactobacillus*, *Bacillus*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, and *Propionibacterium*. Supplementary bacterial DFM have been used to reduce levels of intestinal pathogens and enhance immune response.

More recently, researchers have begun to realize that bacterial DFM may also provide benefits in the rumen. Bacterial DFM may reduce fluctuations in rumen pH throughout the day. *Megasphaeria elsdenii* and propionibacteria ferment rumen lactate to form propionate. By doing this, rumen pH is controlled and energetic efficiency in the rumen is improved. In addition, additional propionate can increase gluconeogenesis, drive milk production, and possibly reduce subclinical ketosis. Additional lactobacillus organisms may positively impact the rumen by helping to maintain a tonic level of lactic acid so that lactate users more consistently perform and are better able to cope when highly fermentable grain creates intervals of high lactate production.

A number of production studies with bacterial DFM in dairy and beef cattle have shown positive responses in milk production and milk production efficiency, especially in early lactation cows. Some studies, however, have shown little response. Positive rumen effects have been measured. It would be beneficial to understand the mode of action of bacterial DFM better and to have more studies assess responses based on diet and animal characteristics.

Introduction

The cow’s digestive tract naturally contains bacteria that colonize soon after birth. Some of these bacteria are beneficial. Some are not. The beneficial bacteria help to digest feed, supply nutrients to the cow, moderate rumen pH and reduce the number of intestinal pathogens. Cows and calves with low numbers of the beneficial bacteria are often less resistant to bacterial infections.

Supplementing bacterial DFM helps to increase the concentration of beneficial bacteria. Direct-fed microbials, formerly called probiotics, are live, naturally occurring microorganisms. Bacteria, yeast, bacterial and fungal extracts, and fermentation byproducts often make up DFM on the market. Many products are combinations of different types of microbial additives. Bacterial DFM approved by the U.S. Food and Drug Administration include species of *Lactobacillus*, *Bacillus*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, and *Propionibacterium*. Bacterial direct-fed microbials (DFM) have often been recommended for reducing diarrhea in stressed calves that do not receive adequate colostrum or for calves that live in an environment with a lot of pathogens. Research is revealing that bacterial DFM may also be beneficial in the diets of high-producing dairy cows.

To effectively perform, bacterial DFM products must be viable, fast growing, and able to survive in stomach acids. Bacterial DFM are often marketed in the form of boluses, powders, pastes, and liquids. The FDA does not regulate bacterial DFM but they must have AAFCO label registration guaranteeing the
levels of viable bacteria at the time of manufacture. A reputable manufacturer will often also show that
the product was produced in compliance with the rigid standards of the international ISO governing
organization.

Intestinal Effects

Pathogen Reduction

Even with good dietary management, high-producing cattle as well as young calves digest a
significant amount of their ration nutrients in their intestines. This situation increases their risk of
intestinal proliferation of detrimental organisms. Microbial additives may improve the balance of
intestinal bacteria providing that they are able to survive passage through the ruminant stomach.

Lactobacillus bacteria normally occupy the intestinal tract. Some supplemental strains can
survive passage through the digestive tract and have beneficial effects in the intestine particularly in
humans and young calves (Sanders and Klaenhammer, 2001). Lactobacillus bacteria produce organic
acids that lower intestinal pH and the oxidation/reduction potential to reduce the growth of undesirable
bacteria. They can also produce specific proteins, such as nisin and bacteriocins that can inhibit the
growth of other bacteria (Thuault et al., 1991). Lactobacillus bacteria may simply out-compete the
undesirable bacteria in digestion rate and rate of attachment to intestinal villi (Rolfe, 2000). Lactobacillus bacteria have been shown to reduce the level of amines (Sanders and Klaenhammer, 2001)
that can irritate the intestinal tract and cause diarrhea.

Lactic acid bacteria have been shown to have positive effects when supplemented to preruminant
calves. Cruywagen et al. (1996) used 40 Holstein-Friesian calves to evaluate the effect of Lactobacillus acidophilus supplementation (5 \times 10^7) in milk replacer from two days to six weeks of age. Overall,
treatment had no effect on growth, intake, or incidence of scour. But, from 2 d to 2 weeks of age, calves fed Lactobacillus acidophilus maintained their weight while control calves lost weight. Japanese
researchers supplemented either Bifidobacterium pseudolongum or Lactobacillus acidophilus to newborn
calves and found that both strains improved gains and feed efficiencies as well as reduced the incidence
of scouring (Abe et al., 1995).

In laboratory cultures, Lactobacillus bacteria have been found to reduce the numbers of Salmonella, E.coli and Clostridium perfringens (Sanders and Klaenhammer, 2001). Lactobacillus acidophilus has also been shown to reduce the shedding of Escherichia coli O157:H7 from feedlot calves infected with the organism (Krebil et al., 2003). Research has been conducted using DFM to reduce
growth of clostridia in other species. Teo and Tan (2005) found that a strain of Bacillus subtilis PB6
isolated from healthy chickens was effective against Clostridium perfringens ATCC 13124. A specific
antclostridial factor was also identified as part of the DFM.

Immune Response

The intestine is constantly being contaminated with antigens from food and microbes. Intestinal immune cells include natural killer cells, macrophages, neutrophils, dendritic cells, and T and B
lymphocytes. When these cells are activated by the presence of an antigen, they produce interleukins (IL-1, IL-6), tumor necrosis factor-α (TNF- α), interferons (IFN), reactive oxygen/nitrogen intermediates, and antimicrobial peptides (Krebil et al., 2003). Consumption of Lactobacilli has resulted in improvements in phagocytosis and natural killer cell activity as well as increased immunoglobulin production (Erickson and Hubbard, 2000; Isolauri et al., 2001). University of Wisconsin-Milwaukee researchers found that
cell-free extracts of Lactobacillus acidophilus enhanced the function of macrophages in in vitro cultures
(Hatcher and Lambrecht, 1993).
Rumen Effects

Originally the focus of supplementing bacterial DFM was to improve intestinal microflora and reduce the problems associated with intestinal pathogens. More recently, researchers have begun to realize that bacterial DFM may also provide benefits in the rumen.

Sub-clinical Acidosis

Sub-clinical rumen acidosis occurs when the pH of the cow’s rumen drops below 5.8. It reduces the growth of the rumen bacteria, especially the fiber-fermenters, and reduces digestibility of the ration. High-producing cows often experience a few hours of high rumen acidity during the day. If this situation is corrected, milk production can be increased.

Lactate is ten times stronger than many of the other volatile fatty acids produced in the rumen, making it a potent reducer of rumen pH. High grain diets and abrupt increases in grain intake encourage the growth of lactate producing bacteria such as *Streptococcus bovis* and *Lactobacilli* (Owens et al., 1998). There are a number of rumen bacteria that ferment lactate to form less acidic products like propionate and acetate. Supplemental bacterial DFM such as *Megasphaera elsdenii* and propionibacteria may be able to increase conversion of rumen lactate to propionate.

One group of researchers suggested that bacterial DFM such as lactobacillus might positively impact the rumen by helping to maintain a tonic level of lactic acid. By sustaining the lactate users in this way, they would more consistently perform and be better able to cope when highly fermentable grain created highs in lactate production (Nocek et al., 2002). In their research with a low level of a combination of *Enterococcus faecium*, *Lactobacillus plantarum*, and *Saccharomyces cervisiae*, fluctuations in rumen pH throughout the day were reduced.

Energy Conservation

Milk production in the high producing cow is often limited by energy. If supplemental bacteria DFM such as *Megasphaeriel dsdenii* and propionibacteria enhance rumen propionate production, overall energy efficiency could be improved. Propionate is used directly as an energy source by the cow and is converted to glucose to produce milk sugar. Propionate at the liver helps to use acetate derived from fat, potentially reducing ketosis.

Bacterial DFM Research

*Megasphaera elsdenii*

*Megasphaera elsdenii* is the primary lactate user in the rumen, aiding the growth of the acid intolerant fiber-digesters. Supplemental *Megasphaera elsdenii* B159 (8.7 x 10⁵ CFU/ml) added to *in vitro* cultures of mixed rumen bacteria has reduced lactic acid accumulation and moderated pH (Kung and Hession, 1995). When supplemented to steers, Megasphaera elsdenii 407A has prevented rumen acidosis (Robinson et al., 1992).

*Propionibacteria*

Propionibacteria normally make up a small proportion of the rumen microbe population. They convert lactate primarily to propionate (Krehbiel et al., 2003). For this reason, supplemental propionibacteria may reduce rumen acidosis and increase gluconeogenesis. *Propionibacterium shermanii* (10⁶ CFU/ml) was shown to increase molar proportions of propionate in batch cultures with mixed rumen
microbes (Kung et al., 1991). Propionibacteria seem to be more efficient producers of propionate than *Megasphaera elsdenii* (Krehbiel et al., 2003). For this reason, more research has been focused on propionibacteria as a bacterial DFM.

Some *in vivo* studies have shown limited response to propionibacteria supplementation. When *Propionibacterium acidipropionici*, strain DH42, was fed to steers on a high concentrate diet at 10^7 CFU/d, rumen propionate concentrations increased and rumen acetate concentrations decreased while rumen pH was unaffected (Kim et al., 2000). Ghorbani et al. (2002) supplemented feedlot cattle on a high concentrate diet with *Propionibacterium P15* (10 g at 10^9 CFU/g) and had no effect on rumenal pH but increased rumen protozoal concentrations. Francisco et al. (2002) supplemented 17 g of propionibacteria culture per day from -2 to 12 wk post-calving and did not affect milk production, percentage milk fat, and reproduction. When *Propionibacteria freudenreichii* (2 x 10^9 CFU/d) were supplemented with other DFM to mid-lactation Holstein dairy cows, production and rumen parameters were not affected (Raeth-Knight et al., 2007).

Others have shown positive responses to propionibacteria supplementation. Stein et al. (2006) fed 38 Holstein cows a control diet (n=13), a diet with a low dose (6 x 10^10 CFU/d) of propionibacteria strain 169 (P169) (n=14), or a diet containing a high dose (6 x 10^11 CFU/d) of P169 (n=11) from -2 to 30 wk postpartum. P169 supplementation increased 4% FCM (29.9, 32.7, and 32.2 kg/d for control, low dose, and high dose, respectively). Multiparous cows fed the high dose of P169 had higher molar percentages of rumen propionate, lower rumen pH, and lower milk fat percentage. In a similar study, Lebloenya et al. (2007) found P169 (6 x 10^11 CFU/d) tended to improve solids corrected milk production but reduced milk fat percentage. Both of these studies showed increases in milk lactose (%) with P169 supplementation, suggesting that P169 may have increased glucose supply by way of enhancing rumen propionate production (Seymour, 2007). In a study conducted at The Ohio State University with P169 supplemented from two weeks before calving to 119 DIM (6 x 10^11 CFU/cow/day), milk production was not increased but production efficiency was improved (milk yield per unit of dry matter intake) (Weiss et al., 2007).

In a recent controlled field study, P169 supplementation (6 x 10^10 CFU/cow/day) increased milk production (P<0.05) (44.3 and 43.1 kg/d for P169 and control diets, respectively) (de Ondarza and Seymour, 2008). This increase was most pronounced in early lactation (0 to 100 DIM) and third lactation and greater cows. However, the production of 3.5% fat-corrected milk was not affected by P169 supplementation. Pregnancy rate tended to be higher for cows supplemented with P169 (23% vs. 15% for P169 vs. control, respectively).

**Lactobacillus Bacteria**

Supplemental lactobacillus bacteria may have a rumen effect if they maintain a tonic level of lactate so that lactate-users in the rumen more consistently perform as suggested by Nocek et al. (2002). If these bacteria DFM survive the rumen and are active in the intestine, they may also enhance post-ruminal digestion and absorption. One study with two groups of 550 cows found that those fed *Lactobacillus acidophilus* (2 x 10^8 CFU/d) produced 1.8 kg more milk (33.6 vs. 31.8 kg/d) without a change in dry matter intake (21.3 kg/d) (Ware et al., 1988). Milk components were not affected. In a study conducted on a commercial dairy in upstate New York, 200 cows were fed either a combination of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus (Streptococcus) faecium* and mannanoligosaccharides (27.27 g/cow/d with 1 billion CFU/g of lactic acid bacteria) or a control diet. The supplemented cows produced 0.73 kg more milk (39.6 vs. 38.8 kg/d) (P<0.06) but ate 0.42 kg less dry matter (24.6 vs. 25 kg/d) (P<0.03) (Gomez-Basauri et al., 2001). Milkfat (%) tended to be improved with the supplemental treatment (4.44 vs. 4.24%).
Propionibacteria and Lactobacillus Combination Research

Scientists have studied the impact of supplementing propionibacteria and lactobacillus in combination to feedlot cattle. Similar studies were conducted with Propionibacterium freudenreichi (PF24) \(10^9\) CFU/g and three different levels and combinations of Lactobacillus acidophilus (LA45 and LA51) \(10^6\) CFU/g to \(10^{12}\) cfu/g using 270 steers at Texas Tech University and 320 steers at Michigan State University (Galyean et al., 2000, Rust et al., 2000). The Michigan State University researchers found that supplementation of these DFM bacteria improved ADG by 6.9% (P<0.02) and feed efficiency by 7.3% (P<0.02) (Rust et al., 2000). The Texas Tech University researchers improved daily gain by 4.3% (P<0.06) relative to controls and feed:gain ratio was numerically increased (Galyean et al., 2000).

Different strains of these bacteria (Lactobacillus acidophilus BG2FO4 \(5 \times 10^8\) CFU/hd/d) and Propionibacterium freudenreichii P-63 \(1 \times 10^9\) CFU/hd/d)) were fed either alone or in sequence in a 126-day experiment with 450 finishing heifers (Huck et al., 2000). Part of their objective was to find out which part of the feeding period would benefit the most from the supplementation of these bacterial DFM. Intake was not affected by treatment. Feeding Lactobacillus acidophilus BG2FO4 or Propionibacterium freudenreichii P-63 alone for the entire study had no effect on gain or efficiency. However, supplementing Lactobacillus acidophilus BG2FO4 for the first 28 days and then supplementing Propionibacterium freudenreichii P-63 for the rest of the trial resulted in increased daily gain by 5% and feed efficiency by 5.1% (P<0.10).

Oklahoma State University researchers tested the effect of supplementing 75 weaned feedlot calves for 120 days with Propionibacterium strain P-63 \(3.0 \times 10^{11}\) CFU/hd/d) alone or a combination of Propionibacterium strain P-63 \(1.0 \times 10^9\) CFU/hd/d) with Lactobacillus acidophilus strain LA53545 \(1.0 \times 10^8\) CFU/hd/d) (Swinney-Floyd et al., 1999). During the first 10 days when the potential for acidosis was highest due to diet adaptation and the inclusion of cracked wheat in the diet, feed efficiency and ADG were increased in calves fed the combination of the two DFM bacteria compared to calves on the control diet or the P-63 diet. Feed efficiency for the full 120 days of the trial was higher for calves fed the combination supplement although ADG was not significantly improved.

Conclusions

As we challenge dairy cows to produce higher levels of milk, we need to continue to find ways to moderate rumen pH, enhance rumen energetic efficiency, and promote efficient digestion and absorption of nutrients from the intestine. Although bacterial DFM need to be understood better, it appears that they can help in all of these areas. Lactobacillus bacteria are naturally in the intestine and if these organisms can be supplemented as bacterial DFM and survive passage through the rumen, they may enhance intestinal function. Research needs to prove that they can indeed survive the rumen and function in the intestine. Lactobacillus may also help in maintaining rumen pH, however more research on this mode of action is warranted. Positive production and rumen responses to supplemental propionibacteria are exciting. More studies to assess responses based on diet and animal characteristics as well as amount of bacterial DFM provided will help dairy producers determine their value for their herds.

References


Poultry Session
AMINO ACID NUTRITION OF MODERN LAYING HENS

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Summary

Laying hens have a physiological requirement for amino acids, which should be considered when formulating diets. Requirements for all amino acids have been determined and published, but may not be applicable under the specific conditions on individual farms or laying-hen houses within a farm. Hence, amino acid requirements should be determined using the ideal amino acid profile, which employs the concept that the ratios among amino acids vary only little regardless of the absolute amounts needed. Because not all amino acids in feed ingredients are available for protein synthesis after consumption, diets should be formulated using digestible amino acids, which estimate amino acid availability reasonably well. Different methodologies exist to determine amino acid digestibility and, of those, the true digestibility measured at the end of the ileum is considered the most accurate. However, due to the magnitude of data available, true digestibility values from cecctomized birds are more commonly used. The advantages of formulating diets using true digestible amino acids are greatest when diets are formulated using feed ingredients with relatively low amino acid digestibilities. For instance, only small amounts of corn dried distiller’s grains with solubles can be included in diets without adversely affecting production unless diets are formulated on a true digestible amino acid basis.

Introduction

Laying hens have a physiological requirement for amino acids for synthesis of body and egg protein as well as non-protein, amino acid–derived compounds (e.g., serotonin, adrenaline, nitric oxide, glutathione, and carnitine). Nevertheless, diets can be formulated on a crude protein basis as long as only a few protein supplying dietary ingredients are used (e.g., corn, soybean meal, and meat and bone meal). However, if it is accepted that hens need amino acids, not protein, the need to monitor or formulate laying hen diets with a crude protein minimum disappears. Instead, diets can be formulated to meet or exceed individual amino acid requirements, which is done by specifying minimum dietary amino acid contents in the least-cost formulation software (and avoiding specifying a minimum crude protein contents). Essentially, the computer program balances for the second- or third-limiting amino acid using protein-supplying ingredients (e.g., corn and soybean meal) and supplements the now deficient first- and second-limiting amino acid with crystalline amino acids, while at the same time considering ingredient costs. Of course, this approach requires accurate estimates of the amino acid requirements and of the contents of available amino acid in feed ingredients.

Amino Acid Requirements

Amino acid requirements for laying hens are published by the NRC (1994). However, the experiments upon which these requirements are based are dated and do not account for the genetic progress of laying hens in the last 15 years. Amino acid requirements have been reported since the publication of the NRC requirements (Table 1). However, these experiments have been conducted for one amino acid at a time, performed under different experimental conditions with different basal diets, genetic lines, feed consumption rates, egg production rates, dietary energy contents, ambient temperature, cage space, and ages of laying hens, all of which influence the amino acid requirements. Moreover, there is little agreement between amino acid requirements among studies, making it difficult at best to decide which requirement to use in diet formulation.
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Age (weeks)</th>
<th>Requirement (mg hen(^{-1}) day(^{-1}))</th>
<th>Basis</th>
<th>Maximal response (g hen(^{-1}) day(^{-1}))</th>
<th>Genetic line</th>
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<th>Basis¹</th>
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<td>Harms and Russell (2000b)</td>
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¹The amino acid requirement is expressed on a total or apparent fecal digestible amino acid basis.
Because multiple factors affect amino acid requirements, requirements determined under experimental conditions may not be applicable under field conditions. The solution to obtaining reliable amino acid requirements is therefore not to determine the amino acid requirements, but rather to determine the ideal amino acid profile for laying hens. The ideal amino acid profile employs the concept that, while amino acid requirements change drastically due to genetic or environmental factors, the ratio among them is only slightly affected. Thus, once the ideal amino acid profile has been determined, the requirement for a single amino acid (e.g., lysine) can be determined experimentally for a given field situation and the requirement for all the other amino acids calculated. Such an approach has been adopted with success by the swine industry (NRC, 1998) and is finding use in the broiler industry as well (Mack et al., 1999; Baker, 2003; Dari et al., 2005).

The NRC (1994), the Dutch Centraal Veevoederbureau (CVB) (1996), and Leeson and Summers (2005) report amino acid requirements from which the ideal amino acid profile can be calculated (Table 2). However, these profiles were estimated from data compiled from a variety of experiments and, therefore, influenced by genetics and environmental factors as mentioned previously. Coon and Zhang (1999) conducted five separate experiments to determine the amino acid requirements of laying hens and reported the ideal amino acid profile from averages of the five experiments (Table 2). Although better than the NRC (1994) and CVB (1996) approaches, these experiments were still performed under different experimental conditions with different basal diets, ages, and genetic lines of hens. Thus, the ideal amino acid profile (or requirements) reported by Coon and Zhang (1999) is subject to similar criticism as that of NRC (1994).

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<td>101</td>
<td>86</td>
<td>102</td>
<td>89</td>
<td>93</td>
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</table>

¹Amino acid requirements expressed as a percentage of the requirement for lysine.
²Calculated from total amino acid requirements.
³Based on digestible amino acid requirements.
⁴Calculated from total amino acid recommendations for 32-to-45-week-old laying hens.
⁵Based on true digestible amino acid requirements for egg mass in 28-to-34-week-old laying hens.
⁶The arginine:lysine ratio was estimated to be 107 or less (see text).

To ensure a valid measurement of the ideal amino acid profile, the same basal diet, the same genetic line, and the same assay period should be used in all experiments of amino acid requirements used to calculate the profile (Baker, 2003). We have recently re-evaluated the ideal amino acid profile for laying hens (Bregendahl et al., 2008). In this study, seven separate experiments were conducted to determine the ideal ratio of arginine, isoleucine, methionine, methionine+cystine, threonine, tryptophan, and valine relative to lysine for maximal egg mass. The experiments were conducted simultaneously using the same basal diet to which crystalline amino acids were added to create the graded level of the respective assay amino acid and to ensure that the assayed amino acid was first limiting. Hens were fed the experimental diets from 26 to 34 weeks of age, with the first two weeks considered a depletion period. Egg production was recorded daily and egg weight was determined weekly on eggs collected over 48 hours; egg mass was calculated as egg production × egg weight. The requirement for each amino acid
(true digestible basis) was determined using the broken-line regression method and the ideal amino acid profile calculated (Table 2). The hens did not respond to consumption of arginine in the diet, so an arginine requirement and, therefore, an ideal arginine:lysine ratio could not be established. However, if it was assumed that the arginine requirement was met with the lowest arginine consumption, the ideal arginine:lysine ratio would be 107% or less. The ideal amino acid profile determined by Bregendahl et al. (2008) differed somewhat from that calculated from NRC (1994), with the exception of arginine and tryptophan. However, ideal amino acid ratios agreed well with those calculated from requirements listed by CVB (1996) and the recommendations listed by Leeson and Summers (2005). There was, however, little agreement with the ideal amino acid profile suggested by Coon and Zhang (1999). Not included in the ideal amino acid profile are provisions for supplying dietary non-essential amino acids. However, it is implied that the diet also must supply non-essential amino acids. Typically, the non-essential amino acids should make up about half of the dietary protein with the remainder supplied by essential amino acids (Heger et al., 1987, 1998; Lenis et al., 1999).

Most nutritionists have a good idea of their hens’ methionine+cystine and lysine needs under the specific conditions on the farms. However, the hens’ need for especially threonine, tryptophan, isoleucine, and valine are more difficult to determine even when the information in Table 1 is considered—knowing these requirements are important when crystalline lysine and threonine are used in the diet formulation. In reality, the dietary content of those amino acids will likely be set by trial and error, because the nutritionist will have less practical experience determining the requirements for those four amino acids than with methionine+cystine and lysine. This is where the ideal amino acid profile is beneficial, because the requirements for all amino acids can be calculated using the hens’ lysine needs. Given a desired lysine consumption of, say, 720 mg/day, the threonine requirement would be (720 mg/day × 77% =) 554 mg/day, the tryptophan requirement would be (720 mg/day × 22% =) 158 mg/day, the isoleucine requirement would be (720 mg/day × 79% =) 569 mg/day, and so on. The methionine+cystine needs of the hens (677 mg/day) can also be determined in a similar fashion. Of course, the hens’ lysine requirements will still have to be determined, but at least the requirement for only one single amino acid must be considered instead of that of multiple amino acids.

**Amino Acid Availability in Feed Ingredients**

Not all of a given amino acid in a feed ingredient or diet is available for protein synthesis after consumption. A portion of the amino acid may not be absorbed and is instead excreted in the feces (‘digestibility’), or amino acids may be absorbed, but in a form that cannot be incorporated into protein, and are therefore is excreted in the urine (‘availability’). Thus, although often used interchangeably, there is a difference between digestible and available amino acids. Methods for estimating amino acid availability include the slope-ratio assay (Batterham et al., 1979), where the availability of one single amino acid in a feed ingredient is compared to the amino acid availability in another feed ingredient, and digestibility assays, in which the amino acid availability is estimated. Digestibility is measured as the disappearance of amino acids in the gastro–intestinal tract. However, not all of the disappearance can be attributed to absorption—some amino acids are fermented by microorganisms in the large intestines, resulting in an overestimation of the amino acid digestibility. To counter this overestimation, birds can be cecectomized and amino acids measured in the excreta, or amino acids can be collected from the end of the small intestine (ileum). Of the three methods (i.e., fecal, fecal from cecectomized birds, and ileal digestibility), the latter method is considered the most accurate estimate of amino acid availability (Darragh and Hodgkinson, 2000; Stein et al., 2006). In addition to where the non-absorbed amino acids are measured, amino acid digestibility also needs to be corrected for endogenous losses. These are amino acids that are absorbed from a previous meal, used for protein synthesis (e.g., digestive enzymes, mucosal protein, etc.), and subsequently excreted back into the gastro–intestinal tract. Hence, some of the amino acids found in the ileal digesta or excreta are from the bird itself, not the diet. When endogenous losses are not considered (i.e., apparent digestibility values), there can be large variations in the digestibility values obtained due to the amount of protein (or amino acids) consumed in the experiment (Fan et al., 1999).
1994), making it difficult to accurately estimate the available amino acid content in a diet (Mosenthin and Rademacher, 2003). As a result, digestibility values, corrected for endogenous losses (i.e., true or standardized digestibility values) should be used when formulating diets (Mosenthin and Rademacher, 2003; Stein et al., 2007). Table values of true digestibility coefficients for various feed ingredients are available from multiple sources (e.g., NRC, 1994; Ajinomoto, 2006) and there are laboratory tests available that predicts the amino acid digestibility of individual feed ingredients with reasonable accuracy (Fiene et al., 2006; Schasteen et al., 2007).

It is especially important to formulate laying hen diets on a true digestible amino acid basis when cereal coproducts are included, because these often have varying and lower amino acid digestibility compared to other ingredients. For example, the amino acid digestibilities in corn distiller’s dried grains with solubles (DDGS) have been reported to vary substantially between ethanol plants (Batal and Dale, 2006; Stein et al., 2006) and sometimes from batch to batch within the same ethanol plant. In particular, the digestibility of lysine varies because of its susceptibility to heat damage during the drying process. Because of the relatively lower amino acid digestibilities in corn DDGS, only little of the product can be included in laying hen diets unless diets are formulated on a true digestible amino acid basis.

To illustrate the importance of formulating diets on a true digestible basis, three different sets of laying hen diets were formulated (Table 3) using either a crude protein minimum of 17%, on a total amino acid basis, or on a true digestible amino acid basis (with no crude protein minimums or maximums in latter two sets of diets). Diets were also formulated with or without 15% corn DDGS to illustrate the effects of including an ingredient with relatively low amino acid digestibility. The dietary lysine content was set to 0.80% total lysine in the diet formulated on total amino acids and 0.72% true digestible lysine in the diet formulated on true digestible amino acid basis; the requirements for all other amino acids were determined using the ideal amino acid profile reported by Bregendahl et al. (2008). The nutrient contents listed by the NRC (1994) were used for all ingredients, except for corn DDGS, for which nutrient contents reported by Poet Nutrition (2007) and a non-phytate phosphorus content of 54% of total phosphorus (Lumpkins and Batal, 2005). The true digestibility coefficients reported by Ajinomoto (2006) were used for all ingredients. Although the diets were not formulated to be ‘least-cost diets,’ the diet costs were calculated using feed ingredient prices from the December 10, 2007 edition of Feedstuffs Magazine (Minneapolis prices).

If it is accepted that the best estimate of the hens’ amino acid needs are true digestible amino acid requirement, then is it evident from Table 3 that a diet formulated to contain 17% crude protein from corn, soybean meal, and meat and bone meal will be marginally deficient in methionine+cystine and threonine when amino acid digestibilities are considered. These deficiencies were exacerbated when corn DDGS was included in the diet. Formulating on total amino acids also resulted in marginal deficiencies when digestibility was considered, yet there may have been some benefits of a concomitant lowered diet cost (although this benefit is likely to be offset by a reduction in performance due to the amino acid deficiencies). The only scenario in which there were no deficiencies, was when the diets were formulated on a true digestible amino acids, demonstrating the benefits of formulating diets this way. Similar conclusions were reached by our research group after an experiment in which no effects on egg production or egg quality were detected between hens fed a control diet or a diet containing 69% corn DDGS (Pineda et al., 2008). Similarly, Rostagno and Pupa (1995) and Hoehler et al. (2005) demonstrated the superiority of formulating broiler diets on a digestible amino acid basis rather than a total amino acid basis.
Table 3. Laying hen diets formulated on the basis of crude protein, total amino acids, or true digestible amino acids and with or without corn distiller’s dried grains with solubles (DDGS).¹

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</tr>
</tbody>
</table>

¹Amino acid values in **bold type** are below recommended levels.
Conclusion

Formulating diets on a true digestible amino acids basis (as opposed to using a crude protein minimum or total amino acids) has several benefits including meeting more accurately the amino acid needs of the hens and minimizing amino acid excesses, which otherwise can lead to increased nitrogen excretion and ammonia emission. To help predict the amino acid needs of laying hens, the ideal amino acid profile should be used to determine the dietary amino acid contents rather than empirically determined requirements.

Acknowledgements

The research determining the ideal amino acid profile for laying hens was conducted in collaboration with Stacey Roberts (Iowa State University), Brian Kerr (USDA/ARS), and Dirk Hoehler (Evonik Degussa Corporation) with funding provided by Evonik Degussa Corporation and Iowa Egg Council.

References


AMINO ACID NUTRITION: INCORPORATING L-THREONINE IN BROILER FORMULAS

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Summary

Threonine is the third limiting amino acid in broiler feeds and the proper incorporation of L-Threonine can save considerable money in formulation, while providing optimal performance. Broilers do not have a crude protein requirement, per se, but instead a requirement for essential amino acids which are the building blocks of that protein. The proper approach of meeting essential amino acid requirements, as discussed and outlined in this paper, is to place emphasis upon those amino acids rather than upon crude protein. This approach, if properly implemented, will lead to only a minimal reduction in the crude protein content of the diet - thus providing for equal performance to a higher crude protein diet. As emphasis is placed upon the essential amino acids required by the bird, the fourth limiting amino acid becomes the constraint which will hold crude protein up to an acceptable level.

The economic advantage of properly implementing L-Threonine, is particularly evident during times when the price differential between corn and soybean meal is wide and/or when dietary fat costs are high. Through a step-wise process of fully implementing and utilizing some of the features in today’s formulation software packages, this economic benefit can be fully realized. The steps which will be presented include de-emphasizing “crude protein” as a nutrient, implementing and using digestible amino acid values, setting an adequate digestible lysine level, incorporating ideal digestible amino acid ratios for the essential amino acids and removing un-needed constraints on inclusion of synthetic amino acids, which are 100% available.

Introduction

Threonine was determined to be an essential and required nutrient for chicks in the early 1940’s and was first manufactured via fermentation in the 1980’s. Despite it being commercially and economically available in a crystalline form for more than 10 years (Kidd et al., 2007) and the accepted knowledge it is the third limiting amino acid in corn-soybean meal based diets, L-Threonine is still neither fully nor optimally utilized as a feed ingredient within the U.S. broiler industry. An excellent and thorough review and history of threonine research in broiler feeds prior to the year 2000, was written by Kidd (2000a,b). Reviews since that time, regarding the key research conducted with threonine in broilers, include Corzo et al. (2003) and (Tillman, 2007). This paper will add to the previous review papers and presentations as well as incorporate the few published and presented articles since 2007.

The Maryland Nutrition Conference for Feed Manufactures, over the years has been a resource for suggesting amino acid requirements for the current broiler of that era, as papers updating these recommendations appeared periodically (O.P. Thomas et al., 1978, 1982, 1986, 1992 and Austic, 1994 to list a few). In the Thomas et al. publications, the amino acid recommendations were based upon a relationship to the Metabolizable Energy (ME, Mcal / Lb) of the diet with the assumption that there is a proportional correlation between ME and amino acid needs in terms of nutrient intakes. This approach was likely rooted in the notion that broilers eat to meet their energy needs and with increased energy level
in the diet, feed intake would be decreased accordingly; hence, in order to maintain a similar amino acid intake, the amino acid level in the diet needed to be increased along with energy. A similar belief was applied to temperature, as the ratio of each amino acid to energy was increased as temperature increased. There may be a new paradigm regarding modern high-lean genotypes and how they respond to altered dietary ME versus altered amino acid density. It appears that today’s bird responds more to amino acid density and less to ME changes in regards to both bodyweight gain and feed conversion. Because of this existing scenario, basing amino acid densities upon the ME content of the diet should be considered with some caution as this correlation may not exist to the degree that it had in the past. Additionally, if this approach of tying amino acid levels to ME is implemented, the possibility arises that amino acids which should be related to each other, in terms of the ideal protein concept, could become disconnected as multiple variables, which impact the final individual amino acid levels in the feed, are being independently altered.

The ratios for lysine and threonine, relative to ME, are shown in the table below, for four of the years in which the Maryland Nutrition Conference values were reported. Lysine ratio recommendations were identical for the four years reported below; whereas values for the threonine to ME ratio during the grower phase (22-42 days), as reported in 1992, were reduced drastically from previous years, to the point that they were only slightly higher than those reported in the finisher phase (43-52 days). Logically, this may not make the most sense as protein deposition is quite high during the grower phase.

<table>
<thead>
<tr>
<th>Suggested Maryland Amino Acid / Metabolizable Energy Ratios for Broilers Reared at Different Temperatures.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Year</td>
</tr>
<tr>
<td>Lysine (Identical values for years 1978, 1982, 1986 and 1992.</td>
</tr>
<tr>
<td>Threonine / ME (Mcal / Lb) ratio, based upon suggested energy ratios.</td>
</tr>
<tr>
<td>Year</td>
</tr>
<tr>
<td>1978</td>
</tr>
<tr>
<td>1982</td>
</tr>
<tr>
<td>1986</td>
</tr>
<tr>
<td>1992</td>
</tr>
</tbody>
</table>

As ratios for each essential amino acid relative to energy were reported (above), each amino acid could also be calculated in a relationship to lysine. The calculated amino acid ratios based upon the individual threonine and lysine to ME recommendations, are shown below.

<table>
<thead>
<tr>
<th>Calculated Threonine / Lysine Ratios using Suggested Maryland Ratios for Broilers Reared at Different Temperatures.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Year</td>
</tr>
<tr>
<td>Threonine / Lysine Ratios, based upon suggested energy ratios.</td>
</tr>
<tr>
<td>Year</td>
</tr>
<tr>
<td>1978</td>
</tr>
<tr>
<td>1982</td>
</tr>
<tr>
<td>1986</td>
</tr>
<tr>
<td>1992</td>
</tr>
</tbody>
</table>

Because the suggested ratio for threonine to ME for the grower phase in 1992 was reduced relative to prior years, the ratio of threonine to lysine during this phase calculates to be lower than that determined for either the starter or the finishing phase. This value appears to be illogical as the threonine needs relative to lysine should at least remain constant or perhaps increase with age. This is because of
increased demands for protein deposition, feather development and maintenance and gastrointestinal mucin production (which contains a high percentage of threonine). If the lysine requirement is determined relative to energy, using the values from 1992, and subsequently the threonine requirement is determined using the same published information, then the potential exists that a threonine deficiency could occur during the grower phase. As such, it is imperative that current research and knowledge be used when setting both lysine levels and amino acid ratios. While the reported values, as previously published in the Maryland Nutrition Conference proceedings, were highly regarded in their time, current recommendations will help avoid under-feeding of particular amino acids during critical periods of time.

<table>
<thead>
<tr>
<th></th>
<th>0 - 21 Days</th>
<th>22 - 42 Days</th>
<th>43 - 52 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME 1.430 Mcal / Lb</td>
<td>80°F, 90°F</td>
<td>80°F, 90°F</td>
<td>80°F, 90°F</td>
</tr>
<tr>
<td>ME 1.445 Mcal / Lb</td>
<td>80°F, 90°F</td>
<td>80°F, 90°F</td>
<td>80°F, 90°F</td>
</tr>
<tr>
<td>ME 1.460 Mcal / Lb</td>
<td>80°F, 90°F</td>
<td>80°F, 90°F</td>
<td>80°F, 90°F</td>
</tr>
</tbody>
</table>

Threonine (% of Diet), based upon suggested energy ratios.

<table>
<thead>
<tr>
<th>Year</th>
<th>0 - 21 Days</th>
<th>22 - 42 Days</th>
<th>43 - 52 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>0.73% 0.76%</td>
<td>0.77% 0.78%</td>
<td>0.63% 0.66%</td>
</tr>
<tr>
<td>1982</td>
<td>0.84% 0.87%</td>
<td>0.77% 0.78%</td>
<td>0.63% 0.66%</td>
</tr>
<tr>
<td>1986</td>
<td>0.73% 0.76%</td>
<td>0.77% 0.78%</td>
<td>0.63% 0.66%</td>
</tr>
<tr>
<td>1992</td>
<td>0.73% 0.76%</td>
<td>0.64% 0.66%</td>
<td>0.63% 0.66%</td>
</tr>
</tbody>
</table>

Perhaps a better approach, relative to threonine requirements and lysine levels would be to either fix the ratio of threonine to lysine (between 65 and 70), or have it increasing slightly with age (65 – 70). Then, as the lysine level declines across the stages of production, the relative quantity of threonine provided would stay in a more correct relationship to lysine. This approach reduces some of the ambiguity of having three independently changing variables (ME, lysine to ME and threonine to ME) and fixes it so only the variables related to each other or those dependent upon each other remain in their proper relationship.

Additional information presented at the MD Nutrition Conference included an update on amino acid requirements and ratios for broilers (Austic, 1994) evaluated in a diet containing 23% crude protein and 3,200 kcal / kg of metabolizable energy. It was stated that the NRC (1994) lysine recommendation of 1.20% of the starter diet (23% crude protein) was too low and 1.3% lysine would be more adequate. It is highly likely with today’s genotype, that the lysine requirement is even greater. In regards to threonine, it was viewed at that time that the NRC recommendation of 0.8% of the diet was adequate in a 23% crude protein diet. However, it was also stated the threonine requirement as a percent of protein tended to decline with increasing protein. Because of this inverse relationship, tying threonine and perhaps other amino acid levels to crude protein is likely unsound. Based on their suggested levels for lysine and threonine, the reported ratio for total threonine to lysine was proposed to be 62, when the crude protein content of the diet was 23%. Because of the inverse relationship noted above, as crude protein declines in the diet, the ratio recommendation of threonine to lysine would increase. As noted above, amino acid “requirements” can be expressed in several manners: as either an absolute total levels or as absolute digestible levels. Then, from either of these absolute levels, they could either be expressed in a relationship with metabolizable energy, in a relationship with crude protein or as will be discussed in more detail below – in a relationship (ratio) with lysine. This latter approach may be the best option with today’s genotypes which tend to respond to increased amino acid density and to a lesser degree to metabolizable energy density.
Lysine

The importance of Lysine should not be underestimated as it is the second limiting amino acid in practical broiler diets and is used as the denominator in the ideal protein concept as the amino acid to which others are set using a ratio. Lysine is primarily used for protein synthesis and has a large impact upon breast meat yield – particularly in modern high-lean genotypes with a genetic predisposition for enhanced protein deposition. Lysine does not interact metabolically with any of the other amino acids, is not used as a precursor for any biochemical pathways (other than protein accretion) and is relatively easy and uncomplicated to assay since interferences are extremely minimal. In addition, the use of L-Lysine as an ingredient is highly economical and minimizes the inclusion of intact amino acid sources, which have lower amino acid digestibilities. Based upon these reasons, and the inherent fact that digestible amino acids represent what the animal can utilize, the concept of using a ratio of digestible amino acid levels relative to digestible level lysine, will be presented as the recommended means of setting the essential amino acids within a broiler formulation.

Waguespack et al. (2007a,b) presented and wrote of the results of five experiments with Ross X Ross 708 broilers from day 0 to 18. In the first three experiments, L-Lysine HCl inclusion was evaluated in a corn-soybean meal diet, containing added DL-methionine (targeting 0.91% total TSAA), threonine (targeting 0.88% total threonine) and glycine (targeting 2.32% total glycine +serine). L-Lysine HCl inclusion was forced in incrementally so as to maintain a targeted 1.26% total lysine. It was determined that lysine can easily be added up to 0.25% (5 pounds per ton) without any consequences on bird performance. It was further noted that 1.26% total lysine might be marginal to deficient and that if the diet were formulated closer to the bird’s requirement, additional lysine could be added.

Plumstead et al. (2007) in two trials, evaluated four incremental levels of digestible lysine along with energy levels from 3,000 to 3,300 kcal / kg in 0-21 day (trial 1) or 0-20 day (trial 2) broilers. Bodyweight gains and feed utilization improved with increasing dietary digestible lysine and the requirement was determined to be 1.19% (1.30% total lysine) of the diet in a 22.4% crude protein diet. The digestible lysine requirement had a linear trend and hence was determined to be even higher if crude protein or amino acid concentrations were increased. Observed responses from digestible lysine were independent across the changes in ME levels.

Most recently, Corzo et al. (2008) and Dozier et al. (2008) presented lysine requirement information for the Ross TP-16 female and male broiler, respectively for the 14-28 day period. Corn, soybean meal, poultry by-product meal and peanut meal diets were fed to broilers in 192 floor pens; with 10 replicate pens per treatment per sex. Nine dietary digestible lysine levels were evaluated from 0.85% to 1.25%, in increments of 0.05, by blending of the low and the high lysine containing diets. Linear, quadratic and broken-line quadratic models were fit to the data. While feed intake followed a quadratic curve, digestible lysine intake showed a linear response. In their determination of lysine requirements it was reported that the optimal digestible lysine level for minimizing feed / gain was 1.13% and 1.06% for the male and female TP-16 respectively, using the quadratic model. Body weight gain in the males was optimized at 1.12% digestible lysine as determined using the quadratic broken-line model. It was noted that these predicted lysine requirements were higher than those previously reported for the Ross 508 and 708 broilers (Dozier et al., 2007).
Optimal Digestible Lysine for Male TP-16
(Bodyweight Gain & Feed / Gain)

<table>
<thead>
<tr>
<th>Digestible Lys, %</th>
<th>Bodyweight Gain (d28)</th>
<th>Feed / Gain (d 14-28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quadratic P ≤ 0.0001</td>
<td>Quadratic P ≤ 0.0006</td>
</tr>
<tr>
<td></td>
<td>R² = 0.95</td>
<td>R² = 0.98</td>
</tr>
<tr>
<td></td>
<td>SEM = 0.019</td>
<td>SEM = 0.010</td>
</tr>
<tr>
<td></td>
<td>Quadratic (95%) Req. = 1.10%</td>
<td>Quadratic (95%) Req. = 1.13%</td>
</tr>
<tr>
<td></td>
<td>Quadratic broken-line Req. = 1.12%</td>
<td>Quadratic broken-line Req. = 1.19%</td>
</tr>
</tbody>
</table>

Threonine

Threonine is an essential nutrient and is the third limiting amino acid in most practical broiler diets. As such, considerable importance should be placed upon this amino acid for a variety of reasons as it is required for many key functions within the body. These include its’ essential involvement in body protein synthesis, its’ vital requirement to optimize gut health and integrity through its incorporation into mucin glycoproteins and also its important role in glycine and serine metabolism. L-Threonine is also a key component in feather development as 4-5% of the crude protein is made up of threonine. The ingredient L-Threonine is of great benefit as it allows the nutritionist to most closely meet the bird’s amino acid requirements while minimizing amino acid overages which are detrimental to performance. As added L-Threonine is 100% digestible to the animal, a 5 to 20% reduction in intact protein sources occurs when it is incorporated into feeds formulated on a digestible amino acid basis. In addition, fat incorporation, which can also be expensive, may also be reduced significantly. L-Threonine utilization will allow for a slight decline in crude protein levels. Obviously, reducing un-utilized excess nitrogen is a benefit not only to the birds in the barn, but also to the workers who oversee those flocks. L-Threonine utilization can lead to lower feed costs as expensive ingredients are minimized or allow one to increase the nutritional density of the diet when offered, at the same cost.

The impact of environment on the L-Threonine requirement was examined in Ross X Ross 708 male broilers (Corzo et al., 2006, 2007). In their trial, either clean (new) or previously used (during 4 growouts) wood shavings litter was incorporated into the treatment pens. The trial was conducted from 21 to 42 days of age and digestible lysine was set at 1.0% of the diet. Digestible threonine levels ranged from 0.43% to 0.78% (0.51% to 0.86% total threonine), in increments of 0.07%, via the addition of L-Threonine, so as to provide 6 points along the titration curve. Since the digestible lysine level was set at 1.0% in all of the diets, the digestible threonine level also represents the digestible threonine to digestible...
lysine ratio. Variables measured during or at the end of the trial included: bodyweight gain, feed utilization, carcass weight, carcass yield, and breast meat weight and breast meat yield. For all variables measured, the determined threonine requirement was higher in birds grown in the standard industry scenario (dirty, used litter) than in clean litter. On average, across the six variables measured, the birds raised on the new litter had a digestible threonine to digestible lysine requirement of 65%; whereas those birds raised in the standard industry scenario, of being on used litter, had a ratio “requirement” three points higher at 68%. The optimal total threonine levels or the digestible threonine to digestible lysine ratios for the variables measured are shown in the table below.

<table>
<thead>
<tr>
<th></th>
<th>Clean</th>
<th></th>
<th>Dirty</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dig. THR / Dig. LYS Ratio</td>
<td></td>
<td>Dig. THR / Dig. LYS Ratio</td>
</tr>
<tr>
<td>Bodyweight Gain</td>
<td>0.74%</td>
<td>66</td>
<td>0.77%</td>
<td>69</td>
</tr>
<tr>
<td>Feed / Gain</td>
<td>0.72%</td>
<td>64</td>
<td>0.73%</td>
<td>65</td>
</tr>
<tr>
<td>Carcass Weight</td>
<td>0.72%</td>
<td>64</td>
<td>0.77%</td>
<td>69</td>
</tr>
<tr>
<td>Carcass Yield</td>
<td>0.71%</td>
<td>63</td>
<td>0.78%</td>
<td>70</td>
</tr>
<tr>
<td>Breast Meat Weight</td>
<td>0.73%</td>
<td>65</td>
<td>0.77%</td>
<td>69</td>
</tr>
<tr>
<td>Breast Meat Yield</td>
<td>0.73%</td>
<td>65</td>
<td>0.75%</td>
<td>67</td>
</tr>
<tr>
<td>Average Values</td>
<td>0.73%</td>
<td>65</td>
<td>0.76%</td>
<td>68</td>
</tr>
</tbody>
</table>

Based upon the trials conducted with threonine during the past several years, it appears that the digestible threonine to digestible lysine ratio is in the range of 65 to 70.

**Practical Application of Digestible Amino Acid Values, Ideal Amino Acid Ratios, Threonine and Concepts in Formulation Systems**

The practical inclusion of L-Threonine was examined (Kidd, et al., 2007) along with the impact on broiler growth, yield and nitrogen excretion. L-Threonine was incorporated into corn-soybean meal-poultry meal diets at either 0.0, 0.5 (made by blending of the two extreme diets) or 1.0 pounds per ton. The trial was conducted from days 25 to 43 of age and digestible lysine was formulated at 1.0% of the diet while the digestible threonine level was kept constant at 0.65 % (digestible threonine : digestible lysine ratio of 65). The other essential amino acids were set with minimum ratios to digestible lysine as follows: TSAA (methionine plus cystine): 75, valine: 75, isoleucine: 66, arginine: 110). No significant differences were noted in performance of birds fed the three different diets for day 43 bodyweights, body weight gain (day 25 to 43), feed intakes, feed utilization or total white meat yield. This indicates that inclusion of L-Threonine up to 1.0 pounds per ton of complete feed does not negatively impact bird performance and as such, there is no reason to limit the inclusion of L-Threonine at these levels. Higher inclusion levels of L-Threonine have been used in the broiler industry with positive results.

A Symposium on “Lessons and Logistics of Application of Digestible Amino Acids in Diet Formulation” was held during the Poultry Science Meetings in 2007. During this symposium, the standardized ileal amino acid digestibility assay was further discussed (Ravindran, 2007). It was also noted that any of the prior digestibility coefficients (apparent, true, etc.) should be implemented versus continuing to formulate on a total amino acid basis (Burnham, 2007) as otherwise, you are over-evaluating plant and protein amino acid availabilities – particularly relative to those synthetic amino acids which are commercially available (DL-Methionine, L-Lysine and L-Threonine).
Only a couple of papers in the published literature address methods and procedures on formulating broiler diets with digestible amino acids (Creswell and Swick, 2001, Dudley-Cash, 2002). To make this approach particularly practical, what I would like to do is show some examples using current feed industry software as to how these basic points of feed formulation can be most easily implemented. The proposed manner of properly implementing digestible amino acids is through the use of existing total amino acids levels, the incorporation of ingredient and amino acid specific digestibility coefficients and then using a calculation to determine the individual digestible amino acid levels for that ingredient. It is highly recommended that all of this be handled completely from within the least-cost formulation software. In setting this up in the two most used formulation packages used in the USA (Feed Management Systems / Brill or Creative Formulation Concepts / Concept 5 (CFC5)), the same basic approach can be employed. Assuming that total amino acids already exist within the ingredient matrix, one should begin by setting up a series of nutrients to represent the digestibility coefficient values for each amino acid as well as a second series of nutrients for the digestible amino acids. It is highly recommended that the digestibility coefficients be entered as whole numbers, rather than their decimal equivalent so as to clearly distinguish them from either total or digestible amino acid levels. Then, by using the built-in global equation option, which will ultimately be applied across all ingredients, the appropriate equations need to be written for each digestible amino acid level from their respective total and digestibility coefficient. Assuming the total (%), digestibility coefficient (whole number) and the digestible amino acid (%) nutrient numbers for lysine are 10, 20 and 30 respectively, the equation to calculate Nutrient 30 (the digestible lysine level) would be written as N10*N20/100. This process is shown in the screen shots below and should be repeated for each amino acid of interest. Then, whenever either the total or digestibility coefficient for an ingredient is changed, the digestible amino acid value is automatically updated. The other benefit of this approach is that when the nutrient specifications for an ingredient are evaluated or printed, all of this information will be clearly visible, without any additional calculations having to be done, as part of the ingredient profile.

With digestible amino acid values having been generated for each ingredient, they can be used to transition from formulating on a total basis to formulating on a digestible basis as they will also now be available for use in the formula. Alternatively, they can also be used to set-up ratios relative to digestible lysine – as the ratios themselves can be calculated on existing feeds, or better yet used for formulation purposes. This is handled quite differently from within the two software packages noted above. From
within the Brill package, these ratios are defined on the “left-hand” side of the linear program as a nutrient where the Factor column description is set as a ratio (using the drop-down box) and Ratio #1 is set as the nutrient number which will be the numerator value in the calculation and Ratio #2 is set as the nutrient number which will be the denominator (digestible lysine in this case). So, if nutrient 30 is digestible lysine and nutrient 31 is digestible methionine, the ratio for digestible methionine to digestible lysine, could be defined in say nutrient 41 by setting the Factor column to Ratio and entering 31 for Ratio #1 and 30 for Ratio #2 (digestible methionine/digestible lysine). From within CFC5, nutrient ratios are set-up on the “right-hand” side of the linear program, or from within the formula itself under the Nutrient Ratios tab. In this case, the numerator is defined as the first nutrient and the denominator is defined as the second nutrient, with the Per column being set at 100 so as to define the appropriate relationship between the two digestible amino acid nutrient levels. It is very important to note that in setting up ratios of one amino acid to the other, the use of global equations to do so will lead to erroneous results. That is why both of these above noted packages offers a special means of setting amino acid ratios correctly.

Now that these nutrients and ratios are all defined correctly, they can be used for the purposes of formulation. For the amino acids, all that need be defined within the formula is the minimum targeted digestible lysine level and then the minimum nutrient ratios relative to digestible lysine (denominator) for TSAA (perhaps methionine too), threonine, valine, isoleucine, tryptophan, arginine and glycine (in a vegetable diet). If these levels are set appropriately, there is little concern for crude protein levels falling too low. An example of this approach to formulation is shown below.

As with the printout of an ingredient after properly setting up digestibility coefficients and digestible amino acids, all of the exact information as to how a given diet was formulated is included in the printout of that formula. Not only can the nutrient levels section show total amino acids, but it can also show the digestible amino acid levels along with their minimum or absolute ratios to digestible lysine.
Conclusions

Employing the concepts discussed in this paper will help optimize broiler performance along with economic return. These key consideration include the following main points:

- Using digestible amino acids for purposes of formulation.
- Setting a proper and adequate minimum digestible lysine level upon which optimal growth and feed efficiency can be achieved.
  o Assuring maximal early growth with correct nutrient density.
- Setting correct essential ideal amino acid ratios on a digestible basis to digestible lysine.
  o Relaxing or removing the crude protein minimum constraint.
- Offering a methionine, lysine and threonine source as ingredients to all poultry diets.
  o Removing constraints on amino acids as ingredients, as they are 100% digestible and therefore should be utilized to their fullest degree.

If these steps are utilized correctly, an acceptable “crude protein” or nitrogen level in the diet will be maintained as the fourth limiting amino acid will provide that assurance. While a reduced protein diet will be formulated, under most circumstances it will not be a low protein diet, as the protein level should fall less than 1.5 percentage points if proper ratios and an adequate digestible lysine level is utilized.

References


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MAXIMIZING PRODUCTION EFFICIENCY BY A BETTER UNDERSTANDING OF THE 4TH LIMITING AMINO ACID IN BROILER FORMULATION

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Summary

In the ongoing effort to maximize profitability, significant emphasis is given to production costs associated with feed considering they represent the majority of production costs. These days, major feed ingredients such as corn, soybean meal, fats, poultry by-product meal, and meat-and-bone meal have experienced increases in their market price, some at a higher rate than others. With the increase of corn usage for ethanol fermentation and fats for biodiesel manufacturing, meeting metabolizable energy needs in formulas has become a much higher pressure point than it has ever been. Until recently, crude protein was arguably the most influential of nutrients in determining the cost of a diet, but it is fair to say that the gap with other nutrients has narrowed. L-threonine supplementation reduces crude protein in formulas, in turn reducing the price of feed by as much as 4 dollars in some cases. With the recent feed ingredient market evolution, supplementation with L-threonine still remains a profitable strategy. But in order for L-threonine inclusion to be most valuable, subsequent limiting amino acids such as isoleucine, valine and arginine must be closely monitored. This vigilance is perhaps the key to maintaining optimum growth, feed conversion and yield of commercial broilers under these dietary conditions. These proceedings will address what we know about marginality and needs of these subsequent limiting amino acids.

Introduction

The US poultry industry is no different than any livestock industry in attempting to take advantage of any opportunity that would result in minimizing dietary costs. One of these opportunities has been through the use of crystalline and synthetic amino acid supplementation as the means for reducing diet cost. This is accomplished by allowing a reduction in dietary crude protein which traditionally has been the pressure point in diet cost. This works by allowing the next supplemental amino acid to be incorporated in the diet, allowing for less proteinaceous feed ingredients to be utilized while concomitantly reducing diet cost. This has shown to provide various advantages in addition to reduction in feed cost, such as a reducing the dietary nitrogen excretion level and improving the dietary protein balance, all this while maintaining performance. Constant oscillating feed ingredient prices make this task a challenge when attempting to remain competitive in the poultry meat market. Additionally, recent trends associated with the generation of biofuels have heightened the volatility in feed ingredient price and availability. Soybean meal, fats and corn prices have all increased in recent years at much higher rates than those associated with inflation, in turn generating additional pressure to generate solutions to offset this problem without having to increase meat and egg prices accordingly.

As previously mentioned, supplemental amino acids have allowed for maximizing profitability by reducing the inclusion levels in diets of expensive ingredients such as soybean meal, and animal by-products such as meat & bone meal, poultry meal, fish meal, and various blends. One of the most recent moves in this strategy has been the inclusion of L-threonine. Less than a decade ago, L-threonine was introduced at a feed grade level with a competitive price, and slowly began being utilized by the US
poultry industry. Nowadays, very few are the integrators that do not offer L-threonine in their formulation, or cases were L-Thr goes unselected by a least cost program. Recent formulas show how L-threonine allows for a reduction of over $2 dollars per ton of feed, and in some cases more than that. However, the key to a successful reduction in crude protein through the use of L-threonine inclusion is to ensure that other marginal amino acids are maintained at adequate levels. These next limiting amino acids are likely to be valine and isoleucine, and to a lesser extent arginine, tryptophan, and glycine (Kidd and Hackenhaar, 2005; Corzo et al., 2007).

Table 1. 4th limiting amino acid prediction in broiler diets during a grower phase formulating with either marginal or high dietary lysine levels (Kidd and Hackenhaar, 2005).

<table>
<thead>
<tr>
<th>Diet type</th>
<th>Digestible lysine (%)</th>
<th>Protein (%)</th>
<th>L-Thr (g/ton)</th>
<th>4th limiting amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn &amp; soybean meal</td>
<td>1.0</td>
<td>18.6</td>
<td>463</td>
<td>Valine</td>
</tr>
<tr>
<td>Corn &amp; soybean meal</td>
<td>1.1</td>
<td>20.4</td>
<td>477</td>
<td>Valine</td>
</tr>
<tr>
<td>Corn, soybean meal &amp; poultry meal</td>
<td>1.0</td>
<td>19.5</td>
<td>392</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>Corn, soybean meal &amp; poultry meal</td>
<td>1.1</td>
<td>21.0</td>
<td>503</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>Corn, soybean meal &amp; meat blend</td>
<td>1.0</td>
<td>20.0</td>
<td>259</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Corn, soybean meal &amp; meat blend</td>
<td>1.1</td>
<td>21.4</td>
<td>428</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Sorghum &amp; soybean meal</td>
<td>1.0</td>
<td>19.4</td>
<td>449</td>
<td>Arginine</td>
</tr>
<tr>
<td>Sorghum &amp; soybean meal</td>
<td>1.1</td>
<td>20.8</td>
<td>608</td>
<td>Arginine</td>
</tr>
<tr>
<td>Sorghum, soybean meal &amp; poultry meal</td>
<td>1.0</td>
<td>20.1</td>
<td>696</td>
<td>Arginine</td>
</tr>
<tr>
<td>Sorghum, soybean meal &amp; poultry meal</td>
<td>1.1</td>
<td>21.5</td>
<td>854</td>
<td>Arginine</td>
</tr>
<tr>
<td>Sorghum, soybean meal &amp; meat blend</td>
<td>1.0</td>
<td>20.5</td>
<td>403</td>
<td>Arginine</td>
</tr>
<tr>
<td>Sorghum, soybean meal &amp; meat blend</td>
<td>1.1</td>
<td>21.9</td>
<td>562</td>
<td>Arginine</td>
</tr>
<tr>
<td>Wheat &amp; soybean meal</td>
<td>1.0</td>
<td>19.8</td>
<td>830</td>
<td>Valine</td>
</tr>
<tr>
<td>Wheat &amp; soybean meal</td>
<td>1.1</td>
<td>21.5</td>
<td>812</td>
<td>Valine</td>
</tr>
<tr>
<td>Wheat, soybean meal &amp; poultry meal</td>
<td>1.0</td>
<td>20.8</td>
<td>788</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>Wheat, soybean meal &amp; poultry meal</td>
<td>1.1</td>
<td>22.3</td>
<td>870</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>Wheat, soybean meal &amp; meat blend</td>
<td>1.0</td>
<td>20.8</td>
<td>852</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>Wheat, soybean meal &amp; meat blend</td>
<td>1.1</td>
<td>22.3</td>
<td>934</td>
<td>Isoleucine</td>
</tr>
</tbody>
</table>

Diets were formulated using ratios to lysine of 72 for total sulfurs, 65 for threonine, 65 for isoleucine, 77 for valine, 105 for arginine and 17 for tryptophan.

Valine

Some small production at the feed grade level is available for this amino acid, but in poultry it has not been utilized. Some nursery pig diets may sometimes include it, but this is not observed in most other swine diets. The next logical question in the effort to relieve dietary crude protein as an economical constraint in broiler diets would be to know which is the next limiting amino acid. The answer to this question is by no means simple, and largely depends on numerous factors. These factors include ingredients being used, digestibility of amino acids of such ingredients, formula type (e.g. broilers, layers, turkeys), age of the birds being formulated for, and obviously the nutrient minimums that the feed formulator is attributing to these next limiting amino acids. For example, Kidd and Hackenhaar (2005) modeled a grower phase broiler diet and predicted what the 4th limiting amino acid would be, using ratios as the means to assign amino acid minimums (Table 1). Resultant predictions labeled valine, isoleucine, tryptophan and arginine, all as possible theoretical candidates to be the 4th limiting amino acid. This specific example depended mostly on the ingredient combination used, as it can be appreciated (Table 1). If a more aggressive digestible lysine formulation is used like in those operations destined to maximize
breast meat yield (Hickling et al., 1990; Moran and Bilgili, 1990; Bilgili et al., 1992), it may be to the
discretion of the feed formulator to increase the nutrient minimums of the other limiting amino acids,
even though that may represent an increase in the inclusion level of soybean meal and meat meals. In this
particular example, the ratios of other limiting amino acids were maintained, thus resulting in the same
limiting amino acid for both dietary lysine levels formulated.

Corzo et al. (2007) observed how in a corn-soybean meal diet where limiting amino acids other
than lysine, TSAA and threonine were formulated without a nutrient minimum value, and subsequently
individually supplementing these other limiting amino acids, it was reported that only valine resulted in
the most significant improvement in body weight gain (Figure 1). A corn-soybean meal diet formulated with
nutrient minimums for all limiting amino acids served as control (+C). When compared to this positive
control, it can be observed how Val supplementation to resulted in the most significant improvement of all
the individually supplemented amino acids. Another all-vegetable diet used by Corzo et al. (2007) showed
how the combination of corn and peanut meal resulted in a significant valine deficiency, and it was
observed how supplementing to L-valine resulted in body weight gain and feed conversion values equal to a
control corn-soybean meal based diet. Thus it is fair to say that formulating using an adequate valine
nutrient minimum is imperative in diets where L-threonine is being supplemented as a means of reducing
crude protein, particularly in the combination of corn and soybean meal and in the absence of an animal
by-product meat meal. Furthermore, based on predictions by Kidd and Hackenhaar (2005), using the all-
vegetable combination of wheat and soybean meal is likely to result in valine becoming the 4th limiting
amino acid.

Displayed on Table 2 are the most recent dietary valine requirement estimates and
recommendations for broilers. It can be observed that all dietary valine recommendations apply only to
males. It is safe to say, however, that if formulating to males, the female valine needs should be easily
met as well as those of all other critical limiting amino acids, considering that males have exhibited higher
amino acids needs than female broilers.

Table 2. Recent valine requirement estimated in broilers.

<table>
<thead>
<tr>
<th>Age</th>
<th>Recommendation</th>
<th>Bird &amp; sex</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-42 days</td>
<td>0.73%</td>
<td>Ross x 508♂</td>
<td>Thornton et al., 2006</td>
</tr>
<tr>
<td>42-56 days</td>
<td>0.73% (0.67% dig)</td>
<td>Ross x 308♂</td>
<td>Corzo et al., 2004a</td>
</tr>
<tr>
<td>20-40 days</td>
<td>81</td>
<td>ISA 220♂</td>
<td>Mack et al., 1999</td>
</tr>
<tr>
<td>8-21 days</td>
<td>78</td>
<td>NH x C♂²</td>
<td>Baker et al., 2002</td>
</tr>
<tr>
<td>21-42 days</td>
<td>78</td>
<td>Ross x 708♂</td>
<td>Corzo et al., 2007</td>
</tr>
</tbody>
</table>

¹Values correspond to the needs for dietary valine as a proportion to dietary lysine.
²New Hampshire x Columbian
Isoleucine

Several ingredients such as gelatin, meat and bone meal, blood meal, and poultry by-product meal have all been reported to be limiting in isoleucine (Boomgaardt and Baker, 1972; Wang et al., 1997; Wang and Parsons, 1998). In all of these reports isoleucine may have not been necessarily the 1st limiting amino acid, but considering the amino acid contributions of an average corn, soybean meal, and feed grade supplemental amino acids to a normal diet, it is very likely that isoleucine would be at the top of the list.

Arguably, the most likely amino acid to be 4th limiting in most commercial poultry diets in the US nowadays is isoleucine. The vast majority of the US poultry industry uses an animal protein of some sort or a blend of various animal sources. Unfortunately, the amino acid composition of such meat blends varies from one source to the next, as do their amino acid digestibility values. Based on predictions by Kidd and Hackenhaar (2005), it was observed that isoleucine could be the 4th limiting amino acid in diets based on corn, soybean meal and poultry meal, or in wheat-soybean meal based diets in combination with either poultry by-product meal or a meat blend (Table 1).

While there seems to be some good values reported recently for isoleucine needs, there also seems to be a need in the literature for recommendations using a high-yield strain. Research using the Ross 708, Cobb 500 and 700, and Hubbard UY broiler strains is warranted. Using Ross x Arbor Acres broilers, Kidd et al. (2000) showed how isoleucine nutrient minimums can impair breast meat yield in a diet were nutrient minimums are neglected. As it can easily occur with valine, failure to formulate and meet an isoleucine nutrient minimum, particularly in diets with L-threonine supplementation, will likely negate the purpose of crude protein reduction as a profitability strategy, and ultimately results in a drop in performance and yields, as observed by Kidd et al. (2000). Recent isoleucine recommendation estimates are displayed in Table 3.

Table 3. Recent isoleucine requirement estimated in broilers.

<table>
<thead>
<tr>
<th>Age</th>
<th>Recommendation</th>
<th>Bird</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-42 days</td>
<td>0.68% (0.64% dig)</td>
<td>Ross x 508 ♀</td>
<td>Hale et al., 2004</td>
</tr>
<tr>
<td>18-30 days</td>
<td>0.71% (0.64% dig)</td>
<td>Ross x 308 ♂</td>
<td>Kidd et al., 2004</td>
</tr>
<tr>
<td>30-42 days</td>
<td>0.66% (0.60% dig)</td>
<td>Ross x 308 ♂</td>
<td>Kidd et al., 2004</td>
</tr>
<tr>
<td>42-56 days</td>
<td>0.63% (0.57% dig)</td>
<td>Ross x 308 ♂</td>
<td>Kidd et al., 2004</td>
</tr>
<tr>
<td>42-56 days</td>
<td>0.66%</td>
<td>Ross x 308 ♂</td>
<td>Corzo et al., 2004b</td>
</tr>
<tr>
<td>20-40 days</td>
<td>71°</td>
<td>ISA 220 ♂</td>
<td>Mack et al., 1999</td>
</tr>
<tr>
<td>8-21 days</td>
<td>61°</td>
<td>NH x C ♀</td>
<td>Baker et al., 2002</td>
</tr>
</tbody>
</table>

1 Values correspond to the needs for dietary valine as a proportion to dietary lysine.
2 New Hampshire x Columbian

Arginine, tryptophan, leucine

Arginine is an amino acid of tremendous importance. In addition of being needed for protein synthesis like any other amino acid, it has functions related to the biosynthesis nitric oxide, collagen and connective tissue generation, and energy muscle reserves in the form of creatine. Fortunately, this is an amino acid that appears to be only a limiting factor in diets containing sorghum as the primary grain source. As predicted by Kidd and Hackenhaar (2005), it appears that no combination of vegetable and/or animal protein by-products may preclude this amino acid from becoming 4th limiting in sorghum-based
type diets. The way that this amino acid has been prevented for becoming deficient in these or any other formulas has been to assign a nutrient minimum, typically done by the imposition of a 105 ratio of arginine to dietary lysine or by imposing a crude protein minimum.

Kidd and Hackenhaar (2005) suggested a potential limitation of tryptophan in a corn, soybean meal, and meat blend based diet. The meat blend these authors used in this prediction exercise was based of poultry meal, meat and bone meal, and feather meal. However, meat blends vary from integrator to integrator. It seems unlikely that tryptophan would become 4th limiting if meat and bone meal or poultry by-product meal were solely used in any grain-soybean meal based diet. Nevertheless, a tryptophan deficiency would result in deleterious consequences, not only from a protein synthesis substrate standpoint, but based on precursor needs for synthesis of important neurotransmitters like serotonin and melatonin. This deficiency, as in the case of arginine, can likely be avoided if imposing a proportion or ratio to lysine of 17 or higher in formulation.

Leucine is an amino acid structurally similar to isoleucine and valine, arguably the two most likely candidates of becoming 4th limiting in most commercial broiler diets in the US. However, the high amounts of leucine in common feed ingredients used in broiler formulation makes it the least likely of becoming 4th limiting. In contrast, it may actually become a problem under certain conditions, exacerbating the needs for isoleucine and valine in poultry, on a metabolic constraint known to nutritionists as the branched-chain amino acid antagonism (D’Mello and Lewis, 1970; Smith and Austic, 1978). It is recommended, therefore, that leucine be simply monitored not for its marginality but for increased spikes in dietary concentration.

Conclusions

The amino acid likely to become 4th limiting in any boiler diet will ultimately depend on what ingredients are being used, their digestibility coefficients and the minimum nutrient levels we impose in formulation. All of the previously discussed amino acids, with the exception leucine, are candidates for this scenario, but valine and isoleucine seem more likely candidates given today’s commercial conditions and the ingredients we utilize in the US for manufacturing of commercial broiler feeds. It is recommended that dietary minimums for valine, isoleucine, arginine, and tryptophan be used if all formulas. Of utmost importance is the use of these minimums when reducing dietary crude protein via L-threonine supplementation, to ensure maintaining good growth and yields.

References


EXCESS CYST(E)INE AND L-METHIONINE PRECURSOR UTILIZATION

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Summary

Five 9 or 12-d chick growth bioassays were done using 2 different Met-deficient diets: an amino acid-based purified diet analyzed to contain 20.3% CP, 0.12% Met, and 0.05% cyst(e)ine, and an amino acid-fortified corn-peanut meal diet analyzed to contain 19.0% CP, 0.22% Met and 0.23% cyst(e)ine. When the purified diet was modified to contain 0.12% Met and 0.12% cyst(e)ine, as little as 0.10% added L-cystine depressed ($P < 0.05$) growth performance. When the purified diet was changed to contain 0.12% Met and 0.40% cyst(e)ine (i.e., single deficiency of Met), multiple linear regression ($R^2 = 0.97$) and slope-ratio evaluation of graded doses of feed-grade DL-Met (99%), i.e., DL-M and feed-grade DL-OH-Met, Ca (84%), i.e., OH-M revealed bioefficacy values for OH-M relative to DL-M of 79.5% (isosulfurous basis) and 66.8% (compound basis). In a subsequent assay, the purified diet was modified to be equally deficient in both Met and cyst(e)ine (0.12% of each), and when isosulfurous supplements of DL-M and OH-M were compared, no differences ($P > 0.10$) in growth performance were evident between the two Met precursor compounds.

The final two bioassays (3 x 2 factorials) employed the Met and sulfur-amino acid (SAA)-deficient corn-peanut meal diet. Isosulfurous DL-M and OH-M supplements were compared in diets with or without 0.10% added bioavailable cyst(e)ine provided as either L-cystine or feather meal. DL-M elicited greater ($P < 0.05$) growth performance than OH-M, but the magnitude of difference was considerably greater in diets containing added L-cystine or feather meal than in diets not containing these supplements. These results suggest that small excesses of dietary cyst(e)ine in Met-deficient diets are both anorexigenic and pernicious to OH-M utilization.

Introduction

Quantification of methionine (Met) precursor utilization has been controversial for at least 40 years (Scott et al., 1966). Our goal in the research reported here was more to evaluate factors affecting DL-Met (DL-M) and DL-OH-Met, Ca (OH-M) utilization than to quantify precursor utilization per se. Dietary cyst(e)ine (i.e., cysteine and/or cystine) level was identified as a critical factor worthy of consideration. Indeed, early studies by Scott et al. (1966), Katz and Baker (1975), Christiansen and Anderson (1980), and Boebel and Baker (1982) showed that OH-M utilization (relative to L- or DL-Met) was greater when Met compounds were supplemented in diets with a low concentration (or ratio) of cyst(e)ine relative to Met in contrast to diets that were singly deficient in Met (i.e., high cyst(e)ine:Met ratio).

The D portion of DL-Met requires two enzymatic steps to be converted to L-Met. DL-OH-Met requires four enzymatic reactions for L-Met biosynthesis: two for D-OH-Met and two for L-OH-Met. Previous research in our laboratory (Dilger and Baker, 2007a) showed that neither cyst(e)ine:Met ratio nor excess cyst(e)ine per se affected utilization of DL-Met relative to L-Met. This suggested that neither the D-Met to Keto Met nor Keto Met to L-Met reactions were influenced by cyst(e)ine level. Therefore, since OH-M also requires a Keto-Met to L-Met reaction, this portion of the DL-OH-Met conversion to L-Met can be ruled out as being influenced by cyst(e)ine level. Because the results presented herein support
the view that excess cyst(e)ine relative to Met in a sulfur amino acid (SAA)-deficient diet reduces OH-M efficacy, one could speculate that excess dietary cyst(e)ine may somehow affect the flux of D-OH-Met to Keto Met via D-hydroxy acid dehydrogenase or L-OH-Met to Keto Met via L-hydroxy acid oxidase (cf. Dibner and Knight, 1984).

Often overlooked in Met dosing studies is the marked difference in response magnitude when Met is supplemented in diets singly deficient in Met compared with diets deficient in both Met and cyst(e)ine (Figure 1). Although this figure displays a theoretical data set, we have unpublished data that very nearly duplicates the plot shown in Figure 1. Clearly, when Met is added to diets containing excess cyst(e)ine, each dose of Met allows an equal dose of cyst(e)ine to be utilized also. Thus, for example, addition of 0.05% L-Met in diets with excess cyst(e)ine produces a growth response actually greater than that obtained with double the L-Met dose (i.e., 0.10%) in diets (equally) deficient in both Met and cyst(e)ine. In fact, in the latter case, only one-half of the L-Met dose is used for Met per se, with the other one-half being used for cysteine biosynthesis via transsulfuration — and cysteine synthesis from Met, while 100% efficient on a molar basis, is only 81% efficient on a weight or concentration basis. Hence, it takes 0.11% L-Met (deficient Met and cyst(e)ine) to give the same response as 0.05% L-Met in diets fed to chicks containing a single deficiency of Met.

**Methods**

All assays were done in batteries using male chicks obtained from the cross of New Hampshire males and Columbian females. Other details of allotment and feeding procedures were the same as those described previously (Dilger and Baker, 2007a,b). Two Met and SAA-deficient diets were employed. The purified basal diet used in Assays 1, 2, and 3 was analyzed to contain 20.3% CP, 0.12% Met, and 0.05% cyst(e)ine, and its complete description has been published (Dilger and Baker, 2007a,b; Dilger et al., 2007). The other SAA-deficient diet (Assays 4 and 5) was an amino acid-fortified corn (57.5%) and peanut meal (27.5%) diet analyzed to contain 19.0% CP, 0.22% Met, and 0.23% cyst(e)ine (Dilger and Baker, 2008). All bioassays except Assay 2 (12 d in length) were of 9-d duration, i.e., from 8 to 17-d posthatch. Ingredients used in all diets were from the same source and batch, and all ingredients were analyzed for CP, Met, cyst(e)ine, and lanthionine. The latter was assumed to have a cyst(e)ine bioavailability of 30% (Robbins et al., 1980; Baker et al., 1981).

The two Met precursor compounds evaluated were feed-grade DL-Met (Degussa) and feed-grade DL-OH-Met, Ca (Novus). Product guarantees of 99% for DL-Met and 84% for DL-OH-Met (Ca) were assumed for these commercially available Met precursor compounds. However, after conducting Assays 1, 2, and 3, analytical and bioassay evaluation of purity for DL-Met (Dilger et al., 2007a,b) indicated that this compound was not different from 100% pure and efficacious relative to pure L-Met. Thus, a 100% value was assumed for feed-grade DL-Met in Assays 4 and 5. We did not have similar information for DL-OH-Met (Ca), so in Assays 4 and 5 we continued to use the 84% value for DL-OH-Met (Ca) in comparisons with DL-Met. In Assays 4 and 5, 0.10% excess cyst(e)ine was evaluated as a factor affecting DL-M and OH-M utilization. Cyst(e)ine was provided by L-cystine in Assay 4 and by 3.54% feather meal in Assay 5. The addition of 3.54% feather meal in Assay 5 furnished 0.160% cyst(e)ine, 0.046% lanthionine, and 0.018% methionine. Based on previous work (Robbins et al., 1980; Baker et al., 1981; Han and Parsons, 1990), we calculated that the cyst(e)ine + lanthionine contributed by 3.54% feather meal provided 0.10% bioavailable cyst(e)ine.
Results and Discussion

Assay 1

Data in Table 1 show clearly that even a small excess of dietary cyst(e)ine in SAA and Met-deficient diets is anorexigenic (Dilger and Baker, 2007b). Additions of 0.08% and 0.28% L-cystine to the basal diet containing an equal deficiency of Met and cyst(e)ine resulted in linear (P < 0.05) depressions in gain, feed intake, and gain:feed. Previous work has also shown this same cyst(e)ine imbalance phenomenon (Featherston and Rogler, 1978; Sell et al., 1980; Hirakawa and Baker, 1985).

Assay 2

Assay 2 was one of two slope-ratio bioassays done using the purified diet, with the basal diet for this assay (Table 2) containing 0.12% Met and 0.40% cyst(e)ine (Dilger and Baker, 2008). Similar results were obtained in both assays, even though the assay shown in Table 2 involved a cyst(e)ine:Met basal diet ratio of 3.33:1 whereas the other assay (data not shown) had a lower cyst(e)ine:Met basal diet ratio, i.e., 1.67:1.

Multiple linear regression analysis (Littell et al., 1997; Kratzer and Littell, 2006) of weight gain and gain:feed as a function of supplemental sulfur intake resulted in an average (gain and gain:feed) OH-M bioefficacy value of 79.5%, equivalent to 66.8% on a supplemental compound basis. Thus, with a single deficiency of Met (i.e., excess cyst(e)ine), OH-M bioefficacy was clearly inferior to DL-M. Other reviews have come to a similar conclusion, although cyst(e)ine:Met ratio was not considered as a factor (Baker, 1994; Lewis and Baker, 1995; Jansman et al., 2003; Baker, 2006).

Table 1. Excess cyst(e)ine for chicks fed methionine –deficient diets (Assay 1).1

<table>
<thead>
<tr>
<th>SAA level (%)</th>
<th>Weight gain, g 3</th>
<th>Feed intake, g 3</th>
<th>Gain:feed, g/kg 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met</td>
<td>Cys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.12</td>
<td>0.12</td>
<td>25a</td>
<td>101a</td>
</tr>
<tr>
<td>0.12</td>
<td>0.20</td>
<td>22ab</td>
<td>93a</td>
</tr>
<tr>
<td>0.12</td>
<td>0.40</td>
<td>16b</td>
<td>79b</td>
</tr>
</tbody>
</table>

SEM 2.0 3.2 15.1

1Values are means of 5 pens of 4 male chicks during a 9-d feeding period from 8 to 17-d posthatch; average initial weight was 83 g; Dilger and Baker (2007b).

2Purified basal diet, with cyst(e)ine supplemented as L-cystine.

3Linear (P < 0.05) decrease. Means with unlike superscript letters are different (P < 0.05).

Table 2. Slope-ratio assessment of DL-OH Met (Ca) relative to DL-Met in chicks fed a purified basal diet containing 0.12% methionine and 0.40% cyst(e)ine (Assay 2).1 4

<table>
<thead>
<tr>
<th>Supplement addition</th>
<th>Weight gain, g 3</th>
<th>Gain:feed, g/kg 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met source 2</td>
<td>Level (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>DL-M</td>
<td>404</td>
<td>82</td>
</tr>
<tr>
<td>DL-M</td>
<td>808</td>
<td>146</td>
</tr>
<tr>
<td>DL-M</td>
<td>1212</td>
<td>242</td>
</tr>
<tr>
<td>OH-M</td>
<td>476</td>
<td>65</td>
</tr>
<tr>
<td>OH-M</td>
<td>952</td>
<td>115</td>
</tr>
<tr>
<td>OH-M</td>
<td>1429</td>
<td>185</td>
</tr>
</tbody>
</table>

SEM 10.5 16.9

1Values are means of 5 pens of 3 male chicks during a 12-d feeding period from 8 to 20-d posthatch; average initial weight was 107 g; Dilger and Baker (2008).


3Multiple linear regression of gain (Y in g) on supplemental sulfur intake (X in mg) from DL-M (X1) and OH-M (X2) was: Y = 40.2 + 2.245 X1 + 1.766 X2; R² = 0.97. The ratio of slopes (percent, relative to DL-M) was 78.7% (95% CI: 70.6 – 86.7) for OH-M.

4Multiple linear regression of gain:feed (Y in g/kg) on supplemental sulfur intake (X in mg) from DL-M (X1) and OH-M (X2) was: Y = 368.8 + 42.15 X1 + 33.86 X2; R² = 0.93. The ratio of slopes (percent, relative to DL-M) was 80.3% (95% CI: 65.2 – 95.3) for OH-M.

5The 3 additions of OH-M were isosulfurous to those of DL-M, assuming purity values of 99% for feed-grade DL-M and 84% for feed-grade OH-M.
**Assay 3**

The purified diet used in this assay was modified to contain 0.12% Met and 0.12% cyst(e)ine (Table 3), and at similar levels of DL-M supplementation, chicks in this assay gained as fast over 9 d as those over 12 d in Assay 2. Under the Met and cyst(e)ine dietary conditions of Assay 3, chicks responded almost the same to both DL-M and OH-M. It is worth noting that the Met and cyst(e)ine dietary conditions of Assay 2 (Table 2) and Assay 3 (Table 3) reflect the dietary conditions shown in Figure 1. Why does OH-M perform well relative to DL-M when the L-Met precursor compounds are forced to provide both Met and cysteine (Table 3) and why does OH-M relative to DL-M perform poorly when the two compounds are used to provide only Met (Table 2). An answer to these questions is not obvious. However, it may be possible that (1) excess cyst(e)ine impedes one or both of the two enzymatic reactions leading to Keto-Met synthesis from D-OH-M and L-OH-M, or (2) OH-M is less efficient than DL-M in allowing efficient utilization of cysteine when the Met supplements are given to chicks consuming diets with deficient Met and excess cyst(e)ine, cf. Figure 1.

**Table 3. Efficacy of Met sources for chicks fed a purified diet that was equally deficient (0.12% of each) in Met and cyst(e)ine (Assay 3).**

<table>
<thead>
<tr>
<th>Supplement addition</th>
<th>Weight gain, g</th>
<th>Gain:feed, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met source</td>
<td>Level (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DL-M</td>
<td>404</td>
<td>88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OH-M</td>
<td>476</td>
<td>84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>4.6</td>
<td>11.1</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means of 8 pens of 3 male chicks during a 9-d feeding period from 8 to 17-d posthatch; average initial weight was 100 g; Dilger and Baker (2008).

**Assay 4**

Assay 4 was done using the SAA-deficient corn-peanut meal diet containing 0.22% Met and 0.23% cyst(e)ine (Table 4). Based on NRC (1994) SAA digestibility estimates, the basal diet for this assay as well as for Assay 5 contained 0.19% digestible Met and 0.19% digestible cyst(e)ine. Statistical analysis of data in Table 4 indicated that the main effects of cystine addition and Met source (DL-M vs OH-M) were significant ($P < 0.05$) for weight gain, but not gain:feed. The growth performance difference between Met sources was greater in the presence than in the absence of the 0.10% L-cystine supplement.

**Table 4. Effect of excess cyst(e)ine on the efficacy of DL-OH-Met (Ca) relative to DL-Met for chicks fed a corn-peanut meal diet (Assay 4).**

<table>
<thead>
<tr>
<th>Supplement addition&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Weight gain, g</th>
<th>Gain:feed, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met source</td>
<td>Met, mg/kg</td>
<td>Cys, %</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DL-M</td>
<td>465</td>
<td>0</td>
</tr>
<tr>
<td>OH-M</td>
<td>554</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0.10</td>
</tr>
<tr>
<td>DL-M</td>
<td>465</td>
<td>0.10</td>
</tr>
<tr>
<td>OH-M</td>
<td>554</td>
<td>0.10</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4.9</td>
<td>12.8</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means of 5 pens of 4 male chicks during a 9-d feeding period from 8 to 17-d posthatch; average initial weight was 103 g; Dilger and Baker (2008).

<sup>2</sup>The corn-peanut meal basal diet contained by analysis 0.22% Met and 0.23% cyst(e)ine. DL-Met (DL-M) and DL-OH-Met, Ca (OH-M) were supplemented at isosulfurous levels; cyst(e)ine was supplemented as L-cystine.

<sup>3</sup>O vs cystine ($P < 0.05$); DL-M vs OH-M ($P < 0.05$); 0 vs Met ($P < 0.01$).

<sup>4</sup>Means with unlike superscript letters are different ($P < 0.05$).
Assay 5 was similar to Assay 4, both containing a 3 × 2 factorial arrangement of treatments, but the cyst(e)ine supplement in Assay 5 was provided by feather meal replacing corn starch in the corn-peanut meal diet. In all cases the 3.54% feather meal addition depressed \((P < 0.01)\) weight gain. It also decreased gain:feed in diets unsupplemented with Met. Both gain and feed efficiency were greater \((P < 0.01)\) in chicks fed DL-M than in those fed an isosulfurous level of OH-M, regardless of cyst(e)ine supplementation. Nonetheless, the weight gain advantage for DL-M over OH-M was greater in the presence (16.7%) than absence (7.7%) of excess cyst(e)ine. A smaller difference existed for gain:feed, with the advantage for DL-M vs OH-M being 7.3% in the presence and 5.7% in the absence of excess cyst(e)ine.

Conclusion

We believe that previous evidence (Scott et al., 1966; Katz and Baker, 1975; Christiansen and Anderson, 1980; Boebel and Baker, 1982) together with the results presented herein point to the conclusion that cyst(e)ine:Met ratio in a given assay diet can have marked effects on the bioefficacy of either free acid or Ca salt forms of DL-OH-Met relative to DL-Met. In 2006, two swine papers were published wherein the free acid form of DL-OH-Met was compared to DL-Met (Kim et al., 2006; Yi et al., 2006). In the Kim et al. (2006) work, their SAA-deficient basal diet contained a cyst(e)ine:Met ratio of 1.90, and they reported a DL-OH-Met bioefficacy value of only 64% (compound basis) relative to DL-Met. Yi et al. (2006), on the other hand, used a SAA-deficient basal diet that was approximately equal in bioavailable Met and cyst(e)ine – and they reported a DL-OH-Met bioefficacy value of over 100% (compound basis) relative to DL-Met. Vazquez-Anon et al. (2006) used a multiple linear regression model to evaluate DL-OH-Met and DL-Met in broiler chick experiments. They found that dietary cyst(e)ine level was a statistically significant parameter in the model – for DL-OH-Met but not for DL-Met. It is our opinion that cyst(e)ine:Met ratio is a more important consideration than diet type (purified vs practical) or degree of Met deficiency (severe vs marginal) in comparisons of DL-OH-Met vs DL-Met bioefficacy. More work is needed on both source and level of excess dietary cyst(e)ine concerning their effects on DL-OH-Met utilization.

| Table 5. Effect of excess cysteine provided by feather meal on the efficacy of DL-OH-Met (Ca) relative to DL-Met for chicks fed a corn-peanut meal diet (Assay 5). |
| Met source | Met, mg/kg | Cys, % | Weight gain, g | Gain:feed, g/kg |
| DL-M | 465 | 0 | 130 | 528 |
| OH-M | 554 | 0 | 120 | 498 |
| SEM | 0 | 0.10 (FM) | 60 | 398 |
| DL-M | 465 | 0.10 (FM) | 108 | 531 |
| OH-M | 554 | 0.10 (FM) | 90 | 492 |

1 Values are means of 5 pens of 4 male chicks during a 9-d feeding period from 8 to 17-d posthatch; average initial weight was 99 g; Dilger and Baker (2008).
2 The corn-peanut meal basal diet contained by analysis 0.22% Met and 0.23% cyst(e)ine. DL-Met (DL-M) and DL-OH-Met, Ca (OH-M) were supplemented at isosulfurous levels; cyst(e)ine was supplemented as .10% bioavailable cyst(e)ine provided by feather meal (FM).
3 0 vs Met (P < 0.01); DL-M vs OH-M (P < 0.01).
4 0 vs Met x 0 vs Cys as FM (P < 0.01).
5 0 vs Met x 0 vs Cys as FM (P < 0.08).
6 Means with unlike superscript letters are different (P < 0.05).
References


Dilger, R.N., and D.H. Baker. 2007a. DL-methionine is as efficacious as L-methionine, but modest L-cystine excesses are anorexigenic in methionine deficient purified and practical-type diets fed to chicks. Poult Sci. 86:2367-2374.


COLLABORATIVE RESEARCH: A TRIAD FOR STRENGTH
(POULTRY INDUSTRY, ALLIED INDUSTRY AND UNIVERSITIES)

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Summary

Collaborative research has the potential to improve poultry meat and egg production, quality, price and profitability for U.S. consumers and poultry companies. A discussion will address the potential for collaborative research limited to the poultry industry, allied industry and universities. Questions will be raised regarding the types of research that can be conducted and under what circumstances. Each of the three components of the triad brings value to the discussion and each is actually dependent on the other. However, each has its own set of barriers to prevent true collaboration from taking place. The poultry industry has potentially millions of birds to learn from in “real world” practical conditions. However, they generally need immediate, low-cost, proprietary solutions. Allied industry brings newly developed products to the marketplace that, hopefully, provides true economic value to the industry but is mainly focused on sales of their products. Universities bring an unbiased, independent, scientific viewpoint but generally deal with small bird numbers in their experiments that have been criticized as not being “real world”. Academic researchers often have good facilities for replicated studies, but overhead and labor costs can be high and there is the requirement for publication in refereed journals. While there is nothing wrong with any of these individual goals or constraints, they do cause barriers for collaboration. Notes will be taken during the discussion and summarized at the end of the conference, so please come and share your perspective.
FEEDING DISTILLERS DRIED GRAINS WITH SOLUBLES (DDG/S) TO POULTRY

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Summary

Grain to fuel has driven a massive increase in ethanol production, resulting in an increase in supply of DDG/S. Ethanol for fuel had its roots in the potable distilling industry. Old product was variable. New generation DDG/S from the dry milling industry is also variable in comparison to many common feedstuffs yet is far superior in many ways to product produced prior to the 1990’s. The basics of ethanol for fuel are conversion of starch to sugar with enzymes, fermentation of the sugar to ethanol, distillation of ethanol, and drying of the co-products. Ethanol plants operating in the U.S. today number 137, with over 60 under construction. Corn use for ethanol from the 2007 crop will approach 2.5 billion bushels. Nutrient variability of DDG/S remains a factor. Vendors should be closely scrutinized for variability. Maillard component formation during drying is a major contributor to differences in amino acid digestibility. Color (Hunter L*) is highly correlated with amino acid, especially lysine, digestibility and it is fast and economical to quantify. DDG/S is high in available amino acids and phosphorus for poultry and has high levels of choline, linoleic acid, and xanthophyll. Generally, feeding up to 15% of the diet as DDG/S has minimal effects on broiler or layer performance. Enzyme feeding with DDG/S is an area that may deserve more investigation. Formulation on a digestible nutrient basis is critical for deriving maximum financial benefit from DDG/S.

Introduction

The North American thirst for energy has driven a massive increase in the grain to fuel business (RFA, 2006). This paper will examine some of the recent and continuing changes in ethanol production; briefly discuss ethanol production processes and will address the feeding value of co-products for poultry from the fastest growing segment of that industry – dry grind ethanol production.

Ethanol Industry and Co-product Overview

All industrial ethanol production in the U.S. has the same basic underlying principal: using yeast to convert sugars to ethanol. Ethanol production had its roots as the potable distilling industry. The new corn to fuel ethanol business is focused on producing ethanol efficiently in large quantities. Each potable distillers plant is producing its own unique product. Potable brewers achieve product differentiation, in part, by altering the “mash bill” prior to fermentation. These and changes in fermentation technology yield a co-product that may be widely variant in color and composition. This is easily understood given the goal of the potable industry distiller. It should be pointed out that the potable spirits industry remains a sizable industry. However, the co-product generation of the potable industry is becoming increasingly insignificant in view of the growth of the industrial ethanol industry and further discussion will focus on production from the ethanol for fuel industry.
Ethanol for fuel, as an industry, is not a new one. Ethanol fueled some of the earliest produced automobiles and helped meet the demand for fuel during the world wars. Co-products from older plants is most aptly characterized as widely variable and often dark and poorly suited as a non-ruminant feedstuffs. This has been elegantly demonstrated by several, in particular Cromwell et al., 1993 and Fastinger and Mahan, 2006. It should also be noted that until the information about “new generation” distiller’s co-products from Dr. Jerry Shurson’s laboratory at the University of Minnesota broke upon the industry the reputation of the “old” ethanol industry and its co-products (NRC, 1994, 1998) stood as an effective barrier to entry of ethanol co-products into the non-ruminant feed ingredient market.

Fuel Ethanol Processes

Discussion at this point will turn to a description of the corn for energy process. Ethanol plants can be widely grouped into two processes: wet milling and dry grind. Wet milling plants in the U.S.A. are almost exclusively millers of corn. Their products are varied including, among others, corn starch, high fructose corn syrup, dextrose, glucose, and of course – ethanol. In this process, corn is first steeped in dilute sulfurous acid. The resultant steep liquor is separated from the grain and is an available co-product. The steeped corn is milled and separated into starch, germ, gluten and bran. The starch is then cooked (to gelatinize), is converted into sugars by enzymes, and converted into ethanol by yeast during fermentation. The germ may be dehydrated and sold as a co-product. It is often de-oiled to produce corn oil with the resultant co-product being germ meal. The proteins and other material from the endosperm are marketed as gluten meal. Most commonly the bran, germ meal and steep liquor are combined and sold wet or dehydrated as gluten feed. The wet milling process has seen little growth - the last commissioning of a new plant occurred in 1995. However, feeding of wet milling plant co-products has been stimulated by the massive increase in the dry grind plants and their resultant co-products.

Unlike the wet milling process, in the dry-grind ethanol process the entire cereal kernel is milled and fermented. Also dissimilar to wet milling, the dry grind process is not exclusive to corn but is readily and commonly adapted to cereal grains other than corn. To begin the process, grain is ground and then mixed with water and cooked to gelatinize the starch. The starch is then converted to glucose by enzymes and then into ethanol by yeast. After distillation, the distiller’s grains are separated by centrifuge and may be sold wet or dry. The resultant thin stillage (after centrifugation) is condensed by evaporation to produce condensed distiller’s solubles, commonly know as syrup. In corn form this product is properly termed Corn Condensed Distillers Solubles or CCDS. This syrup is combined with distiller’s grains to produce distiller’s grains with solubles. This may be sold wet or dry or in various combinations of grains and syrup of varying moisture commonly known as modified distiller’s grains. The AAFCO definition for distiller’s grains requires that the grain of majority inclusion be listed as the source (AAFCO, 2007). Thus corn distiller’s grains could be from as much as 49% of from some other grain, such as sorghum or wheat.

Dry Grind Growth

Almost all of the recent growth in the ethanol industry has come in dry grind plants. In 1996 the ethanol industry produced about one million tons of dried distiller’s grains. In 2007 the industry produced approached 15 million tons. That figure is expected to double again within the next 8 to 10 years. The reasons for this growth are readily apparent in the media and have been summarized elsewhere (Gibson and Karges, 2005). To further emphasize the growth of the industry, ethanol was estimated to consume over 15% of the 2006 U.S. corn crop. As of the writing of this report 137 ethanol plants are in production (the number changes weekly) and about half that many are under construction or expanding. With ethanol production nearing 8 billion gallons, usage of corn for the 2007 corn crop will approach 2.5 billion bushels. The USDA has projected that more than 12 billion bushels will be needed from the 2007 corn crop to meet the resultant total demand. All these factors emphasize this fact; ethanol feed co-products will find their way into animal feeds as never before - at unprecedented levels of consumption. A
corollary to this is that the informed user of distiller’s products has a tremendous opportunity for profitability from judicious use, particularly over the next few years.

**Accounting for Variability**

Despite awareness (and progress) of the emerging industry in quality control substantial variation in color and composition of DDG/S yet remains. Contributing factors are many including: process differences between systems, plant-to-plant variation, intra-plant variation and annual and regional variations in grains. In our industry we are accustomed to products that are physically and/or chemically processed in a continuous flow system such as ground corn or soybean meal. These processes can produce very uniform product. Fermentation is neither continuous nor physical/chemical; it is a batch system that depends upon a live entity (yeast). Therefore fermentation and the resultant co-products, despite diligent QA/QC, are inherently prone to variability. Spiehs, et al. (2002) and Robinson (2004) have demonstrated some of these differences. For proper formulation it is important for a nutritionist to know not only mean nutrient values but also variation about that mean, if possible. Reduction of that variation by careful selection of vendors is strongly encouraged. However, even well managed systems, because of the biological component of ethanol production, may experience substantial intra-plant variation in DDGS quality at times.

It is easy to blame the “old industry” for dark distiller’s grains. The truth is that even today’s new generation plants can produce dark DDG/S. What makes DDG/S dark and why is it important? The darkening (or caramelization) of DDG/S is due to formation of Maillard reaction components. This occurs when sugars and carbohydrates react with proteins (primarily lysine). This process is accelerated by heat. This reaction, which darkens DDG/S, is also the same type of reaction that results in nicely browned bread, a wonderful brown caramel, and even for the intense caramelization that takes place under the right conditions for hay that is put up too wet. Unfortunately, this process reduces the digestibility of lysine. The first persons to demonstrate the relationship between color and quality were Kevin Herkelman and Gary Cromwell at the University of Kentucky (Cromwell et al., 1993). Batal and Dale (2006) and Stein et al. (2005) have demonstrated that color is highly correlated with lysine digestibility for broilers and growing pigs. Extreme browning of DDG/S can reduce energy digestibility as well as amino acid digestibility. This may be one reason why the existing NRC values for metabolizable energy for swine are so low in comparison to recent evaluations. Contrary to energy and lysine, P digestibility for poultry is increased by heating (Kalbfleisch and Robinson, 2004; Martinez Amescua et al., 2004). The complexities in producing quality DDG/S have been elegantly demonstrated in research published by Noll et al. (2006). In this work the nutritional value of DDG with varying additions of CCDS was examined. As more syrup was added and the product was dried available energy content increased but amino acid content was relatively stable and amino acid availability declined.

How do we best characterize variable product? As has been discussed earlier color “lightness” as measured on the Hunter L scale is well correlated with amino acid digestibility. It should be pointed out that use of this measure across plants, especially those that differ in process, introduces substantial variation and is not recommended. As of this date, accurate prediction equations for lysine digestibility are not existent. Other methods to predict nutrient availability are under investigation. The IDEA assay by Novus shows promise in this regard, especially in poultry (Schasteen et al., 2005).

**Co-product Composition**

Fermentation of corn results in an approximate yield of one third mass in ethanol, DDG/S and CO2. Therefore an approximate tripling of nutrient value of corn is achieved. DDG/S composition from a single DDG/S marketer is given in Table 1. Mycotoxins present in the corn are not destroyed by fermentation. Therefore, monitoring of mycotoxins in DDG/S is a necessary process that should be
employed by all users. Whereas knowledge of amino acid level and availability are becoming more commonplace, few are aware that DDG/S is high in choline and also low in pH. DDG/S is also a good source of xanthophyll and linoleic acid. Nutrient availabilities for poultry are given in Table 2. Whereas poultry NRC (1994) values for energy are roughly equivalent to new dry-grind plant DDG/S, lysine availability can be substantially higher than that listed in NRC (Batal and Dale, 2006).

<table>
<thead>
<tr>
<th>Table 1. Nutrient Composition of DDG/Sa</th>
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<tbody>
<tr>
<td>Nutrient % of DM¹</td>
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<td><strong>Proximate Analysis</strong></td>
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<td>Crude Fiber</td>
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<tr>
<td>Ash</td>
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<tr>
<td><strong>Amino Acids</strong></td>
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<td>Arginine</td>
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<tr>
<td>Aspartic Acid</td>
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<td>Cystine</td>
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<td>Xanthophyll</td>
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</table>

a Provided by Dakota Gold Research Association, Sioux Falls, SD  
b All values expressed as a percentage of dry matter unless otherwise noted.
Table 2. Nutrient Availability of Dakota Gold BPX™ DDG/S for Poultry

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Availability</th>
</tr>
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<tbody>
<tr>
<td>Amino Acids&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Aspartic Acid</td>
<td>79 %</td>
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<td>Threonine</td>
<td>76 %</td>
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<td>Serine</td>
<td>83 %</td>
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<tr>
<td>Glutamic Acid</td>
<td>88 %</td>
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<td>Proline</td>
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<td>Alanine</td>
<td>89 %</td>
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<tr>
<td>Cystine</td>
<td>81 %</td>
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<td>83 %</td>
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<td>Methionine</td>
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<td>Arginine</td>
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<td>Tryptophan</td>
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<td>3108 kcal/kg</td>
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<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>75%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Amino acid digestibility for cecectomized cockerels compiled from Batal and Dale (2006) and internal data.
<sup>b</sup> Metabolizable energy for cecectomized cockerels based on Batal and Dale (2006) and internal data.
<sup>c</sup> Phosphorus availability in comparison to calcium phosphate compiled from Martinez Amescua et al. (2004) and Kalbfleisch and Roberson (2005).

Feeding DDG/S to Poultry

The evidence continues to mount that DDG/S can be incorporated into poultry feeding programs without deleterious effects, particularly at modest inclusion rates. In actuality, thanks to the support of the Distillers Grains Council, the feeding of DDG/S to poultry has a more positive history than that of swine (Noll, 2004). More recent data summarizing performance of growing birds fed varying levels of DDG/S are summarized in Figures 1 and 2. It is evident that DDG/S has only modest effects on poultry performance and that ADG and feed conversion are largely unaffected by inclusion rates up to 15% of the complete diet. Data for broilers also indicate inclusion rates higher than 15% of the complete diet may reduce the rate and efficiency of weight gain but performance is largely unaffected prior to that level (Roberson et al., 2003; Noll et al., 2001; Lumpkins et al., 2004; and Mateo, 2006. In contrast to swine, there is very little evidence of negative effects of DDG/S on feed intake in growing birds.

Roberson et al. (2005) and Lumpkins et al. (2005) have suggested that DDG/S inclusions of up to 15% will result in similar layer performance. Recent results at the University of Nebraska and Iowa State suggest that inclusions higher may be acceptable (S. Scheidler, K. Bregendahl personal communication). Roberson et al. (2005) also demonstrated that yolk color could be improved in as little as 4 weeks by feeding 10% DDG/S. Recent research published by researchers at Iowa State University (Roberts et al., 2007) suggested that the inclusion of DDG/S in layer diets may reduce the emissions of ammonia from layer manure. Currently additional studies are underway to further investigate this response.
An interesting area of investigation is the use of enzymes with DDG/S feeding. Hruby et al. (2007) reported that the use of phytase during the ethanol production process resulted in improved energy and amino acid digestibility of the resultant DDG/S. Work at Auburn University (Edwin T. Moran, Jr. personal communication) has shown dramatic responses to commercially available enzymes in feeds containing DDG/S.

**Formulation Fundamentals**

Proper formulation constraints when utilizing DDG/S contribute to improved profitability. Nutrients which are commonly of value are amino acids, fat (or ME) and P. Likewise, choline can contribute value in swine and poultry diets. However, if DDG/S is to contribute to the profitability of the diet the preexisting sources of these nutrients must be allowed to decrease or at least change in comparison to diets without DDG/S. Specifically, available P sources, including phytase, must be allowed to change in diets to fully capitalize on value. Likewise a full understanding of digestible amino acid ratios and lack of constraint on inclusion of crystalline amino acids is also essential. In other words, conservative, rigid constraints on ingredients are likely to result in a loss of potential profitability from feeding DDG/S.

**Conclusion**

In summary, the rapid growth of dry grind ethanol plants makes a massive amount of product available to the market. Although the “new” methods of producing DDG/S result in generally superior in nutrition to the old, a large amount of variation in product still exists. Knowledge of your supplier and variability within their system is warranted. Color is a convenient, fast, economical method of characterizing DDG/S quality, lighter being better. Other methods are under investigation. DDG/S can easily be incorporated into broiler and layer diets with little impact on performance at inclusion rates of 15% or lower. Utilizing all positive characteristics of DDG/S and formulating with available nutrients is important to maximizing profitability.

**References**


ENERGY EVALUATION IN POULTRY: TOWARDS A NET ENERGY SYSTEM?

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Summary

Feed costs are the major costs for poultry production. As approx. three quarters of these feed costs are related to dietary energy, it is economically very important to accurately estimate the content of utilisable energy in feeds to 1) justify feedstuff prices; 2) be able to predict the animal’s production performance; and 3) prepare least costs formulation with adequate utilisable energy contents.

Several methods have been introduced to evaluate the utilisable energy content in feeds, like the apparent (AME) and true metabolisable energy (TME) content and net energy (NE) systems. Rapid methods to determine the AME or TME value of feeds depend on the use of adult cockerels. As these adult animals have limited value for fast growing broilers, also tabulated data are available which are based on broiler assays as target species. More recently also experiments are done using laying hens to obtain a AME-layer energy table. Preferably, the AME and TME values are corrected for a zero nitrogen balance (AME\textsubscript{N} and TME\textsubscript{N}) to correct for differences in production performances between broilers, laying hens and cockerels and in case of TME also for endogenous losses. In the Dutch AME system an additional correction factor has been introduced to take the difference in energy utilization between fat and carbohydrate into account.

In pigs net energy systems are introduced that improve the accuracy of the estimations of the utilisable energy content. These net energy systems take a lower energy value for protein and a higher value for fat relative to carbohydrates into account. It can be debated whether poultry feeding would also benefit from a net energy system. The added value of net energy systems for poultry have been topic of research in many countries. Progress on net energy systems is presented as well as the consequence of the different feed evaluation systems for practical feed formulation.

Introduction

Different energy systems for poultry have been presented and discussed by Farrell (1999), being the apparent and true metabolisable energy system and net energy systems. Energy utilization is summarized in Figure 1. Different systems will be briefly presented.

1. The apparent metabolizable energy system

The apparent metabolizable energy (AME) content of diets is based on the difference between ingested gross energy and energy excretion in droppings (faeces and urine). This method is widely adopted to determine the utilisable energy content in poultry and enables the use of modern fast growing broiler chickens in feedstuff evaluations (Bourdillon \textit{et al.}, 1990). Since Farrell introduced the use of trained adult cockerels as a more rapid method to determine the AME value of test diets (Farrell, 1978), it
is also used as a high throughput system. However, it should be realized that adult cockerels are fasted for 36 h prior to the supply of the test diets, and these birds are fed close to maintenance. These test circumstances might affect the digestibility of nutrients in the gastro-intestinal tract and consequently the metabolisable energy content.

According to the Dutch CVB (2005) the AMEn content can be calculated from the digestible nutrient concentration according to the following equations for adult poultry (Equation 1.1) and broilers (Equation 1.2).

(1.1) \( \text{AME}_N (\text{kJ/kg}) = 18.03 \text{ dCP} + 38.83 \text{ dC.FAT} + 17.32 \text{ dNfE} \)

(1.2) \( \text{AME}_N (\text{kJ/kg}) = 15.56 \text{ dCP} + 38.83 \text{ dC.FAT} + 17.32 \text{ dNfE} \)

For young broilers a lower energy value for digested crude protein is used based on a regression equations between the measured AMEn content and the faecal digested nutrients. The physiology behind this lower energy value for digested crude protein is not clear. Other AME formulas take differences of the energy value of the different carbohydrate fractions into account, like the Rostock equation (Equation 4.6).

2. The true metabolisable energy system

Sibbald (1985) introduced the true metabolisable energy (TME) system, using tube-fed cockerels with a small quantity of an experimental diet or a single test feedstuff. Digestibility values are corrected for endogenous losses measured with cockerels that were fasted or fed a highly digestible starch/sugar/oil mixture. It should be realized that when using just a test feedstuff instead of a complete experimental diet interactions between feedstuffs do not occur which might overestimate the energy value of a feedstuff as part of a complete diet. E.g. it was shown by Van der Klis et al. (1995) that when wheat was added to a basal diet with 7% added fat, the estimated AMEn value for wheat was 3 MJ/kg lower than estimated based on a low fat basal diet. Also feed intake level would potentially affect the energy value of a diet.

![Energy utilization in poultry](image)

**Figure 1. Energy utilization in poultry.**

![Delta AMEn value](image)

**Figure 2. The difference between the AMEn value as determined in adult cockerels and in growing broilers for energy- (●) and protein-rich (○) feedstuffs, (CVB, 2005)**
3. Points of discussion on the AME and TME system

In general both the AME and TME values are corrected for zero nitrogen balance based on the assumption that all N retained will be excreted as uric acid. The factor used in literature for this correction varies from 34.4 kJ/g to 36.5 kJ/g N retained. This implies that in productive animals the corrected AMEn value will be lower than the AME value as measured, and underestimates the actual utilizable energy content. Although the AMEn is corrected for differences in N retention, Lopez and Leeson (2007) demonstrated a clear age effect on the AMEn: The dietary AMEn was increased by 200 kcal/kg between 7 and 35 days of age. Moreover, this correction reduced the bird to bird variation resulting in a more reliable AME value.

Dutch tabulated values (CVB, 2005) clearly indicate large differences between the AMEn values obtained with adult cockerels and those with broilers as indicated in Figure 2. In general, as indicated in the CVB table, nutrient digestibilities were higher in adult cockerels compared to broilers, resulting in lower AMEn values for the latter. This difference might be due to age of the birds, to fasting or to the lower feed intake level in cockerels, resulting in increasing relative importance of the endogenous secretions. The lower AMEn values in broilers compared adult cockerels are consistent with results from Bourdillon et al. (1990). Effects of endogenous secretions are partly overcome by correction for endogenous losses as was clearly shown by Askbrant and Khalili (1990), indicating that the TME value was more or less independent of feed intake, whereas AMEn was reduced at lower feed intakes.

It should be noticed in Figure 2 that the discrepancies in energy value between boilers and adult cockerels is not due to the lower energy value for digested crude protein in broilers (see Equation 1.1 and 1.2), as can be seen in Figure 2 as differences between energy- and protein-rich feedstuffs are small.

4. Net energy systems

Metabolisable energy systems estimate the potentially utilizable energy in diets, but do not predict the efficiency of energy utilization for maintenance and production. Due to ingestion, digestion, absorption, metabolism and excretion of nutrients heat production is increased (heat increment of feeding). After correction of the metabolisable energy for this heat increment, net energy remains (Figure 1), which can be used for maintenance and production. The heat increment depends on the nutrient composition of a feedstuff. McLeod (2002) indicated that in general terms the net energy values for fat and protein per unit of ME are respectively 20% higher and 20% lower compared to carbohydrates. As a net energy system takes into account these differences in the metabolic utilization efficiency of protein, fat and carbohydrates, an energy evaluation system based on net energy is to be preferred (De Groote, 1999).

De Groote (1999) summarized two equations to estimate the net energy value for poultry as obtained from Hoffmann and Schiemann (1971) and De Groote (1975):

\[
\begin{align*}
\text{ME} &= 17.8 \text{dCP} + 39.8 \text{dCFAT} + 17.7 \text{dNFE + dCF} \\
\text{NEf} &= 10.8 \text{dCP} + 33.5 \text{dCFAT} + 13.4 \text{dNFE + dCF} \\
\text{NE} &= 10.9 \text{dCP} + 35.1 \text{dCFAT} + 13.8 \text{dNFE + dCF}
\end{align*}
\]

Hoffmann and Schiemann (1971)

De Groote (1975)

The equation by Hoffmann and Schiemann (1971) was based on fat deposition in adult cockerels using a NE/ME quotient of 0.60 for protein, 0.84 for fat and 0.76 for carbohydrates, whereas in growing broilers De Groote (1974) used the following quotients: 0.61 for protein, 0.88 for fat and 0.78 for carbohydrates.
Beyer et al. (2003) presented the Rostock Feed Evaluation System and indicated the efficiency of energy utilization for poultry as follows:

\[
\begin{align*}
\text{GE} &= 23.6 \text{CP} + 39.8 \text{CFAT} + 17.3 \text{STARCH} + 16.0 \text{SUGAR} + 18.9 \text{NFR} \\
\text{DE} &= 23.6 \text{dCP} + 39.8 \text{dCFAT} + 17.3 \text{dSTARCH} + 16.0 \text{dSUGAR} + 17.2 \text{dNFR} \\
\text{ME} &= 18.8 \text{dCP} + 39.8 \text{dCFAT} + 17.3 \text{dSTARCH} + 16.0 \text{dSUGAR} + 17.2 \text{dNFR} \\
\text{NER} &= 10.8 \text{dCP} + 29.0 \text{dCFAT} + 13.5 \text{dSTARCH} + 12.4 \text{dSUGAR} + 10.5 \text{dNFR}
\end{align*}
\]

GE: Gross energy; DE: Digestible energy; ME: Metabolizable energy; NER= Net Energy Retention; CP: crude protein; CFAT: Crude fat; NFR: N free residue (organic matter - CP - CFAT - STARCH - SUGAR); d: digestible

The Rostock approach, which is based on the ATP generating capacity, was also followed by the CVB, developing an ATP-based net energy system. Energy coefficients for digested nutrients relative to starch were more or less similar to the NER formula. Currently a validation study is in process to test whether the NE system has added value over the Dutch AME system.

Based on a literature dataset with growing chickens Porgozliev and Rosen (1999) showed that the dietary AME value overestimated the NEp (net energy for production) content of protein-rich feedstuffs of animal origin, but not for high protein vegetable feedstuffs. Moreover, Noblet et al. (2007) failed to show an increased heat production in broilers fed high protein diets. In their experiment, the NE/ME ratio was approx. 68% for broiler diets irrespective of the dietary crude protein level, which varied from 22.5% to 27.3%. It can therefore be questioned whether the efficiency of protein utilization is indeed 20% overestimated compared to carbohydrates in fast growing broilers as suggested by MacLeod (2002).

On the other hand the extra caloric value of fat has been clearly demonstrated in laying hens. Scheele (1985, as cited by Van der Klis and Fledderus (2007)) measured the energy retention in laying hens fed isocaloric diets with an increasing fat content at the expense of carbohydrates. Results are indicated in Figure 3. Based on their experiment it was concluded that the energy value of digested fat should be increased by approx. 15% in comparison to digested carbohydrates. Detailed calculations are given by Van der Klis and Fledderus (2007). Huyghebaert (1989, as cited by De Groote, 1999) confirmed this extra caloric value of fat for broilers, using a similar technique in growing broilers from 3 to 6 weeks of age.

If net energy systems indeed predict the energy available for maintenance and production more accurately than metabolisable energy systems, it should have a closer relationship to production performance than the metabolisable energy systems. De Lange and Birkett (2005) indicated that in broiler chickens NE systems only marginally improved the accuracy of predicting broiler growth performance and energy retention as compared to an ME system. Adapted AMEn equations additionally taking into account the extra caloric value of fat might already be an adequate energy evaluation system in broilers. Effects of the different energy systems on feed composition and price will be presented.

Figure 3. The energy retention (in body and egg) in laying hens fed diets differing in AMEn content (13.0 MJ/kg (●) and 13.5 MJ/kg (○)), each containing three levels of dietary fat which were isocalorically exchanged for carbohydrates.
References


Dairy Session
FEEDING FOR AND COST OF PRODUCING MILK COMPONENTS: MILK PROTEIN

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Summary

Milk pricing systems have a direct impact on nutrition and management decisions made by dairy producers. Protein has surfaced as the milk component of greatest value. Research and field experience indicates that both carbohydrates and protein are important to maximizing protein yields. There are three principal strategies in feeding for high milk protein yields: 1) feed to maximize synthesis of microbial protein; i.e., balance for carbohydrates, evaluate starch and fiber digestibility, provide adequate effective fiber, and provide adequate rumen degradable protein (RDP), 2) balance diets for rumen undegraded protein (RUP), and 3) select high quality, high lysine (Lys) protein supplements and a methionine (Met) supplement to achieve the desired levels of Lys and Met in metabolizable protein (MP) of 6.6-6.8% and 2.2%. The benefits of balancing rations for RDP and RUP are usually reduced dietary CP concentrations, usually lower feed costs, increased milk and milk protein production, and increased herd profitability. The benefits of balancing for Lys and Met in MP are increased milk and milk components, more efficient use of MP for milk protein synthesis, more predictable changes in milk and milk protein production to changes in RUP supply, reduced metabolic disorders and increased herd profitability.

Introduction

Multiple component pricing (MCP) programs continue to impact the way producers are compensated for their milk. Protein has surfaced as the most valued milk component. Other than in 2001 when butterfat and protein prices were similar, protein prices have been higher than butterfat prices each of the last 8 years (Table 1).

The average annual component value of protein has steadily increased over that time, from $1.69 per lb in 2000 to $3.31 per lb in 2007 (Table 1). During this time, annual butterfat prices varied between $1.19 and $2.05 per lb (Table 1) but have always been lower than protein. Unlike protein, there has been no consistent pattern in the value of butterfat.

Table 1. Annual Federal Milk Marketing Order (FMMO) component prices beginning January, 2000 when the current 10 FMMO were established.

<table>
<thead>
<tr>
<th>Year</th>
<th>Butterfat</th>
<th>Protein</th>
<th>Other solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>$1.2522</td>
<td>$1.6938</td>
<td>$0.0509</td>
</tr>
<tr>
<td>2001</td>
<td>$1.8480</td>
<td>$1.9613</td>
<td>$0.1343</td>
</tr>
<tr>
<td>2002</td>
<td>$1.1928</td>
<td>$1.9735</td>
<td>$0.0593</td>
</tr>
<tr>
<td>2003</td>
<td>$1.2099</td>
<td>$2.3770</td>
<td>$0.0129</td>
</tr>
<tr>
<td>2004</td>
<td>$2.0510</td>
<td>$2.6035</td>
<td>$0.0751</td>
</tr>
<tr>
<td>2005</td>
<td>$1.7105</td>
<td>$2.4602</td>
<td>$0.1228</td>
</tr>
<tr>
<td>2006</td>
<td>$1.3252</td>
<td>$2.0912</td>
<td>$0.1745</td>
</tr>
<tr>
<td>2007*</td>
<td>$1.4789</td>
<td>$3.3131</td>
<td>$0.4532</td>
</tr>
</tbody>
</table>

Protein prices began to skyrocket in April, 2007 and have remained over $4.00 per lb since then. In May, 2007, protein accounted for about 50% of the milk check based on milk containing 3.7% fat, 3.1% protein, and 5.8% other solids. In December, 2007 it accounted for about 65% of the milk check. Whether such a large disparity in the market value of protein and fat will continue to exist is unknown, but it will likely be in the best interest of dairy producers to have the feedstuffs and nutritional expertise available to maximize milk protein synthesis. About 90% of the U.S. milk supply is marketed to buyers who offer MCP. In 2006, the 6 Federal Milk Marketing Orders (FMMO) paying for components accounted for 83% of the FMMO receipts (Table 2).

In addition to the FMMO supported MCP system, there are also industry sponsored MCP programs. These programs allow for producers outside of the 10 FMMO areas to receive payment for components other than butterfat. And finally, CA, which maintains its own milk marketing program and is not part of the FMMO system, has required MCP since 1962.

### Table 2. 2006 milk production statistics (millions of pounds) for the 10 Federal Milk Marketing Orders (FMMO).

<table>
<thead>
<tr>
<th>FMMO</th>
<th>Order #</th>
<th>Paid for protein and fat</th>
<th>Paid for milk weight and fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northeast</td>
<td>1</td>
<td>22,679</td>
<td></td>
</tr>
<tr>
<td>Appalachian</td>
<td>5</td>
<td>6,243</td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td>6</td>
<td>3,126</td>
<td></td>
</tr>
<tr>
<td>Southeast</td>
<td>7</td>
<td>8,055</td>
<td></td>
</tr>
<tr>
<td>Upper Midwest</td>
<td>30</td>
<td>26,855</td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>32</td>
<td>13,917</td>
<td></td>
</tr>
<tr>
<td>Mideast</td>
<td>33</td>
<td>17,189</td>
<td></td>
</tr>
<tr>
<td>Pacific Northwest</td>
<td>124</td>
<td>7,570</td>
<td></td>
</tr>
<tr>
<td>Southwest</td>
<td>126</td>
<td>11,600</td>
<td></td>
</tr>
<tr>
<td>Arizona-Las Vegas</td>
<td>131</td>
<td>3,383</td>
<td>99,810</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20,807</td>
<td>83%</td>
</tr>
</tbody>
</table>

Feeding for Milk Protein

Feeding for high protein yields requires feeding for both high milk yields and milk protein concentrations because farmers are paid for lbs, not percentages. A cow producing 72.5 lbs with 3.2% protein (cow A) returns the same income from protein as a cow producing 80 lbs of milk with 2.9% protein (cow B). Each cow produces 2.32 lbs of protein. However, changing the diet of cow B so that she produces 80 lbs of milk with 3.2% protein (instead of 2.9%) increases protein yield to 2.56 lbs, or an additional 0.24 lbs of protein. If the protein is valued at $2.50 a lb, that’s an additional 60 cents of income. If the milk protein is valued at $3.30, the average for 2007, then the additional income would be 79 cents. If the protein is valued at $4.00 a lb, then the additional income would be 96 cents. Given that feeding for higher milk protein concentrations often increases milk yield and milk fat concentrations as well, it becomes clear why there is a growing interest in balancing diets to achieve higher milk protein concentrations.

### Feeding for High Milk Yield

Feeding for high milk yield means, first and foremost, feeding for high lactose yields. Lactose is the most constant constituent in bovine milk (about 4.7%); it is the principal milk component that determines milk yield. As the major osmole in milk (accounts for about 50% of the osmotic pressure), and the most hydrophilic (water loving) of the organic milk components, it has a greater effect on milk volume than either protein or fat. Lactose is a small molecule consisting of glucose and galactose and is synthesized in the mammary gland. Most of the galactose is synthesized from glucose. Therefore, the primary factor affecting lactose synthesis is glucose supply to the mammary gland. Most of the circulating plasma glucose in ruminants is synthesized in the liver from propionate, amino acids, and glycerol by
gluconeogenesis. About 45-60% of the glucose is synthesized from propionate, and smaller amounts of glucose are absorbed from the small intestine, largely from rumen unfermented starch.

Because most of the glucose is synthesized from propionate, high milk yields require healthy cows that can consume large quantities of fermentable carbohydrates. Achieving high intakes of fermentable carbohydrates, while maintaining healthy cows, requires excellent herd management, excellent feeding practices, and high quality, well-balanced diets. Some of the most important aspects of feeding that are needed to achieve high milk yields follow:

1) Provide ready access to ample supplies of fresh water, always provide fresh feed, and practice good feed bunk management. Cows eat more feed when bunk surfaces are smooth, feed is pushed up frequently, sorting is minimized, and bunks are cleaned daily.

2) Monitor DM contents of forages so that a constant forage-to-grain ratio in diet DM is maintained, and analyze feeds as frequently as needed to ensure that the composition of the diet that is being fed is similar to the diet that has been formulated.

3) Feed high quality forages. This is the most common factor among high producing herds. High quality grass and legume hays and silages are characterized by low NDF, low lignin, and moderate to high CP content. High quality corn silage is characterized by high NFC and low fiber content. Forage quality affects palatability and ruminal digestibility, the two most important factors affecting feed intake and milk production.

4) Balance diets for carbohydrates. This is often easier said than done because we don’t routinely determine the concentrations of the individual carbohydrates in our feeds, and for many of the carbohydrate fractions we may or may not know very well their rate or extent of ruminal digestion. One example of carbohydrate intake recommendations for lactating dairy cows is presented in Table 3.

Table 3. Tentative carbohydrate recommendations for lactating dairy cows.1

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total NDF2, % of DM</td>
<td>28-32</td>
</tr>
<tr>
<td>Physically effective NDF (peNDF), % of NDF</td>
<td>22-24</td>
</tr>
<tr>
<td>Forage NDF, % of DM</td>
<td>21-24</td>
</tr>
<tr>
<td>Fermentable NDF, % DM</td>
<td>&gt;10%</td>
</tr>
<tr>
<td>Nonfiber carbohydrates (NFC)3, % of DM</td>
<td>30-43</td>
</tr>
<tr>
<td>Soluble fiber4, % of DM</td>
<td>4-10</td>
</tr>
<tr>
<td>Starch, % of DM</td>
<td>23-30</td>
</tr>
<tr>
<td>Fermentable starch, % DM</td>
<td>18-24</td>
</tr>
<tr>
<td>Sugars5, % of DM</td>
<td>4-8</td>
</tr>
<tr>
<td>Sugar:soluble protein ratio</td>
<td>1.5:1</td>
</tr>
<tr>
<td>Fermentable total carbohydrates, % of DM</td>
<td>40-44</td>
</tr>
<tr>
<td>Total volatile fatty acids (VFA), % of DM</td>
<td>0-5</td>
</tr>
</tbody>
</table>

1 C.J. Sniffen (personal communication).
2 Includes cellulose, hemicelluloses and lignin.
3 Includes organic acids, sugars, starch, and soluble fiber carbohydrates.
4 Includes fructans, pectic substances, β-glucans, and galactans.
5 Includes glucose, fructose, sucrose, other oligosaccharides that are from 2 to ~20 carbons in length, and lactose in milk products.
performance of lactating cows. Nonetheless, there is growing evidence that the profile of non-
fiber and soluble fiber carbohydrates in the diet can affect animal performance, and that by
achieving a balance of these, in concert with adequate physically effective fiber (pNDF), rumen
fermentation is optimized, and feed intake and synthesis of microbial protein is maximized.
Recommendations as provided in Table 3 will continue to be refined as more information
becomes available. Some of the differences that exist among carbohydrates are summarized.

- Sugars tend to ferment very rapidly, and therefore, provide energy for microbial growth
shortly after feeding and complement the rapidly degraded non-protein nitrogen (NPN)
fraction of the diet. Rates of hydrolysis of ~1400%/hour and 540%/hour have been
reported for the conversion of sucrose to fructose and glucose, and of lactose to glucose
and galactose by rumen microbes, respectively (Weisbjerg et al., 1998). Organic acid
products of sucrose and lactose fermentations can include lactic acid (Thivend and
Ehouinsou, 1977; Strobel and Russell, 1986). These sugars have been reported to yield
more butyrate than other non-fiber carbohydrates and similar to slightly lesser amounts of
propionate (Strobel and Russell, 1986; DeFrain et al., 2006). Research has indicated that
microbial protein yield is greater when starch is fed as compared to when sucrose is fed
(Hall and Herejk, 2001, Sannes et al, 2002) and that feeding lactose results in greater
yields of microbial protein than feeding sucrose (Hussain and Miller, 1999). Sugar
supplements can depress fiber digestion but not always (Hall, 2005). Common sources of
sugars include molasses, citrus pulp, sugar beet pulp, almond hulls, bakery waste,
soybean meal, and fresh forages or hays (Hall, 2002). Substitution of starch with sugars
has led to mixed results. Substituting some corn with sucrose has increased milk fat yield
and decreased milk protein percentage (Nombekela and Murphy, 1995), increased milk
fat content and yield and decreased feed efficiency (milk/DM intake and milk N/intake
N) linearly (Broderick et al., 2000), increased lactose and milk urea N (MUN)
concentrations (Cherney et al., 2003), and decreased yield of milk and milk components
with no change in percentages of components (Sannes et al., 2002). Others have found no
changes in milk composition or yield with sucrose supplementation (McCormick et al.,
2001).

- Starch is composed of chains of glucose linked together by alpha-bonds and is stored in
crystalline granules in plants. Starch can be digested both by microbes and the cow.
Starches typically have slower rates of ruminal digestion and are less digestible than
sugars. Starch fermentation may also yield lactic acid; however, unlike sugars, there is
greater variation in the rate of fermentation (4 to 40%/h) (Hall, 2002). Factors affecting
starch digestion rates include type of grain (oats > wheat > barley > corn > sorghum;
Herrera-Saldana et al., 1990), grain processing (steam flaked and high moisture > dry and
fine grind > dry and coarse grind), and storage method. Other sources of variation in
starch digestibility for corn are endosperm type for dry corn; maturity, moisture content,
length of storage and endosperm type for high moisture corn; and maturity, moisture
content, length of storage, kernel particle size, and endosperm type for corn silage.
Common starch sources include grains, silages and by-products, bakery waste, and
potatoes. Feeding several of these sources of starch allows for rapid and continuous
fermentation after the sugar is digested. Increasing rumen starch availability by plant or
kernel processing, heat treatment, or moisture content can all increase ruminal
fermentation, synthesis of microbial protein, and efficiency of use of the starch. Many
studies have reported these benefits (Hutjens and Dann, 2000; Hutjens, 2007).

- Soluble fiber carbohydrates (fructans, pectic substances, β-glucans, and galactans) appear
to be fermented at least as rapidly as starch (20 to 40%/h, except soyhulls at 4%/h). These
carbohydrates are not part of NDF because they are soluble in neutral detergent solution.
Unlike starch and sugars, these carbohydrates cannot be digested by mammalian
enzymes. With the exception of fructans, soluble fiber fermentation yields little or no lactic acid, and its fermentation is reduced at lower pH. Soluble fiber carbohydrates have received considerable attention because they appear to have a rapid rate of degradation but the end-products of fermentation are similar to that of NDF. Because they don’t make the rumen acidic like the fermentation of sugars and starches, soluble fiber appears to be beneficial in controlling acidosis when high quality forages are fed. Common sources of soluble fiber include legume forages (15-20%), citrus pulp (25-45%), beet pulp (15-30%), soy hulls (15-20%) and soybean meal (15-20%).

- Neutral detergent fiber, as provided by byproduct feeds, generally has a slower rate of digestion than starches or soluble fiber carbohydrates and usually is not as digestible. For example, Varga and Hoover (1983) and Hsu et al. (1987) reported NDF digestibility values of 69, 52, 52, 45, 42, 14, and 9% for beet pulp, wheat middling, soy hulls, hominy, corn gluten feed, peanut hulls, and cottonseed hulls, respectively. The range in digestion rates of NDF in some byproduct feeds overlap with the digestion rates of starches in some feeds and of NDF in some forages. This is why carefully selected byproducts can be used as partial substitutes for both grains and forages.

- Neutral detergent fiber as provided by forages generally has the slowest rate of digestion and is generally the least digestible. Although of low digestibility (usually 30 to 50%), forage NDF is important as a source of effective fiber. A minimum amount of forage NDF is needed in the diet to maintain good rumen function. The Dairy NRC (2001) recommends minimum dietary forage NDF concentrations of 19, 18, 17, 16, and 15% when minimum dietary non-forage NDF concentrations are 6, 9, 12, 15, and 18%, respectively. The resulting minimum total dietary NDF concentrations are 25, 27, 29, 31, and 33%, respectively. These minimum forage and dietary NDF values are not fixed values and depend on factors such as particle size of forages, forage NDF digestibility, and non-forage NDF digestibility.

5) Balance diets for RDP. Rumen degraded feed protein is the second largest requirement for rumen microorganisms. It supplies the microorganisms with peptides, amino acids, and ammonia that are needed for microbial protein synthesis. The amount of RDP required in the diet is determined by the amount of fermentable carbohydrates in the diet. Diet evaluation models differ slightly in their estimates of RDP in feeds. The NRC (2001) model typically predicts RDP requirements of 10 to 11% of diet DM. Monitor feed intake, fecal consistency, milk/feed ratios and MUN to make the final decision. Our target value for MUN is 11-12 mg/dl. Don’t short-change the cows on RDP…you can negate perfect carbohydrate balancing with inadequate supply of RDP. A deficiency of RDP will suppress the ability of the microorganisms to reproduce, but they can continue to ferment carbohydrates. This results in higher feed intake, but milk/feed ratios will be low because of inadequate synthesis of microbial protein.

It remains unclear as to what the optimum balance is for peptides, free amino acids and ammonia in the rumen. What is known, however, is that: 1) all forms of N contribute to synthesis of microbial protein (Wallace, 1997), 2) the mixed rumen microbial population has essentially no absolute dietary requirement for amino acids or peptides as cross-feeding among bacteria can meet individual requirements (Virtanen, 1966), 3) amino acids and peptides are stimulatory to growth rate and yield of rumen microorganisms, particularly those growing on rapidly degraded energy sources (NRC, 2001; Chikunya et al., 1996), 4) the proportion of microbial protein derived from ammonia varies according to the availability of other N sources (NRC, 2001), and 5) ammonia concentrations can become first limiting for microbial protein synthesis in lactating dairy cows, particularly when corn silage is the primary forage in the diet.
Ammonia concentrations can easily become limiting for microbial protein synthesis. This has been observed in numerous experiments dating back 40 years where studies demonstrated the benefits of urea supplementation. In a recent experiment, Boucher et al. (2007) evaluated the effect of incremental urea supplementation (0, 0.3, 0.6, and 0.9%) of a diet containing 32% corn silage, 16% mixed mostly grass silage, 4% high protein alfalfa hay (25% CP), 19% finely ground corn, 6% rolled barley, 5% soybean hulls, 3% each of dried citrus pulp and beet sugar pulp, 7% soybean meal, 1% blood meal, 0.3% feather meal, 2% Megalac, and 3% minerals and vitamins. Feeding increasing amounts of urea quadratically increased ruminal ammonia N concentrations (9.0, 11.9, 12.8, and 17.4 mg/dL), passage of microbial protein to the small intestine (1519, 1700, 1769, and 1400 g/d, respectively), microbial N in duodenal digesta as a percentage of nonammonia N (40.6, 45.6, 49.4 and 39.3%, respectively), and efficiency of microbial protein synthesis (74, 86, 84, and 65 g/kg of DM intake, respectively). The RDP content of the 4 diets was 9.2, 10.0, 10.8, and 11.6% of DM, and RDP balances were -167, 8, 179, and 356 g/d (NRC, 2001). Microbial protein synthesis was maximized at an average ruminal ammonia N concentration of 12.8 mg/dL when urea was added to the diet at 0.6% in diet DM, and RDP was 10.8% of DM.

In another recent experiment, Brito et al. (2006) incrementally decreased RDP concentrations in diet DM (12.9, 11.9, 11.0, and 10.2% of DM) by varying dietary ratios of alfalfa silage (AS) and corn silage (CS) in diets for high producing cows. The dietary concentrations of AS and CS in the 4 diets were: 1) 51% AS and 0% CS, 2) 37% AS and 13% CS, 3) 24% AS and 27% CS, and 4) 10% AS and 40% CS. The other ingredients in the diets were high moisture shelled corn and solvent soybean meal. In this experiment, with early lactation cows consuming 26.2 kg/d of DM, the authors observed that microbial protein synthesis was maximal at 11.9% RDP and 37% AS. This is in contrast to results reported by Boucher et al. (2007) who observed that microbial protein synthesis was maximized at 10.8% RDP and 32% CS. In the study of Boucher et al. (2007), late lactation cows were used and DM intake averaged 20.8 kg/d. Reports from field nutritionists who use the NRC (2001) model corroborate the observation that diet RDP concentrations need to be higher in high producing early lactation cows than in lower producing late lactation cows.

6) Balance diets for RUP. Many factors affect RUP requirements. Cow factors include stage of lactation, milk yield, feed intake, and milk protein content. Feed factors include intestinal digestibility and the amino acid composition of RUP. According to NRC (2001), the RUP requirements for large breed lactating cows can vary from 5 to 9% of diet DM. Do not over-feed RUP. There are two reasons for this. The first is that excess RUP typically replaces fermentable carbohydrates in the diet, which means less end-products of rumen fermentation. Less propionate production may mean decreased lactose synthesis. The second reason is that the surplus RUP can result in a less desirable profile of amino acids in MP due to a generally less desirable amino acid composition than that of ruminally synthesized microbial protein. This will reduce the efficiency of use of MP for milk protein synthesis.

**Feeding for High Milk Protein Concentrations**

Amino acids are the building blocks of protein. If MP is the limiting nutrient, then protein production is limited by the amino acid that is in shortest supply in MP relative to the cow’s requirement. This amino acid is the first limiting amino acid. The second limiting amino acid is the amino acid supplied in the second smallest amount relative to requirements. Methionine is most often the first limiting amino acid for milk protein production in the US, and Lys is most often second limiting. The limiting amino acid theory is perhaps described best by the barrel and stave example. If the staves of a barrel are of different heights, relative to the full length of the barrel, then the volume of liquid that the
barrel can hold will be determined by the length of the shortest stave. The shortest stave is the most limiting, because it determines the capacity of the barrel; Met is usually the shortest stave and Lys is usually the second shortest stave. If the supply of Met (i.e., length of the stave), relative to requirements, is increased such that it is co-limiting with Lys, then Met and Lys will be co-limiting. Increasing the supply of Met such that it is equally limiting with Lys will increase the efficiency of use of absorbed amino acids (i.e., the barrel has a greater capacity). It is well documented, both by researchers and field nutritionists that increasing concentrations of Lys and Met in MP to optimum levels will result in higher milk protein concentrations, and milk yields. Varying concentrations of Met and Lys in MP from observed low levels of 1.7 and 5.2%, respectively, to achievable levels of 2.2 and 6.6% (using NRC, 2001), respectively, has been shown to increase milk protein concentrations by 0.40 percentage units. Following are some feeding strategies for maximizing concentrations of Lys and Met in MP

1) Balance diets for carbohydrates. This has already been discussed for maximizing milk yield. When carbohydrates are balanced and fed in amounts to achieve high feed intakes, the ability for rumen bacteria to reproduce is optimized and synthesis of microbial protein is maximized. This is important, because microbial protein is the preferred source of protein (has an excellent amino acid profile) and because of more microbial protein, the cows’ requirement for RUP is reduced. Rates of NDF and starch digestibility also make a difference. Two examples that highlight the impact that this could have on microbial protein synthesis and milk protein concentrations follow.

- Rates of NDF digestibility - This was demonstrated by Miller et al. (1990) who compared a diet formulated to have a slow rate of ruminal degradation of NDF with one formulated to have a fast rate of NDF degradation. Both diets contained 34% NDF, 17% CP, and 6% soluble CP. Feeding rapidly degraded NDF resulted in a 4% increase in feed intake, a 10% increase in milk yield, a 2% increase in milk protein percentage (3.03 to 3.10%), and a 14% increase in milk protein yield.

- Rates of starch digestibility - Many studies have been conducted to evaluate the benefits of fineness of grind and steam-flaking of corn and other grains on animal performance. Steam flaking increases starch digestion, both in the rumen and small intestine. In a summary of their work, Theurer et al. (1999) reported that substituting steam-flaked corn for dry rolled corn increased starch digestion in the rumen from 35 to 52% and digestion in the small intestine from 61 to 93%. The increased starch digestion in the rumen resulted in an 18% increase in passage of microbial protein to the small intestine (2.3 to 2.7 lb). In a summary of studies in which steam-flaked corn was substituted for steam-rolled corn, the same researchers reported a 6% increase in milk yield (79 to 84 lb), a 2% increase in milk protein percentage (2.99 to 3.06%), and an 8% increase in milk protein yield (2.5 vs. 2.6 lb).

2) Balance diets for adequate RDP. A shortage of RDP can adversely impact carbohydrate fermentation and feed intake, as well as reduce microbial protein synthesis. None of these outcomes are desirable. Because microbial protein has a more desirable amino acid composition than dietary RUP, any feeding strategy that creates a greater reliance of the cow for RUP and less microbial protein for absorbed amino acids is likely to decrease milk protein concentrations. Several published research studies and field experiences support this.

3) Balance diets for amino acids. Based on current knowledge and available feeds and supplements, this requires selecting high Lys protein supplements (e.g., blood meal, fishmeal and soybean products) and a Met product (using a reasonable estimate for its contribution to MP) that will allow one to achieve the desired predicted ratio of Lys to Met in MP of 3.0-3.1:1.0 and to get their concentrations as close as possible to 6.6-6.8% and 2.1-2.2%, respectively.
4) Don’t overfeed RUP. Let your cows tell you how much they need. Cows are more responsive to changes in diet RUP content when RUP has a good amino acid balance vs. when the balance is not good. This makes sense because the nutritional potency of the RUP is greater when it has a good amino acid balance vs. a poor amino acid balance.

**Cost of Feeding for Higher Milk Protein**

Feeding for higher rather than lower milk yields is nothing short of feeding high quality feeds and diets that are more nutritionally balanced. While the focus of this paper is on balancing diets for carbohydrate and protein fractions, we acknowledge that feeding for higher milk yields requires attention to all of the cows’ nutrient requirements and in many cases, strategic use of feed additives and supplements. Feeding for higher vs. lower milk production has usually been part of the solution for increasing dairy farm profitability. The economics of feeding for higher milk yields still exist so no attempt will be made in this paper to consider the cost of feeding for higher milk yields. Instead, the focus is to consider the cost of optimizing concentrations of Lys and Met in MP without changing MP supply and its effects on lactation performance and return on investment (ROI).

The benefits of balancing for Lys and Met in MP are increased milk and milk components, more efficient use of MP for milk protein synthesis, more predictable changes in milk and milk protein production to changes in RUP supply, reduced metabolic disorders and increased herd profitability. See Schwab et al. (2007) for a more detailed discussion of these benefits. The cost of increasing concentrations of Lys and Met in MP to the recommended concentrations of 6.6 and 2.2%, respectively, if using NRC (2001) or 6.6-6.8% and 2.2%, respectively, if using CNCPS or CPM-Dairy, depends on one’s “starting point”. A point sometimes not appreciated is how low, or imbalanced, model predicted concentrations of Lys and Met in MP are when decisions about protein supplementation are made without regard to their content of Lys and Met. The authors have seen predicted concentrations of Lys as low as 5.7% and as high as 6.9% and Met concentrations as low as 1.7% and as high as 2.1% without Met supplementation. If model predicted flows of MP to the small intestine are 2,800 g/d (the approximate requirement for 90 lbs of milk), then these observed concentrations of Lys and Met in MP equate to predicted MP-Lys and MP-Met flows of 160 to 193 g/d, and 48 and 59 g/d, respectively. Clearly, these differences in flows of MP-Lys and MP-Met to the small intestine will impact milk protein synthesis if MP is limiting milk production. Thus, it becomes clear as to why increasing concentrations of Lys and Met in MP will increase milk protein synthesis and thus, increase milk protein concentrations and often milk yields.

The data in Table 4 is presented to highlight 3 diets, all balanced for 2,800 g/d MP, and the effect that differences in NRC (2001) predicted percentages of Lys and Met in MP have on predicted flows of MP-Lys and MP-Met to the small intestine and predicted yields of milk and milk protein. The primary differences in the diets were the protein supplements that were used and whether a rumen protected Met supplement was used. The primary protein supplements in the first diet were solvent soybean meal and heat processed soybean meal. The second diet contained a blend of soybean products and distillers’ grains. The third diet contained blood meal, solvent soybean meal, and a rumen protected Met product. Milk and milk protein yields were calculated using the regression equations of Schwab et al. (2003). The authors do not suggest that production increases of this magnitude will be observed, but that significant increases in milk and milk protein yields can be expected when concentrations of Lys and Met in MP are brought into balance and increased to concentrations of 6.6-6.8% and 2.2%, respectively.

The cost of increasing concentrations of Lys and Met in MP to the recommended concentrations of 6.6-6.6% and 2.2% depends on ones “starting point”. It should also be noted where it is possible, field nutritionists with experience in balancing for Lys and Met will also lower dietary RDP and/or RUP if the previous diets allow. This has the benefit of reducing the usual added expense of replacing low Lys
protein supplements with high Lys protein supplements and the additional cost of adding one or more ruminant protected Met sources to the diet. In reducing dietary CP, it is important, for the reasons previously stated, not to cause a deficiency of RDP. When employing these feeding strategies, the results of a 10-herd field study in 2006 indicated a ROI ranging from 1.1 – 5.5, with an average ROI of 3.35:1 (Driver, 2007). We also typically observe average ROI of 2.5 – 5.0 as well. Increases in butterfat content and milk yields are also very common, and contribute to the favorable ROI. Milk protein prices at $3.00 per lb or higher, even with high feed costs, make balancing diets for Lys and Met an attractive option for increasing dairy herd profitability. It is no longer uncommon to hear reports of increases in milk protein and milk fat concentrations of 0.20 - 0.25 and 0.10 – 0.15 percentage units, respectively, and 3 - 5 lb more milk as a result of balancing for amino acids.

Table 4. Effect of NRC (2001) predicted percentages of Lys and Met in MP on protein utilization, milk yield, and milk protein yield.

<table>
<thead>
<tr>
<th>Lys/Met in MP</th>
<th>Flows¹</th>
<th>Used for Prot. Syn.²</th>
<th>Milk yield³</th>
<th>Milk protein yield³</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>g/d</td>
<td>g/d</td>
<td>g/d</td>
<td>g/d</td>
</tr>
<tr>
<td>6.4/1.7</td>
<td>179</td>
<td>48</td>
<td>35.6</td>
<td>1025</td>
</tr>
<tr>
<td>5.7/1.9</td>
<td>160</td>
<td>53</td>
<td>38.9</td>
<td>1132</td>
</tr>
<tr>
<td>6.6/2.2</td>
<td>185</td>
<td>62</td>
<td>43.5</td>
<td>1304</td>
</tr>
</tbody>
</table>

¹Calculations are based on predicted MP supply of 2,800 g/d.
²Based on the assumption that the optimum Lys:Met ratio in MP is 3:1 and the understanding that any amino acid supplied in excess of need for protein synthesis is not used for protein synthesis and therefore, is catabolized and used for energy.
³Predicted yields using regression equations developed by Schwab et al. (2003) from plots of measured milk and milk protein yields vs. NRC (2001) predicted MP-Lys and MP-Met flows
⁴Requires feeding high-Lys protein supplements along with a ruminant Met supplement.

References


FEEDING FOR AND THE COST OF PRODUCING MILK COMPONENTS: MILK FAT

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The Value of Milk Components

In 2000, the USDA Federal Milk Marketing System adopted a multiple component pricing (MCP) system for dairy producers. This change shifted producer payments for milk from a historic system based on the volume of milk produced (adjusted for milk fat content) to one where payments are based almost entirely on the amounts of milk fat and protein produced. Adoption of the MCP system reflected the gradual shift over the last 50 years in the use of milk for fluid consumption toward its current use in manufactured dairy products such as cheese, butter, etc. Calculation of butterfat price in the MCP system is straightforward:

\[
\text{Butterfat Price} = (\text{Butter price} - 0.1202) \times 1.20.
\]

Where:

- Butter price = the current average wholesale price for butter as determined by survey by the USDA Agricultural Marketing Service (USDA-AMS, 2008).
- 0.1202 = $0.1202 per lb "Make Allowance" which accounts for butter manufacturing costs that are subtracted from the wholesale price for butter.
- 1.20 = Adjustment factor to account for the fact that butter contains 83.3% milk fat. (The remainder is water and other milk solids.)

For example, the most recent wholesale survey price for butter was $1.20 per lb. By formula, the producer butterfat price would be: $(1.20 - 0.1202) \times 1.20 = \$1.30 \text{ per lb}$. Prices for milk protein and other solids are more complex. However, the bottom line is that milk components and not milk volume are driving producer milk prices.

The relative importance of individual milk components in the MCP system used during the past 8 years (2000-present) is further illustrated in Figure 1. Milk fat and protein contribute the lion’s share of producer payments for milk produced with other milk solids (lactose and minerals) having only minimal impact. Intuitively, this should be providing powerful economic incentives for dairy producers to produce high value milk components, namely fat component contributions to the Uniform Federal Order Milk Price by year and month (2000-2007) (AMS-USDA, 2008).
and protein. Yet when you talk with producers, the subject of milk price always revolves around $/cwt milk, not $/lb fat or $/lb protein. Apparently, these ingrained traditions die hard!

There were two major driving factors behind record milk prices observed during 2007. First, record cheese prices which is the primary factor driving producer protein price, increased the value of milk protein during the last quarter of over $4 per pound, nearly double the historic average (2000-2006) of $2.17 (AMS-USDA, 2008). Secondly, world demand for dried whey and skim milk powder drove prices for non-fat-non-protein milk solids (NFPMS) to $.42/lb for 2007, more than four times the historic average of $.09/lb. It remains to be seen if those trends will continue but already the price of NFPMS has fallen to $.08 per lb (AMS-USDA, March, 2008) which is slightly below the average historical price.

Since fat and protein remain the driving factors behind milk prices, two critical questions emerge: “What is the relative cost of producing milk fat as compared to milk protein?” and “How do I produce more milk fat and protein?” The focus of the remainder of this manuscript with is on the answers to those questions.

**Marginal Feed Costs of Fat Production**

Current diet formulation and nutrient requirement models account for the major milk components (fat, protein, and lactose) in calculating nutrient requirements of the cow, primarily through net energy (milk fat, lactose and protein) and metabolizable protein (milk protein only) requirements. Nutrient requirement models (NRC, 1989 & 2001; CPM, Moate *et al.*, 2006) are excellent representations of absorbed nutrient supply and gross nutritional needs (NE, metabolizable protein and metabolizable amino acids) for milk component production. More recent versions of CPM incorporate prediction of absorbed fatty acids (FA).

In theory, one could estimate the marginal feed cost of producing an individual component using existing feed formulation models by varying the concentration of one milk component at a time and looking at changes in feed cost. However, the accuracy of this approach would depend on whether nutrients in an existing diet were limiting for the production of the specific milk component being evaluated. Alternatively, the cost of milk fat production might be estimated from its energy value and the feed cost per unit energy. This method was used by Hillers *et al.* (1979).

These approaches are not sophisticated enough to account for subtle differences in metabolic precursors required for synthesis of individual milk components. Here an understanding of the basic metabolic precursors required for milk component synthesis is required. Baldwin (1968) was the first to propose the use metabolic precursor requirements for milk fat, protein, and lactose synthesis. These theoretical principles laid the groundwork for subsequent metabolic models such as “Molly” (Baldwin, 1995) and more recently, the Nordic cow model “Karoline” based on earlier work of Danfaer (1990).

Milk fat consists primarily of triglycerides which include a glycerol backbone and three ester-linked fatty acids (FA). Of the milk FA, it is generally thought that about half of the palmitic acid (C16), and all of the short and medium chain fatty acids (C<16) (SMCFA) are synthesized de novo from rumen VFA (acetate and butyrate). The remaining long chain FA (LCFA) including the other 50% of the palmitate and all of the C18 FA (stearic, oleic, and linoleic acids) are derived 18C FA absorbed from the diet. Because of rumen biohydrogenation of unsaturated FA in the diet, oleic acid in milk is produced primarily by desaturation of absorbed stearic acid through delta-9 desaturase in the mammary epithelial cell. In addition to VFA precursors, de novo synthesis requires a source of reducing equivalents (NADPH). Since ruminants have only negligible activities of citrate cleavage enzyme and malic enzyme, a common pathway to transfer reducing equivalents to the cytosol from the mitochondrial, the cow utilizes both the pentose pathway and isocitrate dehydrogenase to generate NADPH for de novo FA synthesis. While most glucose use in milk fat synthesis (for glycerol and NADPH from pentose pathway),
undoubtedly some glucose is derived from gluconeogenic amino acids, introducing a probable need for metabolizable protein even for milk fat synthesis.

Table 1 below illustrates the fundamental problems with the use of nutritional formulation models (NRC, 1989; 2001; CPM 2008) in determination of marginal feed costs of production of milk components. Namely, the failure to account for metabolic precursors unique to each individual component. While this may seem minor at first, if any of these components are limiting, then milk fat production will be restricted. Failure to account for metabolic precursors and other factors (enzyme activity, metabolic capacity of the animal, etc.), utilization other nutrients will be reduced and therefore marginal cost of milk fat production will be increased. While Table 1 illustrates the problems with fat synthesis, similar precursor limitations occur for protein and lactose synthesis.

Table 1. Comparisons of nutrient formulation and metabolic models of requirements for milk fat synthesis.

<table>
<thead>
<tr>
<th>Model</th>
<th>NEₖ for milk fat</th>
<th>Reducing Equivalents for de novo Synthesis</th>
<th>Glycerol precursors (glucose &amp; amino acids)</th>
<th>16&amp; 18C LCFA precursors Absorbed/Needed</th>
<th>VFA for de novo FA Synthesis (Ac &amp; But)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRC, 1989</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No/No</td>
<td>No</td>
</tr>
<tr>
<td>NRC, 2001</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No/No</td>
<td>No</td>
</tr>
<tr>
<td>CPM</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes/No</td>
<td>No</td>
</tr>
<tr>
<td>Molly (Baldwin, 1995)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes/Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

There have been several methods described to estimate the marginal feeding costs of milk component production. In early approaches (Hillers et al., 1979; Dommerholt and Wilmink, 1986) the cost of component production was based on the energy value of each component and the feed cost per unit energy required. This approach ignored the differences in the metabolites needed for individual component production as amino acids for milk protein synthesis, fatty acids for fat synthesis, and glucose required for lactose production. In addition, it completely ignores other required metabolites such as ATP, NADPH, glycerol etc.

Dado and Mertens (1993), building on the earlier theoretical approach of Baldwin (1968) developed an extensive metabolic model of precursor requirements for milk component production including details such as the amounts of individual FA and amino acids required for fat and protein synthesis. In order to apply this approach to practical feed requirements, Mertens et al. (1993) converted these metabolic precursor requirements into NEₖ and absorbed protein (metabolizable protein, MP) requirements from the diet. While admittedly this is somewhat of an oversimplification, this approach at least took into account protein needs for milk protein synthesis and the use of metabolizable protein as a glucose precursor required for milk lactose and fat production. A comparison of approaches to estimating marginal feed costs for milk fat production is in Table 2.

Figure 2 shows the marginal feed cost estimates of milk fat, protein, and lactose production using several approaches and scenarios. Feed cost per component estimates of Hillers et al. (1979) and Dado et al. (1994) represent the costs estimated at the time of publication. For Dado et al. (1994) this was a 25 average price for corn and soybean meal (1964 to 1988). Dado-2008-Adj represents the method of Dado et al. (1994) using February 2008 prices for corn and soybean meal. Finally, Dado-2008-ML utilized the NEₖ and MP requirement estimates for each component (Dado et al., 1994). However, costs to supply NEₖ and MP were estimated by maximum likelihood methods as described by St Pierre and Glamocic (2000)
using the feed value estimator SESAME®. Using February 2008 feed prices; costs of NE\textsubscript{L} and MP were estimated at $0.083 per Mcal and $1.57 per kg, respectively.

Table 2. Comparisons of methods for estimating marginal feed costs of milk fat production.

<table>
<thead>
<tr>
<th>Model</th>
<th>NE\textsubscript{L} for Fat</th>
<th>Reducing Equiv. for de novo Synthesis</th>
<th>Glycerol precursors (glucose &amp; amino acids)</th>
<th>16&amp; 18C LCFA precursors Absorbed/Needed</th>
<th>VFA for de novo FA Synth. (Ac &amp; But)</th>
<th>Theoretical vs. Practical Efficiency</th>
<th>Marginal Cost of Feeding</th>
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</thead>
<tbody>
<tr>
<td>Baldwin (1968)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No/Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Hillers et al. (1979)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Practical only</td>
<td>Yes, energy only</td>
</tr>
<tr>
<td>Dado et al. (1994)\textsuperscript{1}</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No/Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Based on Dado et al. (1993 & 1994) and Mertens et al. 1993.

As is shown in Figure 2, marginal feed costs for component production varied widely according to method used and of course, the time of publication. Of the two general methods, the method described by Hillers et al. (1979) is the least desirable since it bases marginal feeding costs on energy needs alone. As expected, marginal feed costs of fat, protein, and lactose ranked in that order based on their respective heats of combustion. This system fails to take into account the relative cost of dietary protein required for milk protein production.

The method of Dado et al. (1994) takes into account differences in gross nutritional needs (NE\textsubscript{L} vs. MP). As expected, marginal costs for lactose, fat, and protein production adjusted for 2008 feed prices (Dado-2008-Adj) were increased by 50, 56, and 23% respectively reflecting the pronounced changes in feed costs that have occurred since 1994. However, the increase in cost of milk protein production seemed small compared with the recent changes in the price of soybean meal and other protein supplements.

However, when the current maximum likelihood estimates of the cost per unit NE\textsubscript{L} and MP used in the Dado et al. (1994) model, marginal feed costs per component increased by 96, 83, and 119%, respectively. These estimates are more in line with the overall changes in feed costs and are likely to be more representative of the actual marginal feed costs of milk component production.
Table 3 shows a comparison of current milk component prices, the marginal feed costs using the Dado-2008-ML method, and the income over feed costs (IOFC) by component. Clearly, each pound of milk lactose costs $.17 more in feed than the value of the NFMS produced. (Not including minerals). Milk fat and milk protein are of course the components of highest value. While they cost more in feed to produce, their value is such that each is profitable.

Table 3. March 2008 producer prices, marginal feed costs, and income over feed cost (IOFC) by milk component.

<table>
<thead>
<tr>
<th>Item</th>
<th>Lactose</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Price, $/lb</td>
<td>0.08</td>
<td>1.30</td>
<td>4.02</td>
</tr>
<tr>
<td>Feed cost, $/lb</td>
<td>0.25</td>
<td>0.44</td>
<td>0.97</td>
</tr>
<tr>
<td>IOFC, $/lb</td>
<td>-0.17</td>
<td>+0.86</td>
<td>+3.05</td>
</tr>
</tbody>
</table>

How do I produce more milk fat and protein?

*Increasing Milk Production to Increase Fat Yield: It’s not what you expect?*

Component yields are driven by two factors: 1) milk volume; and 2) component concentrations; The dairy cow’s diet has no virtually no effect on milk lactose and mineral content and only modest effects on milk protein concentration (Sutton, 1989). However, diet can and does have large effects on milk fat concentration (Sutton, 1989) which will be discussed later. But just how important is milk volume in driving component yields? Its importance (or lack there of) can be illustrated in two ways: 1) The genetic relationship between milk fat and milk volume yields and 2) the effect of management practices that increase milk yield on milk fat yield. For example, Figure 3 shows the relationship between predicted transmitting abilities (PTA) for milk yield and milk fat yields from the most recent USDA Sire Summary.

While there was a relationship between PTA Fat and PTA Milk, the relationship was modest (Std Y = 21, R² = .25). Further the regression coefficient (.0166) suggested that each 1000 lb increment in PTA Milk resulted in only 16.6 lb added fat production. Since the average fat content of milk produced in federal milk marketing orders has been 3.67 to 3.68% for more than 40 years, increasing milk production by improved genetics results in only 45% of the expected gain in fat yield.

The picture is somewhat different for milk protein (data not shown) where 1000 lb increment in PTA Milk resulted in 21.1 lb in added protein production (Std Y = 9.7, R² = .71) or 69% of the protein yield gain if protein had remained constant (market average for 2000-2006 was 3.03% milk protein). However, unlike milk fat, the market average for milk protein has climbed since from 3.02 to 3.06% over the last 4 years. While increasing milk production does result in an increase in both fat and protein yields, the extent of the increase is not nearly what producers expect!
The observations above reflect the genetic component only. What about management changes that affect milk yield? Examples might include the use of bST, increased milking frequency (3X vs. 2X), and increased photoperiod. Each practice has been shown to increase milk production independent of major changes in nutrition. Table 4 below shows impact of increased milk production through these management changes. Again, the responses are consistent with those observed with genetic effects on milk composition. Fat and protein concentrations go down with increasing milk production. The bottom line is that increasing milk yield alone accompanied by the normal decreases in fat and protein concentration results in a smaller than expected increase in milk component yields.

Table 4. Milk fat responses to management changes including 3X milking (Erdman and Varner, 1995), bST (Thomas et al., 1991), and increased photoperiod (Dahl et al., 2000).

<table>
<thead>
<tr>
<th>Management Change</th>
<th>Δ Milk, kg/day</th>
<th>Δ Fat, %</th>
<th>Δ Protein, %</th>
<th>Δ Fat % / Δ Milk, kg</th>
<th>Δ Protein % / Δ Milk, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased PTA, kg</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>-0.023</td>
<td>-0.011</td>
</tr>
<tr>
<td>3X vs 2X Milking</td>
<td>+3.5</td>
<td>-0.17</td>
<td>-0.06</td>
<td>-0.049</td>
<td>-0.017</td>
</tr>
<tr>
<td>bST</td>
<td>+4.9</td>
<td>+0.03</td>
<td>-0.06</td>
<td>+0.006</td>
<td>-0.012</td>
</tr>
<tr>
<td>Increased Photoperiod</td>
<td>+2.0</td>
<td>-0.12</td>
<td>-0.04</td>
<td>-0.050</td>
<td>-0.022</td>
</tr>
<tr>
<td>Mean</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>-0.029</td>
<td>-0.0155</td>
</tr>
</tbody>
</table>

Strategies to Increase or Maintain Fat Yields:

Controlling Milk Fat Depression

Considerable progress has been made during the last 15 years on understanding the causes of milk fat depression (MFD). Older theories of regarding the influence of rumen VFA (i.e. acetate deficiency, acetate:propionate ratios, butyrate deficiencies, insulin effects, etc.). However, it is now quite clear that milk fat depression is directly related to aberrations in the process of fatty acid biohydrogenation in the rumen whereby intermediates in the biohydrogenation pathways including trans-10,cis-12 CLA and possibly trans containing CLA’s and 18:1 FA are absorbed and directly inhibit milk fat synthesis in the mammary gland. The etiology is extensively discussed in NRC (2001).

Two feed factors are consistent with current MFD theory. First, a source of polyunsaturated fatty acids (PUFA) is required in the diet as a source of biohydrogenation substrate. Second, low rumen pH leads to incomplete biohydrogenation of PUFA causing an accumulation of biohydrogenation intermediates. Diet formulation procedures that control both factors will successfully minimize MFD and maintain normal or even elevated milk fat concentration. These would include:

- Adequate amounts of forage with the correct particle size length to maintain rumination activity which increases salivary buffer flow to the rumen.
- Careful monitoring of PUFA in the diet ensuring that rumen biohydrogenation substrates are kept to a minimum.
- Monitoring of starch and soluble carbohydrate levels in the diet to ensure that rumen fermentability is not too high, driving down rumen pH.
- Maintaining adequate DCAD levels in the diet including the use of sodium and potassium bicarbonate and carbonates to keep DCAC levels at or above 350 meq/kg diet dry matter. It is increasingly apparent that there is a direct relationship between dietary DCAD and rumen pH.

**Feeding Dietary Fat**

Surprisingly, after some initial promising work with oilseed feeding, the use of dietary fat to increase milk fat yield has been at best marginally successful. Feeding long chain fatty acids generally increases the proportion of LCFA and reduces the proportion of palmitate and *de novo* synthesized FA in milk fat (Grummer, 1991), resulting in no net change in fat yield unless total milk production is increased. While addition of fat to the diet is an excellent method to increase the energy concentration, unless dietary fat increases milk production and maintains both milk fat and protein concentrations, there does not appear to a positive impact of fat addition on milk fat yield.

**Managing Seasonal Influences**

Seasonal influences on milk production and fat yields are profound. Low summer time milk fat percent is compounded with typically lower milk yields also making for significantly lower milk fat yields. This is the one example where milk production and milk components (fat and protein) decrease simultaneously. Seasonal influences on trans fatty acids and CLA’s in milk have been reported that correspond with reduced milk fat percent during the summer months. (Precht *et al.* 2000). While some have suggested that this seasonal response is due to grazing of forages high in PUFAs (biohydrogenation substrates) during the early spring and summer, most cows in the U.S. are not grazed and therefore this effect is more likely due to effects of environment on rumen pH. It has been suggested that this may be due to temperature effects on blood bicarbonate levels, reducing salivary bicarbonate flow to the rumen.

There are significant opportunities from both a nutritional and environment management standpoint to manage low fat yields during the summer months. Losses in milk production and fat test due to heat stress can be mitigated by the use of shade, ventilation and evaporative cooling. In addition, reductions in fat percent might also be mitigated by paying proper attention to nutrition including the use of cations such as sodium bicarbonate or potassium carbonate. Figure 4 shows the interaction between season and response to DCAD level (KHCO₃) adapted from work by West *et al.* (1991).

**What about Short Chain Fatty Acids?**

Earlier I had mentioned that it is generally thought that about 50% of the palmitic acid (C16), and all of the short and medium chain fatty acids (C<16) are synthesized *de novo* from rumen VFA (acetate and butyrate). A 12% increase in milk fat yield was reported in lactating cows fed diets supplemented with coconut oil, containing predominantly 12:0 and 14:0 FA (Astrup *et al.*, 1974; Storry *et al.*, 1971). Pure saturated FA (12:0, 14:0, 16:0 or 18:0) fed to lactating cows (Steele and Moore, 1968b) produced variable changes in milk fat content but increased these FA in milk, suggesting that the proportions of FA from treatment supplements were increased in blood triacylglycerols.

![Figure 4. Influence of DCAD on mitigation of the decline in milk fat during hot weather (Adapted from West *et al.* 1991).](image-url)
We hypothesized that in some situations, rates of \textit{de novo} FA synthesis could be limiting milk fat production (Kade Gowda \textit{et al}., 2008). Figure 5 shows the effect of postruminal infusion of short and medium chain FA on Fat synthesis and yield. Treatments consisted of 1) \textbf{Control} (no infusion); 2) \textbf{Butterfat} (400 g/d butterfat as a source of short and long chain FA); 3) \textbf{LCFA} (245 g/d of a LCFA mixture providing 50\% of the 16:0 and equivalent amounts of C18 FA as found in 400 g of butterfat); and 4) \textbf{CLA} (100 g/d of commercial \textit{CLA} mixture providing 10 g of t10c12 CLA/day which served as a negative control.

The infusion of butterfat where the pattern of FA is identical to that of milk fat, increase fat yields by 243 and 142 g/d over Controls and LCFA, respectively. This suggested that SCFA could be limiting milk fat synthesis, a new concept that is contrary to the previously held assumption that adequate precursors and metabolic machinery were available to provide milk SCFA via \textit{de novo} FA synthesis. Previous work by Astrup \textit{et al}., (1974) and Storry \textit{et al}., (1971) and more recent work with postruminal infusion of palmitate Enjalbert \textit{et al}., (2000) suggested that opportunities exist to not only maintain but also increase milk fat percent through targeted delivery of SMCFA.

\textbf{References}


IMPROVING SILAGE QUALITY

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Summary

With sky rocketing feed costs, maintaining and/or improving forage quality should be a major concern for every dairy farm. Harvesting forages at the proper stage of maturity is the first step to having high quality forages to feed to dairy cows. Next, pre-ensiling management techniques (e.g. cutting corn silage high, cutting forages to optimal particle size, processing corn silage, and using a proven microbial inoculant) are also essential to maximizing forage quality. Once in the silo, filling quickly, packing tightly and covering with plastic to keep air out of the forage mass help to ensure a good fermentation. During long term storage and feed out, management to keep silage fresh and away from air (which causes spoilage) will help to maintain forage quality.

Introduction

Harvest and storage management can have marked effects on silage quality. The objective of this paper will be to briefly discuss some recommended management practices to make high quality silages.

Evaluating Corn Forage Hybrids

Much emphasis has been placed on selection of corn hybrids for dairy cattle. There has been a significant resurgence in using brown mid rib (BMR) corn as newer hybrids now come with many stacked traits and yield drags (although still present) have been significantly reduced. A good tool that can help in on farm evaluation is the MILK2006 spreadsheet from the University of Wisconsin at www.uwex.edu/ces/crops/uwforage/dec_soft.htm. The MILK2006 program calculates milk/ton and milk/acre for silage hybrids. This latest version allows for input data related to kernel processing score, degree of starch access and in vitro/in situ starch digestibility. This version also allows one to enter data using either 24, 30 or 48 h in vitro NDF digestibility estimates. Depending on your specific situation, most would try to choose hybrids that give high milk/ton and milk/acre for silage hybrids. This latest version allows for input data related to kernel processing score, degree of starch access and in vitro/in situ starch digestibility. This version also allows one to enter data using either 24, 30 or 48 h in vitro NDF digestibility estimates. Depending on your specific situation, most would try to choose hybrids that give high milk/ton and milk/acre. CornPicker is another program for evaluating corn silage hybrids and is available from Michigan State University (www.msu.edu/~mdr/cornpicker.html). This program calculates partial budgets and compares net farm income between two corn silage hybrids. The program is more complicated than MILK2006 but unlike that program, CornPicker provides a monetary bottom line between hybrids. Some of the inputs that users can manipulate include the cost of SBM and corn, cost of the seed; planting densities, amount of the hybrid fed to various groups of cows and of course NDF-digestibility.

Forage Maturity and DM

Harvesting forages at optimum maturity is important because it sets the stage for the rest of the year. High forage quality drives intake and in turn, this drives milk production. Not even the best nutritionists in the world can make cows maximize their milk production if they are working with poor quality forages. Corn silage should be harvested when the whole plant is at 32 to 35% DM and the kernels...
are at ½ milk line. However, milk line and whole plant DM do not always match up. In all cases, whole plant DM should be the overriding factor for corn silage harvest. To monitor whole plant DM, cut representative samples of corn plants from the field and have them chop them. Collect the chopped material and dry it down with a microwave or Koster moisture tester. Depending on the conditions, corn silage will dry down at a rate of about 0.5 percentage units per day (faster in dry and hot weather). Based on your acres and equipment you may have to start at a lower DM and you may end at a higher DM but the key is to avoid the extremes. Harvesting corn silage that is too wet (typically < 28-30% DM) results in excessive fermentations that produce high concentrations of acids and results in nutrient run off. Specifically, these wet silages are often characterized by high concentrations of acetic acid produced from “wild-type” fermentations. A common problem when feeding large quantities of wet corn silages is a reduction in DM intake because of the high acid content. In contrast, extremely dry corn silage (> 38-40% DM) should be avoided because the low moisture restricts fermentation and this material is more difficult to pack which often leads to poor aerobic stability. In addition, dry corn silage is usually very mature and thus fiber and starch digestibility are low.

One of the biggest challenges for making good alfalfa or grass silage is managing the period of wilting to result in maximum conservation of fermentable sugars and obtaining an adequate dry matter level to prevent the growth of clostridia. During prolonged wilts, sugars are metabolized by the plant in the windrow thus a quick dry down is beneficial. Wet grass and alfalfa silages are highly prone to undergo clostridial fermentations when the dry matter is less than 30-35%. Wilting these crops above this level makes it harder for clostridia to dominate the ensiling process.

**Cutting Height**

Corn silage is normally harvested to leave 4 to 6 inches of stalk in the field. Typically, the only time that cutting height should be higher is during drought years when the potential for nitrate accumulation in the lower third of the stalk may occur. However, some dairymen have been high-cutting their corn silage as a normal practice for years in order to improve forage quality. Leaving more of the stalk in the field that contains high concentrations of fiber and lignin may also help to improve soil conditioning. Research has shown that high cut corn silage (typically leaving 18 to 20 inches of stalk) results in silage with slightly lower concentrations of fiber and lignin, but higher concentrations of starch and net energy (Wu and Roth, 2003). A small yield drag of about 5 to 10% can be expected. Disappointingly, improvements in NDF digestion have been very small. The ultimate success of high cutting corn silage will depend on milk produced per ton of forage and milk produced per acre of forage.

**Particle Size**

Chopping corn silage too fine and too coarse should be avoided. Finely chopped silage reduces the effective fiber and coarsely chopped silage does not pack well and often leads to sorting of the TMR. Recommendations for theoretical chop size usually run between 3/8 to 1/2 inch for unprocessed corn silage and about 3/4 inch for processed silage). In diets where corn silage makes up the majority of the forage, 15 to 20% of the particles should be greater than 1.5 inches long. If using a Pennsylvania State Forage Separator with the fourth box (now with a top, middle, low screens and bottom pan), about 8 % of the corn silage should be retained on the top screen to ensure optimum levels of effective fiber in the diet. If corn silage is not the major forage in the diet, about 3% of the top screen may be sufficient. For corn silage, the middle screen should have 45 to 65% of the particles after shaking and there should be no more than 5% of particles on the bottom pan. If corn silage is processed, a higher proportion of particles can be targeted for the top screen. In measurements that we have taken, some baggers decrease the proportion of corn silage particles on the top screen by about 10 to 15 units so this must be taken into consideration when setting chop length. Instructions for using the new particle size separator can be found at:
www.das.psu.edu/TeamDairy/. In general, if faced with drier forages, one can cut shorter to achieve a tighter pack. If feeding long hay, silages may also be cut a bit shorter.

**Mechanical Processing**

Mechanical processing of whole plant corn has been an accepted method to improve the quality of corn silage (Johnson et al., 1999). Whole plant processing crushes the entire plant through rollers and can be accomplished in the field during harvesting, at the silo but prior to storage, or after ensiling and just prior to feeding. Processing corn silage improves starch and allows for good packing in silos even with a longer length of particle chop. Rollers should be set obtain adequate kernel damage. In drier and more mature corn silage, clearances between rollers will usually need to be tighter. However, care should be taken to monitor the effectiveness of the processing. When large amounts of acreage require harvesting, there may be a tendency to open the rollers more than what is recommended in order to speed up the harvest, reduce energy use and to reduce wears on equipment. As a rule of thumb, adequate processing is occurring if more than 90-95% of the kernels are crushed or cracked and cobs are more than quartered. Many labs currently provide a Corn Silage Processing Score, which is coupled to NIR (near infra-red) analyses of corn silage. A dried corn silage sample is sifted through several screens and particles of corn that are greater than ¼ to ½ of a kernel are retained on a screen and considered difficult to digest. The score provides feedback on processing as “optimum”, “average”, or “inadequately processed”. (One draw back is that the test takes several days for completion). An improvement in starch digestion is greater when more mature corn silage (e.g., black layer) is processed. However, always target harvest for 32-35% DM (whole plant DM). Corn should probably not be processed if harvesting forage that is less than 30% DM and especially if the corn has not dented. Improvements in fiber digestion due to mechanical processing are inconsistent. When there are reasons out of your control (inclement weather, equipment problems, and scheduling problems with a contractor) those results in corn being harvested at later stages of maturity, processing should be considered. A common observation by producers switching to processed corn silage is the reduction in cobs in the feed bunk and a reduction in kernels in the manure.

**Keys to Making Good Silage**

The keys to making quality silage are to 1) rapidly exclude air from the forage mass, which will result in 2) a rapid production of lactic acid and reduction in silage pH, and 3) to prevent the penetration of air into the silage mass during storage. Excessive air, due to slow silo filling or poor packing (overly dry forage or forage chopped too coarsely) allows the plant to respire for prolonged periods of time. This results in utilization of sugars and excessive degradation of plant protein. Air also encourages the growth of undesirable microbes such as yeasts and molds.

Air can be eliminated by fast filling (but not too fast), even distribution of forage in the storage structure, chopping to a correct length and ensiling at recommended dry matters (DM) for specific storage structures. Bunk and pile silos should be filled as a progressive wedge to minimize exposure to air and packed in 6 to 8 inch layers. The recommended optimal packing density for bunk silos is 14 –16 lbs. of dry matter per cubic foot (Ruppel et al., 1995). Silage corers can be obtained from several commercial sources. An Excel spreadsheet can be downloaded from the University of Wisconsin Extension web site that helps with bunker silo filling (www.uwex.edu/ces/crops/uwforage/storage.htm). Users can input silo dimensions, tractor weight, forage delivery rate, forage dry matter, and packing time to estimate packing density. In several recent surveys of bag silos, packing densities are more commonly between 9 top 12 lb of DM/cu ft. Silage in bags should be packed tightly by monitoring the stretch marks on the bags. Tunnel extensions on older units can be added to increase pack density. Silo bags should be vented for about 3 days to rid the bags of excess gas.
Under anaerobic conditions (lack of air) silage fermentation is dominated by microbial activity. Fermentation is controlled primarily by a) type of microorganisms that dominate the fermentation, b) available substrate (water soluble carbohydrates) for microbial growth, and c) moisture content of the crop. Lactic acid-producing bacteria utilize water-soluble carbohydrates to produce lactic acid; the primary acid responsible for decreasing the pH in silage. Unlike alfalfa and other grass silages, corn silage rarely undergoes clostridial fermentation. However, because of its high starch content, preventing the proliferation of yeasts that produce alcohol and cause lower DM recovery is a challenge in corn silage. Yeasts are also responsible for aerobic spoilage of silages during storage and feed out.

**Microbial Inoculation**

Because forage often naturally contains many detrimental types of bacteria, the concept of adding a microbial inoculant to silage was to add fast growing homofermentative lactic acid bacteria in order to dominate the fermentation resulting in higher quality silage. Some of the more common homolactic acid bacteria used in silage inoculants include *Lactobacillus plantarum, L. acidophilus, Pediococcus acidilactici, P. pentococcus,* and *Enterococcus faecium.* Microbial inoculants contain one or more of these bacteria which have been selected for their ability to dominate the fermentation. The rationale for multiple organisms comes from potential synergistic actions. For example, growth rate is faster in *Enterococcus > Pediococcus > Lactobacillus.* Some *Pediococcus* strains are more tolerant of high DM conditions than are *Lactobacilli* and have a wider range of optimal temperature and pH for growth (they grow better in cool conditions found in late Fall and early Spring). When compared to untreated silages, silages treated homolactic acid bacteria are often lower in pH, acetic acid, butyric acid and ammonia-N but higher in lactic acid content and have better DM recovery (Muck and Kung, 1997).

*Lactobacillus buchneri* has been proven to improve the aerobic stability of silages. In the silo, *L. buchneri* results in a “controlled” fermentation that produces moderate amounts of acetic acid which limits the growth of spoilage yeasts. Production of moderate amounts of acetic acid by this organism is not detrimental to intake nor does it lead to excessive amounts of DM loss during ensiling (Kleinschmit and Kung, 2006). *Lactobacillus buchneri* has been combined with traditional homolactic acid bacteria to form “combination” inoculants that are specifically designed to speed up the fermentation process and to improve the aerobic stability (shelf life) of silages. Recently, a strain of *L. buchneri* has been marketed that produces ferulic acid esterase which may help in improving fiber digestion (Nsereko et al., 2007).

The location of applying a microbial inoculant is important. If silage is to be stored in a bunker, pile or pit silo I would recommend that the inoculant be applied at the chopper for a more even distribution. Remember that these bugs don’t have legs, nor do they swim! If all the inoculant gets put on in one spot, it will probably stay there. (Some distribution will occur during tractor movement and packing, but this is not efficient.) For silage that will be stored in a bag silo, application at the chopper or bagger will probably not make a difference. (In a few instances, forage is chopped and harvested far away from where it is ensiled. Under these circumstances, I would prefer to have the inoculant applied at the chopper so that the microorganisms can begin their work right away.) Don’t forget to properly calibrate your applicators to match forage delivery and don’t increase the dilution or reduce the application rate! Also, remember that inoculants in water are stable for about 2 to 3 days but maybe less under very hot temperatures. If for some reason, unused liquid inoculants must be stored, do so in shade and place a few ice packs into the liquid to lower the temperature of the liquid. Do not allow the temperature of water in the applicator tanks to rise above about 100°F as this may decrease the viability of the bacteria (Mulrooney and Kung, 2008). Seal any unused portion of powders tightly to protect from moisture and stored in a cool area.
Sealing Silos and Fermentation

After filling silage should be covered with plastic as soon as possible and weighted down with tires (tires should be touching) or gravel bags to exclude air. Split tires are good alternative because they are easier to handle, do not accumulate water (thus less breeding grounds for mosquitoes that could carry the West Nile Virus), and are undesirable for animals to nest in. The return on investment (labor and plastic) is extremely high for covering bunk and pile silos (Bolsen et al., 1993). Oxygen barrier plastics are also now available for use (Borreani et al., 2007).

When conditions allow for it, silage should ferment for a minimum of 6 to 8 weeks before feeding. A gradual transition over a 10 to 14 day period from old silage to new silage is also recommended. Unfermented feed is the equivalent of feeding green-chop that is high in fermentable sugars and can cause cows to go off feed and have loose manure.

Silage Feedout

Proper management for removal of silage from silos and management at the feed bunk can help producers to maximize profits and production. Enough silage should be removed between facing to minimize aerobic spoilage. Lesser amounts may be removed in areas of the country where ambient temperatures remain cool during the winter months. Removal of silage should be such to minimize loose silage on the ground between feedings. Hot, moldy feeds should not be fed because they are low in nutritive value and digestibility and depress intakes. Feed bunks should be kept full but clean of decaying feed. Face shavers are becoming popular (W. C. Stone: www.anisci.cornell.edu/prodairy/health/reducingpap.pdf) but research is needed on their benefit. Extreme care should be taken to prevent air from penetrating between the plastic and reaching the silage mass.

Conclusions

Great care should be taken to preserve and maintain the nutritive value of forage crops. Management starts in the field with harvesting crops at the optimum maturity and then following this with a quick wilt (for grasses and alfalfa), by chopping to an adequate particle size, treating with a good microbial inoculant, processing the plant (for corn silage), filling silos quickly and packing them tightly and finally managing the silage in the silo with plastic and weights to minimize exposure to air.

References


COPING WITH HIGH CORN PRICES:
LOW STARCH DIETS AND LACTATION PERFORMANCE BY DAIRY COWS

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Summary
Lactating dairy cow diets with more than 24% starch (DM basis) are common, but recent high corn prices have fueled a desire to feed lower-starch diets. The potential for using digestible neutral detergent fiber or pectin from byproduct feeds or sugars to partially replace starch from corn grain in diets fed to lactating dairy cows was reviewed. Potential modifying effects of corn silage, starch digestibility and ionophore on lactation performance by dairy cows fed low starch diets was also evaluated. Lactation performance was reduced for 18% and 20% starch diets (DM basis) formulated using beet pulp and citrus pulp, respectively, to partially replace corn grain. Lactation performance was not reduced for 16%-17% starch diets (DM basis) formulated using soyhulls to partially replace corn grain. Diets containing 21% starch (DM basis) appear to be acceptable when high-fiber, moderate protein byproduct feeds are used to partially replace corn grain and protein supplement. Feeding corn silage at a higher percentage of the forage, high starch corn silage, sugars and ionophore can reduce the percentage of corn needed in high-starch diets or increase the "corn equivalency" of low-starch diets. The cost of using digestible NDF or pectin from byproduct feeds or sugars to partially replace starch from corn grain needs to be evaluated relative to corn and protein supplement prices. The starch in low-starch diets should be highly digestible.

Introduction
The optimum starch content of diets fed to lactating cows is not well defined in the literature, but 24% to 26% starch diets (DM basis) have been suggested as ideal (Staples, 2007). Kaiser and Shaver (2006), from a survey of six high producing (13,500 kg RHA) Wisconsin dairy farms, reported starch concentrations of diets fed to lactating cow groups ranging from 25% to 30% (DM basis). With today’s high corn prices, however, there is much interest in feeding diets that are lower in starch content than what has been the norm. Reducing dietary concentrate and starch concentrations via forage substitution below 36% and 21% (DM basis), respectively on average across lactation, reduced (P < 0.05) 305-d fat-corrected milk yield (FCM; >1,000 kg) and 305-d cheese yield (>120 kg) for multiparous cows fed alfalfa silage based diets (Tessmann et al., 1991). The purpose of this paper is to evaluate the potential for using digestible neutral detergent fiber (NDF) or pectin from byproduct feeds or sugars to partially replace starch from corn grain in diets fed to lactating dairy cows. Potential modifying effects of corn silage, starch digestibility and ionophore on lactation performance by dairy cows fed low starch diets will also be examined.

Partial Replacement of Starch from Corn Grain with Digestible NDF or Pectin from Byproduct Feeds

Summarized in Tables 1 and 2 are the results from five trials on the partial replacement of starch from corn grain with digestible NDF or pectin from byproduct feeds.
Table 1. Literature review on partial replacement of starch from corn grain with digestible NDF or pectin from byproduct feeds (Test feeds, design, cows, and diet ingredient and nutrient composition).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Test Feeds</th>
<th>Trial Design</th>
<th>Cows</th>
<th>Diet Ingredient Composition (DM basis)</th>
<th>Diet Nutrient Composition (DM basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voelker &amp; Allen, 2003</td>
<td>Pelleted Beet Pulp</td>
<td>4x4 LS(^2) 21-d periods</td>
<td>n=8 &gt;79 DIM(^3) Parity&gt;1</td>
<td>40:60 F:C(^4) 50:50 CS(^4) AS(^5) HM corn 0, 6, 12, &amp; 24% BP(^6)</td>
<td>18% CP 17% Forage NDF 24%–32% NDF</td>
</tr>
<tr>
<td>Broderick et al., 2002</td>
<td>Dried Citrus Pulp</td>
<td>RCB(^7) 12-wk period</td>
<td>n=48 Parity&gt;1 &gt;66 DIM</td>
<td>60:40 F:C 83:17 AS: Grass silage Dry cracked corn 0 &amp; 19% DCP(^8)</td>
<td>19% CP 22% Forage NDF 26 &amp; 28% NDF</td>
</tr>
<tr>
<td>Ipharraguerre et al., 2002</td>
<td>Pelleted Soyhulls</td>
<td>5x5 LS 21-d periods</td>
<td>n=15 &gt;112 DIM Parity&gt;1</td>
<td>46:54 F:C 50:50 CS: AS Dry ground corn 0, 10, 20, 30, &amp; 40% SH(^9)</td>
<td>16% CP 19% Forage NDF 29–49% NDF</td>
</tr>
<tr>
<td>Stone, 1996</td>
<td>Soyhulls</td>
<td>RCB(^7) 14-wk period</td>
<td>n=63 Parity&gt;1 10-108 DIM</td>
<td>52:48 F:C 50:50 CS: AS HM corn 0 &amp; 14% SH</td>
<td>18-19% CP 21% Forage NDF 29 &amp; 36% NDF</td>
</tr>
<tr>
<td>Batajoo &amp; Shaver, 1994</td>
<td>WM(^10) BDG(^1) Soyhulls</td>
<td>4x4 LS 28-d periods</td>
<td>n=8 &gt;63 DIM Parity&gt;1</td>
<td>48:52 F:C Alfalfa silage Dry ground corn 0-10% WM 3%-20% BDG 0-9% SH</td>
<td>19-20% CP 21% Forage NDF 28%–43% NDF</td>
</tr>
</tbody>
</table>

\(^1\)Latin square. \(^2\)Days in milk. \(^3\)Forage:concentrate ratio. \(^4\)Corn silage. \(^5\)Alfalfa silage. \(^6\)Beet pulp. \(^7\)Randomized complete block. \(^8\)Dried citrus pulp. \(^9\)Soyhulls. \(^10\)Wheat midds. \(^11\)Brewers dried grains.

Voelker and Allen (2003) fed mid-lactation cows diets containing 35, 31, 27 and 18% starch (DM basis); high-moisture corn was replaced by 6, 12 and 24% pelleted beet pulp (DM basis) to formulate diets with decreasing starch content. Effects of decreasing dietary starch content were linear (P < 0.05) for dry matter intake (DMI) and quadratic (P < 0.07 and 0.03, respectively) for FCM and fat yields. Relative to the average for the 27 and 31% starch diets, feeding the 18% starch diet reduced DMI, FCM yield and fat yield by 9%, 4% and 5%, respectively; true protein (TP) content and yield were numerically reduced by 4% and 5%, respectively.

Broderick et al. (2002) fed mid lactation cows diets containing 31 or 20% starch (DM basis); dry cracked corn was replaced by 19% dried citrus pulp (DM basis) to formulate the low-starch diet. Feeding the low starch diet reduced DMI (P < 0.02), milk yield (P < 0.02), fat yield (P < 0.03), TP content (P < 0.01) and TP yield (P < 0.01) by 8%, 11%, 14%, 4% and 20%, respectively.

Ipharraguerre et al. (2002) fed mid-lactation cows diets containing 28, 23, 17, 13 and 7% starch (DM basis); dry ground corn was replaced by 10, 20, 30 and 40% pelleted soyhulls (DM basis) to formulate diets with decreasing starch content. Decreasing dietary starch content decreased linearly DMI (P < 0.06) by 7% and increased linearly fat content (P < 0.004) and fat yield (P < 0.001) by 8% and 10%, respectively. Yield of TP tended (P < 0.09) to be reduced by 5% for the lowest-starch diet relative to the 28%-starch diet. There were no differences between the 17%- and 23%-starch diets. Comparing the average of the 7%- and 13%-starch diets to the average of the 17%- , 23%- and 28%-starch diets, feeding the low starch diets numerically reduced DMI, milk yield and TP yield by 6%, 3% and 3%, respectively; milk fat content and yield were each numerically increased by 8%. Stone (1996) reported no differences between 25%- and 16%-starch diets fed to early lactation cows with high-moisture corn being replaced by 19% soyhulls (DM basis) to formulate the low-starch diet.
Batajoo and Shaver (1994) fed mid-lactation cows diets containing 30, 26, 21, and 15% starch (DM basis); dry ground corn was replaced by 0-10% wheat midds, 3-20% brewers dried grains and 0-9% soyhulls (DM basis) to formulate diets with decreasing starch content. Decreasing dietary starch content decreased (P < 0.05) linearly DMI, TP content and TP yield by 7%, 4% and 6%, respectively, and increased (P < 0.05) linearly fat content by 3%. Adverse effects of low starch diets on DMI, TP content and TP yield were more apparent for the 15%-starch diet than the 21%-starch diet. Staples (2007), from a review of 14 trials with lactating dairy cows where corn gluten feed partially replaced grains, protein meals or forages with dietary starch concentrations ranging across the trials from 15% to 40% (DM basis), concluded that 21%-starch diets may be acceptable. The cost of using digestible NDF or pectin from byproduct feeds to partially replace starch from corn grain needs to be evaluated for the various high-fiber byproduct sources on a local basis relative to corn and protein supplement prices.

Table 2. Literature review on partial replacement of starch from corn grain with digestible NDF or pectin from byproduct feeds (Lactation performance).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Diet Starch DM basis</th>
<th>DMI kg/d</th>
<th>Milk kg/d</th>
<th>FCM kg/d</th>
<th>Fat %</th>
<th>Fat kg/d</th>
<th>TP' %</th>
<th>TP kg/d</th>
<th>FCM/DMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voelker and Allen, 2003</td>
<td>35%</td>
<td>24.8</td>
<td>36.4</td>
<td>37.4</td>
<td>3.72</td>
<td>3.94</td>
<td>3.21</td>
<td>1.13</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>31%</td>
<td>25.0</td>
<td>36.6</td>
<td>38.4</td>
<td>3.84</td>
<td>4.04</td>
<td>3.81</td>
<td>1.15</td>
<td>1.54</td>
</tr>
<tr>
<td></td>
<td>27%</td>
<td>25.1</td>
<td>35.9</td>
<td>38.0</td>
<td>3.90</td>
<td>3.96</td>
<td>3.22</td>
<td>1.15</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>18%</td>
<td>22.9</td>
<td>35.4</td>
<td>36.8</td>
<td>3.81</td>
<td>3.83</td>
<td>3.10</td>
<td>1.09</td>
<td>1.62</td>
</tr>
<tr>
<td>Broderick et al., 2002</td>
<td>28%</td>
<td>23.8</td>
<td>29.5</td>
<td>29.0</td>
<td>3.60</td>
<td>3.99</td>
<td>3.17</td>
<td>0.93</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>31%</td>
<td>20.9</td>
<td>33.6</td>
<td>32.7</td>
<td>3.25</td>
<td>3.11</td>
<td>2.96</td>
<td>1.00</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>19.2</td>
<td>29.9</td>
<td>28.2</td>
<td>3.40</td>
<td>0.98</td>
<td>2.85</td>
<td>0.80</td>
<td>1.47</td>
</tr>
<tr>
<td>Ipharraguerre et al., 2002²</td>
<td>28%</td>
<td>24.8</td>
<td>29.3</td>
<td>29.0</td>
<td>3.61</td>
<td>1.00</td>
<td>3.15</td>
<td>0.92</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>23%</td>
<td>24.4</td>
<td>29.9</td>
<td>30.1</td>
<td>3.67</td>
<td>1.06</td>
<td>3.18</td>
<td>0.92</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>17%</td>
<td>22.9</td>
<td>29.3</td>
<td>30.7</td>
<td>3.93</td>
<td>1.11</td>
<td>3.12</td>
<td>0.92</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>13%</td>
<td>22.7</td>
<td>28.3</td>
<td>29.7</td>
<td>3.91</td>
<td>1.08</td>
<td>3.13</td>
<td>0.88</td>
<td>1.25</td>
</tr>
<tr>
<td>P &lt; 0.05</td>
<td>L³</td>
<td>Q⁴</td>
<td>Q</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stone, 1996¹</td>
<td>25%</td>
<td>20.7</td>
<td>40.7</td>
<td>41.2</td>
<td>3.58</td>
<td>1.45</td>
<td>2.92</td>
<td>1.19</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>16%</td>
<td>22.6</td>
<td>41.7</td>
<td>41.9</td>
<td>3.56</td>
<td>1.48</td>
<td>2.89</td>
<td>1.20</td>
<td>1.86</td>
</tr>
<tr>
<td>P &lt; 0.10</td>
<td>L²</td>
<td>L²</td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batajoo and Shaver, 1994</td>
<td>30%</td>
<td>27.6</td>
<td>40.2</td>
<td>35.5</td>
<td>3.24</td>
<td>1.29</td>
<td>3.07</td>
<td>1.23</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>26%</td>
<td>27.2</td>
<td>39.7</td>
<td>35.6</td>
<td>3.30</td>
<td>1.32</td>
<td>3.02</td>
<td>1.20</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>21%</td>
<td>26.7</td>
<td>39.7</td>
<td>35.9</td>
<td>3.33</td>
<td>1.33</td>
<td>3.02</td>
<td>1.20</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td>15%</td>
<td>25.8</td>
<td>39.5</td>
<td>35.6</td>
<td>3.35</td>
<td>1.32</td>
<td>2.95</td>
<td>1.16</td>
<td>1.53</td>
</tr>
<tr>
<td>P &lt; 0.05</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹True protein. ²Diet starch concentrations calculated using book values for ingredient starch concentrations. ³Linear effect. ⁴Quadratic effect.

Partial Replacement of Corn Starch with Sugars

Summarized in Tables 3 and 4 are the results from three dose response trials on partial replacement of corn starch with sugars. Dry matter intake and lactation performance parameters were highest for diets containing 6%, 5% and 8% total sugar (DM basis) in trials using dried molasses, liquid molasses and sucrose supplements, respectively, to partially replace corn starch. Increasing total diet sugar to these concentrations coincided with reductions in dietary starch concentrations of 5%-units on average (DM basis). Thus, sugars can reduce the percentage of corn needed in high-starch diets or
increase the “corn equivalency” of low-starch diets by about 7%-units (DM basis). Supplemental sugar options include dry and liquid molasses, sucrose, whey, bakery waste, citrus pulp and beet pulp. The cost of supplementing sugar to partially replace starch from corn grain needs to be evaluated for these various sugar sources on a local basis relative to corn prices.

Table 3. Literature review on partial replacement of corn starch with sugars (Test feeds, design, cows, and diet ingredient and nutrient composition).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Test Feeds</th>
<th>Trial Design</th>
<th>Cows</th>
<th>Diet Ingredient Composition (DM basis)</th>
<th>Diet Nutrient Composition (DM basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broderick &amp; Radloff, 2004</td>
<td>Dried Molasses RCB1</td>
<td>8-wk period</td>
<td>n=40</td>
<td>&gt;128 DIM2 Parity&gt;1</td>
<td>60:40 F:C 33:67 CS3: AS4 HM corn 0, 4, 8, &amp; 12% DMO5</td>
</tr>
<tr>
<td>Trial 1</td>
<td>Liquid Molasses RCB</td>
<td>8-wk period</td>
<td>n=48</td>
<td>Parity&gt;1 &gt;85 DIM</td>
<td>52:48 F:C 40:60 CS:AS HM corn 0, 3, 6, &amp; 9% LMO1</td>
</tr>
<tr>
<td>Broderick et al., 2000</td>
<td>Sucrose RCB</td>
<td>8-wk period</td>
<td>n=48</td>
<td>60:40 F:C 33:67 CS: AS HM corn &amp; starch 0, 2.5, 5.0, 7.5% sucrose</td>
<td>17% CP 23% Forage NDF 29% NDF</td>
</tr>
</tbody>
</table>

1Randomized complete block. 2Days in milk. 3Forage:concentrate ratio. 4Corn silage. 5Alfalfa silage. 6Dried molasses. 7Liquid molasses.

Table 4. Literature review on partial replacement of corn starch with sugars (Lactation performance).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Diet Starch, Sugar (DM basis)</th>
<th>DMI kg/d</th>
<th>Milk kg/d</th>
<th>FCM kg/d</th>
<th>Fat %</th>
<th>Fat kg/d</th>
<th>TP1 %</th>
<th>TP kg/d</th>
<th>FCM/DMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broderick &amp; Radloff, 2004</td>
<td>32%, 3%</td>
<td>25.3</td>
<td>38.0</td>
<td>41.2</td>
<td>4.00</td>
<td>1.52</td>
<td>3.10</td>
<td>1.19</td>
<td>1.63</td>
</tr>
<tr>
<td>Trial 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &lt;</td>
<td>0.05</td>
<td>0.04</td>
<td>0.07</td>
<td>0.07</td>
<td>0.02</td>
<td>0.05</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Broderick &amp; Radloff, 2004</td>
<td>31%, 3%</td>
<td>25.4</td>
<td>43.6</td>
<td>46.0</td>
<td>3.67</td>
<td>1.65</td>
<td>2.96</td>
<td>1.32</td>
<td>1.79</td>
</tr>
<tr>
<td>Trial 2</td>
<td></td>
<td>28.1</td>
<td>45.5</td>
<td>46.7</td>
<td>3.74</td>
<td>1.67</td>
<td>3.21</td>
<td>1.43</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26.1</td>
<td>44.0</td>
<td>44.0</td>
<td>3.54</td>
<td>1.55</td>
<td>3.12</td>
<td>1.37</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26.8</td>
<td>42.4</td>
<td>42.4</td>
<td>3.72</td>
<td>1.52</td>
<td>3.13</td>
<td>1.29</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>C</td>
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<td></td>
</tr>
<tr>
<td>Broderick et al., 20005</td>
<td>32%, 3%</td>
<td>24.5</td>
<td>38.9</td>
<td>40.5</td>
<td>3.81</td>
<td>1.47</td>
<td>3.24</td>
<td>1.24</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.6</td>
<td>40.4</td>
<td>42.2</td>
<td>3.82</td>
<td>1.53</td>
<td>3.22</td>
<td>1.28</td>
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<td></td>
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<td>26.0</td>
<td>40.0</td>
<td>43.9</td>
<td>4.07</td>
<td>1.65</td>
<td>3.27</td>
<td>1.29</td>
<td>1.69</td>
</tr>
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<td></td>
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<td>26.0</td>
<td>39.4</td>
<td>43.2</td>
<td>4.16</td>
<td>1.62</td>
<td>3.30</td>
<td>1.28</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>0.01</td>
<td>0.11</td>
<td>0.01</td>
<td>0.01</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1True protein. 2Linear effect. 3Cubic effect. 4Quadratic effect.
5Diet starch concentrations calculated using book values for ingredient starch concentrations.

The Role of Corn Silage

Corn silage (CS) contains about 25%-units more starch (DM basis) than alfalfa silage (AS; Dairyland Labs, 2007; NRC, 2001). Thus, increasing the forage mixture from 1/3rd:2/3rd to 2/3rd:1/3rd CS:AS in a diet with 50% forage will increase the dietary starch content by about 4%-units in low-starch
diets or can reduce the percentage of corn needed in high-starch diets by about 6%-units (DM basis). Data on corn silage starch concentrations (Dairyland Labs, 2007) for 2002-2006 are presented in Table 5. Across the five years, corn silage starch content averaged 29.3% (DM basis) with an average within year standard deviation of 7.1% (DM basis). Thus, for 2/3rd of corn silage samples the starch content fell between 22% and 36% (DM basis). Feeding the high-starch corn silage versus the low-starch corn silage in diets containing 34% corn silage (DM basis; 50% forage diet with 2/3rd:1/3rd CS:AS) will increase the dietary starch content by about 5%-units in low-starch diets or can reduce the percentage of corn needed in high-starch diets by about 7%-units (DM basis).

Table 5. Corn silage starch data from Dairyland Laboratories, Inc. Arcadia, WI (Dairyland Labs, 2007).

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>Average</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>10,864</td>
<td>29.7</td>
<td>6.3</td>
</tr>
<tr>
<td>2005</td>
<td>13,452</td>
<td>29.6</td>
<td>7.3</td>
</tr>
<tr>
<td>2004</td>
<td>12,540</td>
<td>28.7</td>
<td>7.1</td>
</tr>
<tr>
<td>2003</td>
<td>12,804</td>
<td>28.5</td>
<td>7.2</td>
</tr>
<tr>
<td>2002</td>
<td>12,115</td>
<td>30.0</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Starch Digestibility

If low starch diets are fed, then it seems logical that the starch should be highly digestible. Total tract digestibility of starch by dairy cows is variable ranging from 70% to 100% (Firkins et al., 2001). Various factors, such as particle size (fine vs. coarse grind), grain processing (steam flaked vs. dry rolled), storage method (dry vs. high-moisture corn), moisture content of high-moisture corn, type of corn endosperm, and corn silage maturity and processing, influence the digestibility of starch by dairy cows (Firkins et al., 2001; Johnson et al., 1999; Nocek and Tamminga, 1991). The impact of the digestibility of corn grain starch on lactation performance by dairy cows as summarized by Firkins et al. (2001) is presented in Table 6. Based on regressions from the tabular data, increasing starch digestibility increased milk and protein yields ($R^2 = 0.89; P < 0.01$) and reduced milk fat percentage ($R^2 = 0.58; P < 0.05$) but not yield. When in concurrence with the feeding of low starch diets, however, increasing starch digestibility may not result in reduced milk fat percentage.

Table 6. Total tract starch digestibility and lactation performance by dairy cows fed different corn sources (adapted from Firkins et al., 2001).

<table>
<thead>
<tr>
<th>Corn</th>
<th>n</th>
<th>Starch Digestibility</th>
<th>Milk kg/d</th>
<th>Fat %</th>
<th>Fat kg/d</th>
<th>Protein %</th>
<th>Protein kg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry, cracked or rolled</td>
<td>9</td>
<td>85</td>
<td>30.9</td>
<td>3.59</td>
<td>1.11</td>
<td>3.09</td>
<td>0.96</td>
</tr>
<tr>
<td>Steam-rolled</td>
<td>10</td>
<td>89</td>
<td>31.9</td>
<td>3.49</td>
<td>1.11</td>
<td>3.10</td>
<td>0.98</td>
</tr>
<tr>
<td>Dry, ground</td>
<td>13</td>
<td>91</td>
<td>31.5</td>
<td>3.53</td>
<td>1.11</td>
<td>3.18</td>
<td>1.00</td>
</tr>
<tr>
<td>Dry, ground finely</td>
<td>3</td>
<td>91</td>
<td>32.4</td>
<td>3.49</td>
<td>1.13</td>
<td>3.02</td>
<td>0.98</td>
</tr>
<tr>
<td>Steam-flaked</td>
<td>10</td>
<td>94</td>
<td>32.5</td>
<td>3.36</td>
<td>1.09</td>
<td>3.10</td>
<td>1.01</td>
</tr>
<tr>
<td>High-moisture, rolled</td>
<td>3</td>
<td>94</td>
<td>32.5</td>
<td>3.54</td>
<td>1.15</td>
<td>3.17</td>
<td>1.03</td>
</tr>
<tr>
<td>High-moisture, ground</td>
<td>2</td>
<td>99</td>
<td>33.9</td>
<td>3.37</td>
<td>1.14</td>
<td>3.17</td>
<td>1.08</td>
</tr>
</tbody>
</table>

1 Number of treatment means across studies summarized by Firkins et al., 2001.
2 Least squares means reported by Firkins et al., 2001.
3 Calculated from milk yield and composition least squares means reported by Firkins et al., 2001.

Ionophore

The feeding of Rumensin® improves feed efficiency in lactating dairy cows and has been approved for use by the FDA. Calculations by Thomas (2006) suggest that feeding Rumensin increases diet energy density enough to provide a “corn equivalency” of 0.45 to 0.91 kg. The cost of feeding...
Rumensin at 300 mg/cow/d is $0.03-$0.04/cow/d versus the savings in reduced corn feeding of $0.07-$0.14/cow/d. Issues with milk fat test depression when feeding Rumensin appear to be less of a concern with low-starch than high-starch diets (Thomas, 2006).

Conclusions

Lactation performance was reduced for 18% and 20% starch diets (DM basis) formulated using beet pulp and citrus pulp, respectively, to partially replace corn grain. Lactation performance was not reduced for 16%-17% starch diets (DM basis) formulated using soyhulls to partially replace corn grain. Diets containing 21% starch (DM basis) appear to be acceptable when high-fiber, moderate protein byproduct feeds are used to partially replace corn grain and protein supplement. Feeding corn silage at a higher percentage of the forage, high starch corn silage, sugars and ionophore can reduce the percentage of corn needed in high-starch diets or increase the “corn equivalency” of low-starch diets. The starch in low-starch diets should be highly digestible.

References

Summary

Feeding dairy cows in excess of their nutrient requirements for nitrogen is no longer an acceptable practice. Since the ruminant animal is not very efficient in utilizing nitrogen, overfeeding results in a substantial amount being excreted in manure. The excess nitrogen excreted can result in both water and air pollution. With current and pending regulations regarding the Clean Water Act and the Clean Air Act, a proactive approach is needed to maintain animal performance while minimizing environmental concerns.

Numerous feeding strategies have been evaluated at the Penn State Dairy Teaching and Research Complex with the focus on improving nitrogen efficiency. Forage quality, various forage rations, high inclusion levels of forages, and nutritional focuses on metabolizable protein and carbohydrate sources have been examined. Their affect on milk urea nitrogen, milk nitrogen efficiency, animal performance and income over feed costs have been documented. In the past five years diet protein levels have been maintained between 15 to 16.5% while successfully maintaining animal performance and exceeding the benchmarks for income over feed costs. The key to successfully balancing rations for the cow’s nitrogen requirement is maintaining the basics regarding feeding management coupled with good nutrition.

Introduction

Feeding dairy cows in excess of their nutrient requirements, especially phosphorus and nitrogen, is no longer an acceptable practice. Since the ruminant animal is not very efficient, a substantial amount of these nutrients are excreted in manure. Competition of valuable cropland is leading the dairy industry towards consolidation of large number of animals onto smaller land areas. This has resulted in manure applications occurring on less land increasing the nutrient loads on farms. Regulations at the state and federal level are in place to manage nutrients as it relates to water and air quality. Feeding strategies that can reduce nutrient intake without impeding animal performance are needed to comply with environmental concerns.

Nitrogen (N) is the primary nutrient of concern and the most complex for both the animal and the environment. From the animal perspective it is an important nutrient for maintenance and production. Protein tends to be overfed in rations either deliberately through ration formulation or due to inadequate monitoring of feed management practices. Protein nutrition is challenging because there are various N fractions, especially with ensiled feeds that add complexity when formulating rations and balancing them with carbohydrates. Excess protein fed results in increased N excretion. This is both an air and water quality concern.

Another concern is related to the large amounts of N that can be brought onto the farm in the form of purchased feeds. The problem is that much of the N remains on the farm rather than being incorporated into milk, animal tissue and crops. The end result is an animal operation that is out of nutrient balance. There are several strategies to improve a farm’s nutrient balance. A key factor is improving forage quality. This will allow more farm raised feeds to be fed and minimize the amount of purchased N.
Environmental Concerns

The first environmental issue with nitrogen is ammonia emissions. It can be released directly or indirectly from the degradation of proteins, which may occur within the soil or in the digestive system of the dairy cow and during manure storage. Ruminants excrete nitrogen in their urine and feces. The urea in urine in the presence of the enzyme urease found in fecal material, rapidly decomposes to form ammonia. Ammonia is a very reactive compound and atmospheric ammonia can negatively impact the environment through several pathways.

Ammonia deposition contributes an estimated 35 to 60% of the total nitrogen load to coastal waters (Paerl, 1995). Ammonia deposition can result in excessive build up of nitrogen in soil, leading to crop damage in sensitive plants and soil acidification as ammonia is converted to nitrate. Finally ammonia contributes to the formation of fine airborne particles or liquid droplets, called particulate matter. Particulate matter of this size can penetrate deep into the lungs, contributing to respiratory disease and contributes to haze formation that reduces visibility. Ammonia and PM$_{2.5}$ are air quality concerns. They are regulated under the Clean Air Act, the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and the Emergency Planning and Community Right-To-Know Act (EPCRA).

The second environmental issue with nitrogen is water quality. Eutrophication is the slow, natural nutrient enrichment of streams and lakes and is responsible for the "aging" of ponds, lakes, and reservoirs. Excessive amounts of nutrients, especially nitrogen and phosphorus, speed up the eutrophication process. As algae grow and then decompose they deplete the dissolved oxygen in the water. This condition usually results in fish kills, offensive odors, unsightliness, and reduced attractiveness of the water for recreation and other public uses. However, this condition occurs only when excessive nutrients are present; a certain amount of nitrogen and phosphorus is essential for any life to exist in water. Excessive nitrates (NO$_3$) in drinking water can cause human and animal health problems.

The regulations and environmental issues related to excess nutrients is real. Dairy producers are faced with implementing whole farm strategies that address these concerns. However, practical solutions are needed in order for the dairy industry to survive in the northeast. It is possible to adjust silage based feeding systems to improve nitrogen efficiency of the dairy cow as well as maintain milk volume, components and profitability.

Feeding Strategies

There are numerous feeding strategies that can be implemented to improve nutrient efficiency. Improving and maintaining high quality forage is the key to developing a sound ration program. Forage quality and how animals perform on those forages is more than just entering a few numbers in a ration formulation program. How the dairy cow utilizes ensiled forages is influenced by crop growing environment, cutting date, moisture content and management practices at harvest, storage and feed-out (mycotoxins and spoilage problems). In addition to these factors, the cow’s size, amount of dry matter consumed and the amount of forage in the diet affect rate of passage and digestibility of the forage. Emphasis is always placed on how forage nutrients will be utilized in the rumen environment, however post ruminal digestion should not be overlooked as a critical component in dairy nutrition.

Several feeding strategies have been evaluated at the Penn State Dairy Complex over the past years. A main goal of the research program is to simultaneously conduct intensive small scale studies along with an applied large scale study. This approach provides meaningful information to answer basic research questions as well as how likely a nutritional concept could work in the field. With the emphasis being on N efficiency, the question has been “Can lower protein diets be fed on various forage diets while improving nitrogen efficiency and maintaining or improving animal performance?” The various feeding rates of carbohydrates and protein were taken into consideration when diets were formulated. NRC 2001 and CPM were the models used to evaluate rations.
Feeding Strategy 1 – Heavy Corn Silage Ration

Several studies have been conducted evaluating forage source and reduced protein feeding. The advancement of corn hybrids for silage has allowed the successful feeding of heavy corn silage based diets, where corn silage makes up the majority of forage dry matter (Bal et al., 2000; Dhiman et al., 2000; Onetti et al., 2003). Wattiaux et al. (2004) evaluated varying protein levels in alfalfa and corn based diets. They observed improved milk production when corn silage based rations were fed compared to the alfalfa based diets and observed no production difference by cows fed the lower protein diets (formulated for rumen degradable protein (RDP) and rumen unavailable protein (RUP)). Based on the diversity of the studies and the various incorporation of hybrid type, particle size, and ration components; milk components, especially fat percent, tend to be reduced (less than 3.5%). The feeding strategy of a high corn silage based ration and reduced protein level was initiated in the fall of 2002 at the Penn State Dairy Complex. The objective was to determine if milk yield and components would be enhanced or maintained on rations formulated with reduced protein.

Historically, rations for the Penn State Dairy Herd had been formulated to the industry standard of 17.5 to 18% crude protein on a dry matter basis for a one-group total mixed ration. The average production of the herd when fed the higher protein diet was 76 to 79 pounds on a 3.5% fat corrected basis. When the ration was adjusted to a lower protein level, other changes also occurred. The corn silage was processed to reduce the large corn cobs and cottonseed hulls were added to provide fiber and replace four pounds of western hay. Table 1 shows the ration formulation for the 18% and 16% protein diets. To complement this feeding scenario coarsely ground corn grain was used. Protein sources with a balance of RDP and RUP were used.

Table 1. Ration formulation for the Penn State dairy herd fed an 18% and 16% protein diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>2001-2002 18%</th>
<th>2002-2003 16%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>25.6</td>
<td>26.5</td>
</tr>
<tr>
<td>Alfalfa silage</td>
<td>14.8</td>
<td>14.6</td>
</tr>
<tr>
<td>Hay</td>
<td>9.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>-</td>
<td>6.7</td>
</tr>
<tr>
<td>Shelled corn, coarse ground</td>
<td>14.2</td>
<td>20.3</td>
</tr>
<tr>
<td>Cookie meal</td>
<td>6.8</td>
<td>6.8</td>
</tr>
<tr>
<td>Liquid sugar</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Distillers grain</td>
<td>5.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Wheat midds</td>
<td>4.9</td>
<td>-</td>
</tr>
<tr>
<td>Heat treated soybean meal</td>
<td>4.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Canola meal</td>
<td>4.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Fish meal</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>Roasted soybeans</td>
<td>4.6</td>
<td>6.0</td>
</tr>
<tr>
<td>Min-vitamin mix</td>
<td>1.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

The release of the 2001 NRC has given dairy cattle nutritionists an improved scientific template for designing rations. Balancing for crude protein is becoming an outdated concept. A more precise measure of protein nutrition is formulating for metabolizable protein (MP), RDP and RUP. Metabolizable protein is the true protein that is digested post ruminally and the component amino acids are absorbed by the small intestine. The RDP is the protein broken down in the rumen to microbial protein. The protein that escapes the rumen is the RUP.

In addition to protein, the source and types of carbohydrates are just as important. The balance between sugar, starch and soluble fiber is essential for a healthy rumen. Table 2 presents the nutrient specifications for the 18% and 16% protein diets. Formulating rations for protein and carbohydrate
fractions to improve N efficiency is an important concept, however, what is an achievable goal and is it economical? Feeding strategies that improve nutrient efficiency are more than likely to happen if there is a positive economic incentive. A tool that is readily available to producers to monitor the efficiency of feed N utilization by dairy cattle is milk urea nitrogen (MUN). Several researchers have developed calculations that determine N efficiency. Jonker et al., (2002) developed and evaluated a model to estimate N excretion, N intake and N utilization efficiency for lactating dairy cows. Another tool is the Milk Nitrogen Efficiency calculation (Chase, 2007).

Table 2. Nutrient profile of the 18% and 16% protein diets.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18%</td>
<td>16%</td>
</tr>
<tr>
<td><strong>Dry matter basis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Protein profile</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP, lb/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required</td>
<td>5.71</td>
<td>5.22</td>
</tr>
<tr>
<td>Supplied</td>
<td>6.18</td>
<td>5.65</td>
</tr>
<tr>
<td>RDP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of diet DM</td>
<td>11.1</td>
<td>10.3</td>
</tr>
<tr>
<td>Supplied, lb/day</td>
<td>6.02</td>
<td>5.64</td>
</tr>
<tr>
<td>Balance, lb/day</td>
<td>+0.66</td>
<td>+0.25</td>
</tr>
<tr>
<td>RUP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of diet DM</td>
<td>6.9</td>
<td>5.7</td>
</tr>
<tr>
<td>Supplied, lb/day</td>
<td>3.74</td>
<td>3.11</td>
</tr>
<tr>
<td>Balance, lb/day</td>
<td>+0.59</td>
<td>-0.09</td>
</tr>
<tr>
<td><strong>Amino Acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine, % of MP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001-2002</td>
<td>6.17</td>
<td>6.42</td>
</tr>
<tr>
<td>2002-2003</td>
<td>1.81</td>
<td>1.89</td>
</tr>
<tr>
<td>Lys/Met ratio</td>
<td>3.41</td>
<td>3.40</td>
</tr>
<tr>
<td>MP required (lbs./day)</td>
<td>5.71</td>
<td>5.72</td>
</tr>
<tr>
<td>MP supplied (lbs./day)</td>
<td>6.18</td>
<td>5.65</td>
</tr>
<tr>
<td>RDP (lbs./day)</td>
<td>6.02</td>
<td>5.64</td>
</tr>
<tr>
<td>RUP (lbs./day)</td>
<td>3.74</td>
<td>3.11</td>
</tr>
<tr>
<td>Balance RDP (lbs./day)</td>
<td>+0.66</td>
<td>+0.25</td>
</tr>
<tr>
<td>Balance RUP (lbs./day)</td>
<td>+0.59</td>
<td>-0.09</td>
</tr>
<tr>
<td><strong>Carbohydrate profile</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001-2002</td>
<td>7.4</td>
<td>6.8</td>
</tr>
<tr>
<td>2002-2003</td>
<td>26.5</td>
<td>29.7</td>
</tr>
<tr>
<td>Starch, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001-2002</td>
<td>6.6</td>
<td>4.7</td>
</tr>
<tr>
<td>2002-2003</td>
<td>3.2</td>
<td>3.1</td>
</tr>
<tr>
<td>Soluble fiber, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001-2002</td>
<td>31.0</td>
<td>31.4</td>
</tr>
<tr>
<td>2002-2003</td>
<td>43.8</td>
<td>44.3</td>
</tr>
</tbody>
</table>

1Protein profile based on the 2001 NRC. MP=metabolizable protein; RDP=rumen degradable protein; RUP=rumen undegradable protein; CP=crude protein.

2Carbohydrate profile based on CPM dairy ration analyzer.
Cows improved in milk N efficiency (Chase, 2007) when comparing the average values for the herd on the 18% to the 16% protein diets. It should be noted that when the 18% ration was fed the calculated milk N efficiency was 23.7%, which reflects some opportunities for improvement. The 16% rations had a milk N efficiency of 27%, which is average. The key to achieving improved N efficiencies is feeding cows closer to their requirement for protein, improved milk production and milk protein, and lower MUNs.

**Feeding Strategy 2 – Alfalfa and Grass Silage Based Rations**

The chemical composition of grass and legume are distinctively different. Crude protein content is generally lower for grasses than legumes; however the composition of the crude protein differs. Grasses contain more non-protein nitrogen in soluble protein and legumes contain more amino acids or peptides in soluble crude protein (Glenn et al., 1989). Feeding alfalfa silage as the sole forage for ruminants often results in diets with excessive protein that is poorly utilized. Among the strategies that have been applied to dilute alfalfa crude protein have been to partially replace dietary alfalfa with corn silage for lactating cows. As the soluble nitrogen load is increased from legume sources this additional nitrogen load on the kidneys increases the energy needs of the cow. The added metabolic costs to the animal, inefficient capture of nitrogen as ammonia in the rumen, and the inefficient use of this nitrogen results in greater excretion of nitrogen.

The effect of cereal grain processing on starch fermentability in the rumen has been reviewed (Owens et al., 1997; Theurer, 1986; Yang et al., 2000). Matching ruminal energy fermentation with the various protein fractions can be effective in improving nitrogen efficiency. There are substantial differences among starch sources (Herrera-Saldana et al., 1990) and within grains due to processing, in the rates of energy release in the rumen. Owens et al., (1986) showed that the effects of processing on extent of ruminal digestion of corn starch are much greater than the effects on total tract digestibility. Rumen digestibility of starch decreased from 70% with ground corn to 54% with coarsely rolled corn. Small intestine digestibility of starch was not significantly affected by corn particle size and the amount of starch digested in the small intestine tended to be greater for rolled than ground corn (Remond et al., 2003). Starch digestion in the small intestine has been shown to be energetically more efficient than ruminally fermented starch (Harmon and McLeod, 2001).

Brito and Broderick (2003) assessed the effects of step-wise replacement of alfalfa silage with corn silage. The greatest improvement in nitrogen efficiency, without loss of production of milk, fat and protein, occurred at about 50% of the forage from alfalfa silage and 50% from corn silage. Additionally, replacing some of the dietary starch with rapidly fermenting sugars has been shown to enhance ruminal capture of degraded nitrogen. Another aspect to evaluate is how the balance of forage sources along with balancing carbohydrate and protein sources will affect daily ammonia emissions.

In a 2005 Penn State study we evaluated animal performance and monitored ammonia emissions on alfalfa and grass silage based rations containing either finely ground or coarsely ground corn (Table 3). The objective was to formulate protein levels close to animal requirement and adjust the particle size of corn grain to evaluate effects on milk volume, milk components, N efficiency and ammonia emitted.
Table 3. Ration formulas for the alfalfa and grass based silage rations.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Alfalfa silage based</th>
<th>Grass silage based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa silage</td>
<td>16.5</td>
<td>---</td>
</tr>
<tr>
<td>Grass silage</td>
<td>---</td>
<td>13.4</td>
</tr>
<tr>
<td>Corn silage</td>
<td>16.5</td>
<td>13.4</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>5.7</td>
<td>0.45</td>
</tr>
<tr>
<td>Shelled corn (fine or coarse)</td>
<td>11.1</td>
<td>11.8</td>
</tr>
<tr>
<td>Cookie meal</td>
<td>1.13</td>
<td>1.37</td>
</tr>
<tr>
<td>Liquid sugar (dextrose)</td>
<td>3.0</td>
<td>2.45</td>
</tr>
<tr>
<td>Canola meal</td>
<td>1.95</td>
<td>1.22</td>
</tr>
<tr>
<td>Roasted soybeans</td>
<td>6.1</td>
<td>4.95</td>
</tr>
<tr>
<td>Heat treated soybean meal</td>
<td>2.0</td>
<td>2.35</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>2.14&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.19&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total dry matter intake&lt;sup&gt;3&lt;/sup&gt;</td>
<td>66.1</td>
<td>53.6</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mineral mix contains distiller’s grain as the carrier.
<sup>2</sup> Mineral mix contains distiller’s grain as the carrier and urea.
<sup>3</sup> Actual average dry matter intakes after subtracting refusals.

Diets for the alfalfa and grass silage based rations were formulated for similar nutrient densities (Table 4). In order to achieve similar fiber levels in the diet, cottonseed hulls were used at a higher inclusion level for the alfalfa silage based ration compared to the grass ration. Dry matter intakes were greater on the alfalfa diet vs. the grass diet. Some possible explanations for the higher intakes include the level of cottonseed hulls. Both alfalfa and cottonseed hulls have a high lignin content (rumen unavailable fiber). This tends to allow less rumen fill and increased dry matter intakes. Morales et al. (1989) showed that feeding cottonseed hulls increased voluntary NDF intake as a percentage of bodyweight to 1.4 to 1.5%. This same trend was evident for the PSU study. Both high levels of milk production and components were achieved while maintaining dry matter intake efficiencies around 1.5 (Table 5). Using the Penn State Particle Size Separator, the average particle size distribution of the alfalfa and grass TMRs respectively were, upper: 9%, 24%; middle: 46%, 30%, bottom: 36%, 37% and pan: 9%, 10%.
Table 4. Ration evaluation for the alfalfa and grass silage based diets.

<table>
<thead>
<tr>
<th></th>
<th>Alfalfa silage-based TMR</th>
<th>Grass silage-based TMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry matter basis</td>
<td></td>
</tr>
<tr>
<td><strong>DMI, lb/d</strong></td>
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<td></td>
</tr>
<tr>
<td>Actual</td>
<td>66.0</td>
<td>53.6</td>
</tr>
<tr>
<td>Predicted</td>
<td>61.7</td>
<td>56.9</td>
</tr>
<tr>
<td><strong>Protein profile</strong>^1</td>
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<td></td>
</tr>
<tr>
<td>Diet, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>16.6</td>
<td>16.5</td>
</tr>
<tr>
<td>RDP</td>
<td>10.0</td>
<td>10.1</td>
</tr>
<tr>
<td>RUP</td>
<td>6.6</td>
<td>6.5</td>
</tr>
<tr>
<td><strong>MP, lb/day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required</td>
<td>6.53</td>
<td>5.64</td>
</tr>
<tr>
<td>Supplied</td>
<td>7.16</td>
<td>5.98</td>
</tr>
<tr>
<td><strong>RDP, lb/day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplied</td>
<td>6.60</td>
<td>5.41</td>
</tr>
<tr>
<td>Balance</td>
<td>+0.27</td>
<td>+0.01</td>
</tr>
<tr>
<td><strong>RUP, lb/day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplied</td>
<td>4.33</td>
<td>3.46</td>
</tr>
<tr>
<td>Balance</td>
<td>+0.79</td>
<td>+0.36</td>
</tr>
<tr>
<td><strong>RDP (lbs./day)</strong></td>
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<td></td>
</tr>
<tr>
<td>Required</td>
<td>6.60</td>
<td>5.41</td>
</tr>
<tr>
<td>Supplied</td>
<td>7.16</td>
<td>5.98</td>
</tr>
<tr>
<td><strong>RUP (lbs./day)</strong></td>
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</tr>
<tr>
<td>Required</td>
<td>4.33</td>
<td>3.46</td>
</tr>
<tr>
<td>Supplied</td>
<td>-0.27</td>
<td>-0.01</td>
</tr>
<tr>
<td><strong>Balance RDP (lbs./day)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required</td>
<td>6.60</td>
<td>5.41</td>
</tr>
<tr>
<td>Supplied</td>
<td>7.16</td>
<td>5.98</td>
</tr>
<tr>
<td><strong>Balance RUP (lbs./day)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required</td>
<td>4.33</td>
<td>3.46</td>
</tr>
<tr>
<td>Supplied</td>
<td>-0.27</td>
<td>-0.01</td>
</tr>
<tr>
<td><strong>CP- RDP % dry matter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required</td>
<td>10.0</td>
<td>10.1</td>
</tr>
<tr>
<td>Supplied</td>
<td>6.6</td>
<td>6.5</td>
</tr>
<tr>
<td><strong>CP - % DM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required</td>
<td>16.6</td>
<td>16.5</td>
</tr>
<tr>
<td>Supplied</td>
<td>42.2</td>
<td>42.2</td>
</tr>
</tbody>
</table>

^1Protein profile based on the 2001 NRC. MP=metabolizable protein; RDP=rumen degradable protein; RUP=rumen undegradable protein; CP=crude protein.

The reduced dry matter intake on the grass based diet can also be explained. The grass silage, because it was ensiled very wet, had high levels of butyric acid. It had a less than ideal smell and palatability probably was an issue. The particle size distribution was much coarser compared to the alfalfa diet. Most of the particles in the top box of the Penn State Particle Separator were comprised of the long particles of grass silage. Because of its high moisture content, cows were not able to sort. Particle size and with grass containing higher levels of digestible NDF, rumen fill was probably an issue. Dry matter intake efficiencies tended to be better for the grass based diet vs. the alfalfa based.

The fine grind of the corn appeared to have no added benefit to the alfalfa ration compared to the grass where it appeared there was some benefit in improved components. Income over feed costs was better for the alfalfa based ration compared to the grass based ration (Table 5).
Table 5. Performance results and income over feed costs (IOFC) from the alfalfa and grass silage based TMRs.

<table>
<thead>
<tr>
<th>Month</th>
<th>Milk</th>
<th>Fat</th>
<th>Protein</th>
<th>ECM</th>
<th>DMI-Eff</th>
<th>IOFC</th>
<th>Corn Particle Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lbs</td>
<td>%</td>
<td>%</td>
<td>lbs</td>
<td>$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb</td>
<td>93.1</td>
<td>4.00</td>
<td>3.00</td>
<td>97.7</td>
<td>1.51</td>
<td>10.74</td>
<td>Fine grind</td>
</tr>
<tr>
<td>Mar</td>
<td>93.7</td>
<td>3.97</td>
<td>3.16</td>
<td>99.1</td>
<td>1.46</td>
<td>11.60</td>
<td>Coarse grind</td>
</tr>
<tr>
<td>Apr</td>
<td>95.6</td>
<td>3.83</td>
<td>2.98</td>
<td>98.1</td>
<td>1.53</td>
<td>10.95</td>
<td>Fine grind</td>
</tr>
<tr>
<td>May</td>
<td>103.2</td>
<td>3.94</td>
<td>2.94</td>
<td>107.1</td>
<td>1.76</td>
<td>12.37</td>
<td>Coarse grind</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Month</th>
<th>Milk</th>
<th>Fat</th>
<th>Protein</th>
<th>ECM</th>
<th>DMI-Eff</th>
<th>IOFC</th>
<th>Corn Particle Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lbs</td>
<td>%</td>
<td>%</td>
<td>lbs</td>
<td>$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb</td>
<td>83.2</td>
<td>4.00</td>
<td>3.00</td>
<td>87.3</td>
<td>1.66</td>
<td>10.09</td>
<td>Coarse grind</td>
</tr>
<tr>
<td>Mar</td>
<td>83.1</td>
<td>3.85</td>
<td>3.09</td>
<td>86.2</td>
<td>1.64</td>
<td>10.15</td>
<td>Fine grind</td>
</tr>
<tr>
<td>Apr</td>
<td>85.6</td>
<td>3.69</td>
<td>2.91</td>
<td>85.9</td>
<td>1.61</td>
<td>9.66</td>
<td>Coarse grind</td>
</tr>
<tr>
<td>May</td>
<td>85.4</td>
<td>4.10</td>
<td>3.26</td>
<td>92.3</td>
<td>1.71</td>
<td>10.74</td>
<td>Fine grind</td>
</tr>
</tbody>
</table>

Nitrogen utilization efficiency (Jonker et al., 2002) and milk nitrogen efficiency (Chase, 2007) were calculated for the grass and alfalfa silage based rations. These calculations were compared to results taken from an intensive study (milk N/N intake) conducted at the same time as the large scale study. The milk urea nitrogen averaged 10.3 mg/dl and 11 mg/dl over the four month period on the alfalfa and grass silage based diet, respectively. For the free-stall study, the resulting N utilization efficiency calculated was 38.0% and 34.6% for the alfalfa and grass based diets, respectively. The milk N efficiency calculated for the alfalfa and grass based diets was 29.1 and 31.2 respectively. The results of the milk N efficiency better matched the results from the intensive study. Also, the milk N efficiency calculation (Chase, 2007) showed a slight advantage on the grass based ration compared to the alfalfa, which was the opposite using the N utilization equation (Jonker et al., 2002). A slightly improved milk N efficiency was observed on both the hay-crop forage rations compared to the first feeding strategy on heavy corn silage. The bottom line is not to focus on which diet shows the best N efficiency, but how can profitability be maintained while optimizing N efficiency.

**Feeding Strategy 3 – Heavy Forage Based Ration**

In addition to enhancing N efficiency of the cow, the other critical component related to improving N balance on farm is minimizing the amount of nutrients brought on farm. This can be achieved by feeding more home-grown forages. The third strategy evaluated was feeding a heavy forage based ration consisting primarily of corn silage. Brown mid-rib (BMR) and conventional corn silage were compared at two different inclusion levels.

Much of the research into the effects of feeding BMR has looked at including BMR silage at less than 40% of the ration dry matter and has utilized corn as a source of carbohydrate (Ivan et al., 2004) (Weiss et al., 2006). In today’s grain market and increasing price of corn, there are other more economically feasible options such as bakery waste, molasses, distillers grains, etc. as well as increased forage levels in the diet that may influence the effectiveness and economics of BMR and/or conventional corn silage. Because carbohydrates can be absorbed post ruminally, the source of this energy may play an important role in the effectiveness of high forage diets at increasing milk production. The type and processing of corn grain fed with a high forage based ration may affect performance.
One hundred twenty cows of varying lactation stages were fed a conventional corn silage variety, or a BMR variety, sixty cows on each diet. For four months cows received a 58% forage ration where corn silage made up 35% of the ration dry matter. For the following two months (months 5 and 6) cows received the high forage ration where corn silage made up 50% of the total ration dry matter (Table 6). The metabolizable protein was supplied to the cow’s requirement and resulted in the crude protein of the ration at 16.2% (Table 7). Cows fed the high forage ration with corn silage at 35% showed very similar results in performance regardless which corn silage was fed. The BMR corn silage did show a slight advantage in dry matter intake efficiency and milk N efficiency. When corn silage made up 50% of the total ration, the BMR corn silage fed cows showed a 10% improvement in milk yield compared to the conventional. However, milk N efficiency was identical for both the BMR and conventional corn silage rations.

Table 6. Ration formulas for the heavy forage rations with corn silage (CS) at either 35% or 50% of the total ration dry matter.

<table>
<thead>
<tr>
<th>58% Forage Ration (CS at 35% of DM)</th>
<th>58% Forage Ration (CS at 50% of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lbs.</td>
<td>lbs.</td>
</tr>
<tr>
<td>BMR/Conv CS-39.5%DM</td>
<td>BMR/Conv CS-39.5%DM</td>
</tr>
<tr>
<td>Grass hay/straw</td>
<td>Grass Hay/Straw</td>
</tr>
<tr>
<td>4.75</td>
<td>4.62</td>
</tr>
<tr>
<td>BAG 1 HLG-33.3%DM</td>
<td>Ground Corn-PSU</td>
</tr>
<tr>
<td>8.68</td>
<td>4.00</td>
</tr>
<tr>
<td>Ground Corn-PSU</td>
<td>Cookie Meal</td>
</tr>
<tr>
<td>5.93</td>
<td>4.00</td>
</tr>
<tr>
<td>Cookie Meal</td>
<td>Sugar</td>
</tr>
<tr>
<td>4.00</td>
<td>2.52</td>
</tr>
<tr>
<td>Sugar</td>
<td>Soybeans Cooked</td>
</tr>
<tr>
<td>2.52</td>
<td>4.51</td>
</tr>
<tr>
<td>Soybeans Cooked</td>
<td>Canola Meal</td>
</tr>
<tr>
<td>4.45</td>
<td>4.94</td>
</tr>
<tr>
<td>Canola Meal</td>
<td>Turbo Meal</td>
</tr>
<tr>
<td>3.23</td>
<td>2.30</td>
</tr>
<tr>
<td>Turbo Meal</td>
<td>Min-Vit Mix</td>
</tr>
<tr>
<td>2.45</td>
<td>1.94</td>
</tr>
<tr>
<td>Min-Vit Mix</td>
<td>Urea 45% N</td>
</tr>
<tr>
<td>1.94</td>
<td>0.15</td>
</tr>
<tr>
<td>Limestone GD 38%Ca</td>
<td>Limestone GD 38%Ca</td>
</tr>
<tr>
<td>0.07</td>
<td>0.23</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>58.5</td>
<td>58.5</td>
</tr>
</tbody>
</table>

Table 7. Ration evaluation for the heavy forage rations.

<table>
<thead>
<tr>
<th>58% Forage Ration</th>
<th>35% CS</th>
<th>50% CS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry matter basis</td>
<td></td>
</tr>
<tr>
<td>CP, %</td>
<td>16.2</td>
<td>16.2</td>
</tr>
<tr>
<td>RDP, % of CP</td>
<td>61.0</td>
<td>61.0</td>
</tr>
<tr>
<td>RUP, % of CP</td>
<td>39.0</td>
<td>39.0</td>
</tr>
<tr>
<td>Metabolizable protein (lbs/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required</td>
<td>6.32</td>
<td>6.33</td>
</tr>
<tr>
<td>Supplied</td>
<td>6.39</td>
<td>6.40</td>
</tr>
<tr>
<td>Sugar, %</td>
<td>8.0</td>
<td>8.1</td>
</tr>
<tr>
<td>Starch, %</td>
<td>25.2</td>
<td>28.4</td>
</tr>
<tr>
<td>Soluble fiber, %</td>
<td>4.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Protein profile based on the 2001 NRC. MP=metabolizable protein; RDP=rumen degradable protein; RUP=rumen undegradable protein; CP=crude protein.
Carbohydrate profile based on CPM dairy ration analyzer.
Conclusions

Dairy cattle diets can be adjusted to improve nitrogen efficiency. From the point of view of environmental issues, nitrogen is dynamic in that it is more challenging to manage nutritionally and because it affects both air and water quality. However, it has been demonstrated that through proper feeding management practices and careful nutritional formulation, N inputs can be reduced without compromising animal performance or income over feed costs. Nitrogen utilization efficiency can be improved and MUN is a practical tool for monitoring herd performance. There is more work needed in this area as feeding systems, feeding sequence, times per day can vary dramatically among herds and may require different approaches. The common denominator is that forage quality is a key component in improving nutrient efficiency and strategies involving corn silage, alfalfa silage and grass silage can be successfully manipulated to that end.

References


DAIRY PANEL DISCUSSION:
CHANGING NUTRITIONAL STRATEGIES WITH VOLATILE MILK PRICES

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Summary

While 2007 was an excellent year for mailbox milk prices, milk prices are starting to show some decline. On the other hand, feed costs are extremely volatile and at all-time highs for many ingredients. With corn at over $5/bushel, soybean meal over $350/ton and whole cottonseed in excess of $300/ton, we are in for a wild ride with escalating feed prices and volatile milk prices. In addition, extremely high input costs for fuel and fertilizer could add to the challenge for dairy profitability. Our expert panel will discuss a variety of nutritional strategies for dealing with rising feeds costs during times of volatile milk prices. Come and hear their thoughts on nutritional or feeding management strategies to deal with price volatility. This discussion will delve into intricate strategies for dealing with lactating diets. It will also discuss some nutritional strategies for calf and heifer feeding. Proceedings of the discussion will be recorded and published to the Conference website.
NRCS DAIRY NUTRITION NUTRIENT MANAGEMENT RESEARCH PROJECTS
IN THE MID-ATLANTIC

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Summary

Agriculture has been identified as the leading source of non-point source water pollution in the Chesapeake Bay watershed. In Pennsylvania, almost 4,000 miles of streams are impaired, largely due to excessive loading of sediment and nutrients from livestock manure. Such impacts can be traced down the watershed to Virginia. The Shenandoah River basin, for example, combines intensive animal agriculture and a geophysical structure that makes it vulnerable to ground and surface water contamination. The scientific body of evidence accumulated by the efforts to restore the Chesapeake Bay has clearly established the need to resolve this manure nutrient overload. As such, State Tributary Strategies and the regional “Manure Management Strategy” have all identified precision dairy feeding as a critical component in reducing non-point agricultural water pollution.

Introduction

The National Water Program set forth by the USDA Cooperative State Research, Education, and Extension Service’s (CSREES) Mid-Atlantic region includes Delaware, Pennsylvania, West Virginia, Virginia, Maryland, and the District of Columbia. These five states and the District encompass four major watersheds. In the western end of Region 3, waters flow to the Gulf of Mexico, where a prominent dead zone of about 10,000 square kilometers – resulting from excessive nitrogen loads – appears annually. Similarly, the Chesapeake Bay watershed, which spans the central Mid-Atlantic, flushes enough nitrogen (N) and phosphorus (P) to cause severe eutrophication in the catchment basin. The Delaware Bay receives the region’s eastern waters and its assortment of nutrient and toxic pollutants that are associated with industry and marine trade. Finally, the Roanoke and Chowan River Basins drain the southern part of the region, depositing their collection of nutrient and sediment pollutants into the Ablemarle-Pamlico Sound Estuary. Given these conditions, state reports show that, under the Clean Water Act, all of these watersheds contain stream segments that are “impaired.” Nutrient enrichment is obviously a dominating and critical water quality issue in the Mid-Atlantic Region. Managing these nutrients requires implementing practices that adequately control losses of N and P from agricultural operations.

Since late 2002, the Mid-Atlantic Water Program (MAWP) has addressed the area’s major water quality issues in a uniquely regional way. By developing a cohesive network of extension and research faculty from six 1862 and three 1890 land grant universities, the MAWP has promoted a structure of interstate collaboration founded on the dissemination of water quality science for practical applications in the field and on “the Hill.” Such a coordinated effort to incorporate the best available land-based science at both the policy level and on-the-ground operations was not formerly available or pursued in the region.
Dairy Feed Management

Precision feeding reduces N and P inputs to the levels required to maintain optimum production, resulting in 20-40% reductions in the nutrient content of manure. Pennsylvania, Maryland, and Virginia have all recognized the need to implement this practice and assist producers in adopting it into their operations. Given the MAWP’s involvement in state and regional strategies, several dairy specialists from different states partnered with each other to help dairy farmers adopt precision feeding. Charles Stallings from Virginia Tech (cstallin@vt.edu), Rick Kohn from the University of Maryland (rkohn@umd.edu) and Virginia Ishler from Penn State (vishler@psu.edu) are the region’s dairy nutrition and nutrient scientists, partnering with several external organizations to create a unique research and extension collaboration that addresses state- and operator-specific concerns while meeting regional water quality needs.

Three distinct, but associated, dairy management projects have been implemented through members and partners in Pennsylvania, Maryland, and Virginia. Aided by over $3 million from NRCS Conservation Innovation Grants (CIG), foundation funding, and innovative cost-sharing, these projects provide the foundation for feed management efforts in the region. MAWP members have been a central force in developing the analytical, demonstration, and education efforts. The purpose of these projects is to enhance feed efficiency while reducing N and P pollution in the Chesapeake Bay watershed. While this goal may sound similar to several other projects, the manner in which it is pursued is where these projects separate themselves from the rest.

These projects are founded upon an incentives framework that sets aside funds, supported though an innovative cost-share program with state NRCS offices, that compensates farmers when they have reduced nutrient levels in their feed to approved conditions. Ensuring that these conditions are met requires an intensive amount of research and extension to test the samples; educate farmers, nutritionists and veterinarians about advanced precision feeding and feed management strategies; and then demonstrate the benefits for wide-spread, long-term adoption.

Two additional projects have formed out of this feed management effort. The first is a joint effort with the University of Maryland Center for Environmental Science to develop a voluntary feed management certification program directed at dairy nutritionists and veterinarians to reduce the N and P in dairy manure through more careful management of the daily ration. This certification program is in coordination with the development of a national program but maintains a strong regional focus. The goal of the project is to certify individuals with competency to write feed management plans. As a pilot project, testing and refining will be limited to communities in the Upper Potomac sub-watershed of Maryland, where agriculture has been found to be a significant contributor to water quality impairment. While Maryland NRCS has established feed management practice standards, state agencies do not have the technical expertise to widely implement the practice.

The second project to form out of this larger feed management effort is a joint project with the Chesapeake Bay Foundation and the Maryland Department of Agriculture. This effort, which was recently funded, aims to demonstrate that substantial reductions, up to 30-40%, in N and P nutrient losses to the environment can be achieved through implementing complimentary best management practices (BMPs). Program members will work with farmers in the Maryland portion of the Monocacy Watershed to ultimately achieve a nutrient balance in the watershed by adopting specific BMPs that will work in combination with precision feeding activities.

Partners:
- Chesapeake Bay Foundation
- Cumberland Valley Analytical Services
- Maryland Dept. of Agriculture
Penn State University (via the Dept. of Dairy and Animal Science and Cooperative Extension)
Pennsylvania’s Center for Dairy Excellence
Pennsylvania Dept. of Agriculture
Pennsylvania Dept. of Environmental Protection
University of Maryland Center for Environmental Science
University of Pennsylvania
USDA Natural Resources Conservation Service
VA Cooperative Extension
VA Dept. of Conservation and Recreation
VA Tech (via the Dairy Dept. and the VA Waste Solutions Forum)

Maryland CIG Project

The 300 dairy herds in the watershed have been contacted. Enrollment has not taken place yet. The Pennsylvania component of the Monocacy watershed project in collaboration with Maryland has enrolled 6 out of the 10 viable dairy farms in Adam’s county. An educational program was provided for the dairy producers on the project. Four farm visits have been made to date and reports have been sent out to the dairy producers evaluating their protein and P status on their farm. The herd results have been evaluated and goals will be set for 2008.

Pennsylvania CIG Project

The Chesapeake Bay Foundation, in partnership with veterinarians from the University of Pennsylvania’s New Bolton Center, dairy nutritionists from the Pennsylvania State University, the Natural Resources Conservation Service (NRCS), and the Pennsylvania Department of Agriculture is pursuing ambitious goals to bring about significant changes in the dairy industry’s standard feeding practices and subsequently improve water quality. This project’s three-year goals under this grant are as follows:

- Initiate precision dairy feeding on 60 farms that will receive cost-share assistance to cover the necessary laboratory analyses, technical assistance in interpreting data and adjusting rations and management. To date, 48 dairy farms are participating in the project.
- Develop and distribute educational materials on precision dairy feeding to over 3,000 Pennsylvania dairy farmers. Educational materials have been developed and provided on a CD during the educational workshops. They are also available on the web at http://www.das.psu.edu/dairynutrition/.
- The Profitability Assessment Dairy Tool and the precision feeding drill down tools are available on the web at http://dairytool.psu.edu.
- Develop and conduct 12 workshops on precision dairy feeding to reach 300 veterinarians, animal nutritionists and feed industry representatives. To date, 12 workshops have been held with more scheduled for 2008-2009. One hundred forty-five consultants have participated in the educational program: Profitability Assessment Dairy Tool, with 114 going through the drill downs to precision feeding.

The project partners will implement a program to obtain the necessary farm information, conduct the relevant analyses, design the appropriate feed ration, and implement that ration on Pennsylvania dairy farms. Each participating farm will receive intensive technical assistance to ensure that herd health and production are maintained or improved. The program will also include a comprehensive education strategy to reach farmers, animal nutritionists, veterinarians, and livestock feed industry staff.
The project partners will work with the dairy operations to implement the strategies and procedures in their operation to ensure that the formulated ration is reaching the animals, including the use of MUN analysis. Follow-up data collection of manure and urine will document the reduction in nutrient output from the dairy herd.

**Virginia CIG Project**

Almost 2 years after project initiation, the final group of collaborator farms was added to the project, bringing the total number of participating herds to 215, with 35,064 cows. Project herds represent 29% of Virginia dairy farms and 35% of Virginia dairy cows. One-hundred fifty-one (151) of these herds and 22,348 of the cows are in the Chesapeake Bay watershed. These herds have been signed up in groups, with staggered start dates for logistical reasons. During this reporting period, four groups completed the first year of the project and a yearly summary was provided to each farm that completed the requirements for payment calculation. To accomplish this, at least 5 feed samplings had to be reported for the year. Ninety-one farms have completed year 1 (groups, A, B, C and I) and 46 of these herds qualified for incentive payments totaling $33,545. The other groups will have yearly summaries provided as they complete their first year. Educational programming has involved sending a newsletter to all participating herds to update them on items related to the project, especially results from the “P Report.” Both producer and nutritionist meetings have been conducted. The addition of herds in all areas of the state will result in a more comprehensive educational effort that will have direct impact on all Virginia dairy herds.

**Dairy Feed Management Certification Program**

Specialists from the Mid-Atlantic region in collaboration with the national feed management education project held a training session in Pennsylvania on November 12, 2007. The objective was to hold an educational workshop on how to develop a feed management plan to support the implementation of the NRCS Feed Management 592 Practice Standard. One hundred and three consultants participated in the training. Fifty-two people took the feed management planner’s exam. Fourteen people took the ARPAS exam with the intention of taking the feed management exam at a later date.

Consultants are taking the first steps to become certified feed management planners. Dairy producers enrolling in the NRCS environmental quality incentive program (EQIP) and that are interested in the feed management program, should soon have certified planners available to write and oversee feed management plans. This program provides financial assistance for producers wanting to improve their nutrition as well as water and air quality.

**Conclusion**

Dairy feed management and nutrition alone will not solve the nutrient loading problems experienced by the various watersheds in the Mid-Atlantic Region. It is one of several strategies that when combined can have significant impact on water and air quality. The dairy feed management projects being implemented throughout the region are focusing on research, education and producer incentives to reduce the amount of P and N entering surface waters. The continued collaboration and partnering with various agencies is necessary if the goals to reducing P and N loads in soil, water and air are to succeed.
Equine Session
ANTIOXIDANT SUPPLEMENTATION OF EQUINE DIETS

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Summary

Oxidative stress occurs when the balance of pro-oxidants out-weighs the capacity of antioxidants within the body and can result in damage to biomolecules. Several lines of antioxidant defenses exist within the body, many of which are nutrients (e.g., vitamins E and C) or are influenced by nutrients (e.g., Se). This presentation associated with this paper discusses supplementation of vitamin E, vitamin C and selenium of equine diets emphasizing effect of supplementation level and source on antioxidant status and subsequent health effects.

Introduction

Metabolism is defined as the sum of all chemical reactions in the body. Several of these reactions yield substances known as pro-oxidants, which if not contained have the potential to damage all classes of biomolecules (e.g., DNA, RNA, proteins, lipids). The body contains several lines of antioxidant defense against pro-oxidants. Under normal circumstances pro-oxidants are either prevented from forming or accumulating by the antioxidant defense system. However in some instances (e.g., accelerated metabolism, stress, and disease) the production of pro-oxidants may overwhelm the body’s antioxidant defense system damaging cells/tissues/organ systems, a condition referred to as oxidative stress (Sies, 1993). Oxidative stress has been implicated in several diseases/conditions of the horse, including equine motor neuron disease, equine Cushing’s disease, joint disease, exercise induced muscle damage, recurrent airway obstruction disease (RAO; heaves), yet much remains unknown regarding its role in these diseases/conditions (Soffler, 2007).

Many of the antioxidant defense mechanisms within the body are influenced by nutrients that have direct antioxidant effects (e.g., vitamin E, vitamin C, beta-carotene) or are components of compounds having direct antioxidant effects such as antioxidant enzymes (e.g., selenium as a component of glutathione peroxidase). The purpose of this paper/presentation is to discuss the effect of dietary antioxidant supplementation on antioxidant status and subsequent health effects.

Pro-oxidants

Pro-oxidants are generated in the body during metabolism under normal conditions (Halliwell, 1996). Cells require energy to fuel numerous biochemical reactions (e.g., maintenance of ion gradients, biosyntheses, and muscle contraction). Energy required for these processes is provided by adenosine triphosphate (ATP). The majority of ATP is synthesized by the oxidation (loss of electrons) of energy substrates (e.g., glucose, long-chain fatty acids, volatile fatty acids and amino acids) and the subsequent reduction (gain of electrons) of oxygen to water. This process is known as oxidative phosphorylation. During oxidative phosphorylation oxygen is reduced to water by accepting four electrons. However, a small percentage of oxygen is incompletely reduced (i.e. accepts less than 4 electrons). The incomplete reduction of oxygen results in the formation of a group of compounds known as reactive oxygen species.
Reactive oxygen species consist of oxygen free radicals (e.g., superoxide (O$_2^-$), hydroxyl radical (-OH)) and their derivatives (e.g., hydrogen peroxide (H$_2$O$_2$)). The immune system is another source of ROS. The respiratory burst, a mechanism by which phagocytes (e.g., macrophages and Neutrophils) use to degrade and kill ingested pathogens also produces O$_2^-$ and H$_2$O$_2$.

**Antioxidant defenses**

The body’s antioxidant defense system involves three different strategies: prevention, interception and repair. These strategies are discussed in detail by (Sies, 1997), and are only given general discussion in this paper/presentation.

Preventing the formation of ROS is the first line of antioxidant defense. Metal ions such as Cu and Fe are capable of initiating reactions forming ROS. Metal binding proteins such as ferritin, transferrin and ceruloplasmin serve to sequester metal ions and prevent them from participating in ROS generating reactions. Several enzymes generate ROS as intermediates; however the conformation of these enzymes sequesters the ROS and prevents reactions with susceptible biomolecules.

Interception of ROS and metabolism to harmless end products is the second line of antioxidant defense. This strategy incorporates both antioxidant enzymes and non-enzymatic antioxidants. The primary antioxidant enzymes are superoxide dismutase, glutathione peroxidase and catalase. Superoxide dismutase catalyzes the metabolism of O$_2^-$ into H$_2$O$_2$. Superoxide dismutase is located in the mitochondria and cytosol. Mitochondrial superoxide dismutase contains Mn in its active site while in the cytosol contains Cu or Zn. Glutathione peroxidase is a Se-containing enzyme involved in the metabolism of H$_2$O$_2$ and lipid peroxides to water and lipid aldehydes, respectively. Catalase is hemoprotein that also catalyzes the metabolism of H$_2$O$_2$ to water.

The primary non-enzymatic antioxidant defenses are alpha-tocopherol (vitamin E), beta-carotene (pro-vitamin A) and ascorbate (vitamin C). Vitamin E’s lipophilic nature allows it to incorporate into cell membranes where it serves to protect unsaturated lipids and other susceptible membrane components against oxidative damage. Vitamin E donates a hydrogen atom from its phenolic group to lipid peroxyl radicals produced during autooxidation of membrane polyunsaturated fatty acids forming a more stable lipid peroxide and stable tocopheryl radical. The subsequent lipid peroxides are further degraded by Se-dependent glutathione peroxidase. A detailed description of vitamin E’s antioxidant function is described by Pryor (2001). Beta-carotene and ascorbate are free radical scavengers capable of donating electrons to free-radicals thus metabolizing them into harmless end products. Additionally, ascorbate is thought to regenerate alpha-tocopherol from tocopheryl radicals, thus conserving alpha-tocopherol.

The final antioxidant defense mechanism is repair of oxidative damage. This strategy involves several enzyme systems involved in removal and repair of biomolecules damaged by pro-oxidants.

**Antioxidant Supplementation**

**Vitamin E**

Current vitamin E requirements (NRC, 2007) are based on intakes associated with maximized tissue vitamin E (alpha-tocopherol) concentrations (Roneus *et al.*, 1986) and enhanced humoral immune response (Baalsrud and Overnes, 1986). The requirements range from 1 IU/kgBW (maintenance) to 2 IU/kgBW (very heavy exercise or lactation), which expressed as a dietary concentration, assuming dry matter intakes of 2 and 2.5% of BW, is approximately 50 and 80 IU/kgDM. Feedstuffs vary considerably in vitamin E activity. Forage contains concentrations ranging from 30 to 100 IU/kgDM; whereas cereal grains contain lesser concentrations (20 to 30 IU/kgDM) (NRC, 2007). As a result of this variability
vitamin E is commonly supplemented in equine diets. Alpha-tocopheryl acetate is the most common form of supplemental vitamin E. Alpha-tocopheryl acetate is available as either synthetic form (all-rac) or natural source (RRR). Synthetic alpha-tocopheryl acetate (all-rac-alpha-tocopheryl acetate) contains 1 IU/mg; whereas natural source (RRR-alpha-tocopheryl acetate) contains 1.36 IU/mg. Dietary supplementation with natural source vitamin E has been reported to be more effective at increasing serum alpha-tocopherol concentrations as compared to all-rac-alpha-tocopheryl acetate in short-term studies ranging in length from 14 d (Pagan et al., 2005) to 6 wk (Gansen et al., 1995).

Serum alpha-tocopherol reflects tissue (muscle, liver, adipose) alpha-tocopherol status and is used as an indicator of equine vitamin E status (Roneus et al., 1986). Serum alpha-tocopherol concentrations of > 2 µg/ml are thought to reflect adequate status; whereas those between 1.5 and 2 µg/ml are considered marginal and < 1.5 µg/ml are considered deficient (Craig et al., 1992). Serum alpha-tocopherol concentration has been reported to vary considerably within a single horse throughout the day (mean within-horse CV ranged from 7 to 17% over 72-hr period) making single sample interpretations of status difficult (Craig et al., 1992). However, other data (Siciliano, unpublished) suggest that the within-horse CV calculated from 8 hourly samples is < 7%. The ratio of alpha-tocopherol to dietary polyunsaturated fatty acids (PUFA) may influence vitamin E status. A ratio of 0.6 mg α-tocopherol to 1 g PUFA was predicted as a minimum to protect against vitamin E deficiency based on work in animals and humans (Harris and Embree, 1963); however, the ratio of α-tocopherol:PUFA required to maintain vitamin E status may be even greater as the degree of fatty acid unsaturation increases, (Muggli, 1989). Supplementation of soybean oil (6.4% of diet or 20% of required DE) resulted in a alpha tocopherol to PUFA ratio of 1.7 and did not negatively impact vitamin E status of horses (Siciliano and Wood, 1993). Exercise conditioning over time can decrease vitamin E status of horses (Petersson et al., 1991; Siciliano et al., 1997). Vitamin C and selenium have been shown to influence vitamin E status in other species (Lauridsen and Jensen, 2005; Halpner et al., 1998; Combs, 1996), but their effect on equine vitamin E status is not documented.

Vitamin E supplementation in horses has been evaluated for its effect on reproduction in mares and stallions, exercise induced muscle damage, humoral immune response and passive transfer of immunoglobulins in foals. Vitamin E supplementation (100 IU/d; alpha-tocopheryl acetate) improved reproductive efficiency in barren mares having relatively low vitamin E status (< 2 µg/ml)(Stowe, 1967); however supplementing (46 IU/d; alpha-tocopheryl acetate) a diet already containing 15 IU/kg DM did not affect rebreeding efficiency in mares (Ott and Asquith, 1981). Supplementing 5,000 IU/d (alpha-tocopheryl acetate) did not affect stallion libido or seminal characteristics as compared to a base diet of grain and grass hay containing no supplemental vitamin E (Rich et al., 1983). Vitamin E supplementation does not appear to consistently reduce lipid oxidation or decrease indicators of exercise induced muscle damage (McMeniman and Hintz, 1992; Siciliano et al., 1997; Williams et al., 2004; Williams and Carlucci, 2006) in exercising horses, but is effective at preventing declines in vitamin E status associated with exercise conditioning (Petersson et al., 1991; Saastamoinen and Juusela, 1993; Siciliano et al., 1997; Williams et al., 2004). Additionally, white blood cell apoptosis was reduced (supplemented = 1.52% vs unsupplemented control = 16.5%) in horses fed diets supplemented with 5,000 IU/d (alpha-tocopheryl acetate) (Williams et al., 2004). Humoral immune response to vaccination was improved in horses supplemented with 50 IU/kg DM as compared to an unsupplemented control diet containing 18 IU/kg DM (Baalsrud and Overnes, 1986). Vitamin E supplementation (160 IU/kg DM; alpha-tocopheryl acetate) of diets fed to gestating mares has been shown to have a positive influence on passive immunoglobulin transfer to their foals as reflected by a tendency for increased serum IgG concentration (Hoffman et al., 1999).
Vitamin C (Ascorbic Acid)

No dietary vitamin C requirements have been determined for horses (NRC, 2007). Horses are assumed to synthesize ascorbic acid from glucose (Pearson et al., 1943; Stillions et al., 1971) similar to other species (Chatterjee, 1973). Vitamin C has been supplemented in equine diets using ascorbic acid, ascorbyl palmitate and calcium ascorbyl-2-monophosphate; however, ascorbyl palmitate is more efficient at raising plasma ascorbic acid concentrations (Deaton et al., 2003; Snow and Frigg, 1990; Snow and M.Frigg, 1987).

Several factors have been reported to decrease equine plasma or serum concentrations of ascorbic acid suggesting that endogenous synthesis is not keeping pace with ascorbic acid consumption. These factors include disease (Jaeschke, 1984), transport (Baucus et al., 1990b; Baucus et al., 1990a), recurrent airway obstruction (Deaton et al., 2004b). Initial reports suggested that old age (> 20 years of age) (Ralston et al., 1988), and endurance exercise (Hargreaves et al., 2002; Marlin et al., 2002) decreased plasma ascorbic acid concentration; however, more recently, endurance exercise has been reported to increase plasma ascorbic acid concentrations (de Moffarts et al., 2005; Williams et al., 2004) while old age had no effect (Deaton et al., 2004a). Dietary supplementation of ascorbic acid in combination with vitamin E and selenium has been reported to improve exercise tolerance and inflammatory score of horses affected with recurrent airway obstruction (RAO; heaves) (Kirschvink et al., 2002).

Selenium

The current selenium requirement is 0.1 mg/kg DM and is based on intakes associated with the absence of classical deficiency symptoms (e.g., white muscle disease) and maintenance of blood selenium concentration (NRC, 2007). Naturally occurring selenium concentrations in feedstuffs commonly fed to horses varies considerably (< 0.05 to 0.4 mg/kg DM) depending upon geographical region and associated soil selenium concentration. Selenium is supplemented in equine diets as sodium selenite or selenium enriched yeast. Selenium enriched yeast contains a relatively large (~40%) proportion of selenium as selenomethionine (Kelly and Power, 1995), which is metabolized differently than sodium selenite (Sunde, 2001). Selenium enriched yeast has been reported to be better retained as compared to sodium selenite (27.8 vs 20.4% of selenium intake; Pagan et al., 1999) and more effective at increasing serum selenium concentrations (Janicki et al., 2001; Pagan et al., 1999). Plasma selenium concentration tended to be higher in yearlings fed zinc-selenomethionine (~3.5 mg/d) for 28 d as compared to those fed a similar amount of selenium from sodium selenite; however, by 56 d of supplementation plasma selenium concentration was similar between horses fed the two different sources (Richardson et al., 2006). Selenium supplementation can increase the glutathione peroxidase activity of red blood cells, but the effect of selenium source on this end point is unclear (Richardson et al., 2006).

Selenium supplementation has been shown to influence immune system parameters. Humoral immune response to pig red blood cell vaccination was enhanced in ponies fed diets containing with 0.22 mg/kg DM (sodium selenite) as compared to those fed an unsupplemented control diet containing 0.02 mg/kg DM (Knight and Tyznik, 1990). Passive transfer of immunoglobins from mare’s to their foals was improved when mares were fed diets containing approximately 0.3 mg Se/kg DM as compared to foals from mares fed diets containing approximately 0.1 mg Se/kg DM (Janicki et al., 2001).

Selenium can be toxic to horses. The maximum tolerable concentration for horses is 2 mg/kg DM (NRC, 2007). Signs of toxicity include loss of mane and tail hair, separation of hoof capsules at the coronary band, apparent blindness, head pressing, perspiration, abdominal pain, colic, diarrhea, increased heart rate and increased respiration (NRC, 2007).
Conclusion

Vitamin E, C and selenium supplementation level and source influences antioxidant status of horses. Vitamin E and selenium supplementation may have important practical applications for enhancing humoral immune response and passive transfer of immunoglobins from mares to their foals. The effect of vitamin C supplementation on equine health remains largely undetermined, but may have application in horses with RAO.

References


CONSIDERING DIRECT-FED MICROBIALS AS A SUPPLEMENT FOR EQUINE

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Summary

In recent years, researchers have become more acutely aware of the normal flora that inhabit the gastrointestinal tract of mammals and the benefits it can exert upon the host. Of primary interest is to understand, characterize and optimize the fermentative action of the normal flora for benefit of mammalian species. An accelerated thrust of research efforts have recently focused on enhancing the normal flora through direct-fed microbials (DFM) for application across species. While DFM research conducted in other mammalian and avian species shows promise for enhanced nutritional and health benefits, equine DFM application research is still in its infancy. In general, there is a paucity of literature published on DFM administration to horses. The literature that is available renders only disjointed information and leaves little room for a solid recommendation for the application of DFM as a supplement for equine. Largely, researchers report a lack of effect when DFM are supplemented in the equine diet. This lack of response could be attributed to (among others): 1) the use of microorganisms with no beneficial properties to equine, 2) inadequate dosing, or 3) poor quality control of DFM preparations. This review focuses on recent peer-reviewed literature involving bacterial and fungal DFM application in equine and is intended to provide equine professionals with a basic understanding of the nature of the dynamic normal flora of the equine hind-gut, the substrate specificities of general classes of bacteria, and an assessment of the next step forward in equine DFM research. It is the author’s belief that future research in DFM application would most benefit from substrate-based application because microbial growth, viability, and metabolic activity are substrate-driven processes. Further investigation on the efficacy and application of bacterial DFM in equine is needed.

Introduction

Probiotics were first recognized by Metchnikoff (1907), who speculated that the longevity of Bulgarian peasants was achieved by organisms, present in the fermented milk (yogurt) they were consuming, inhibiting pathogens that were otherwise causing disease. Later, Rettger and Chaplin (1921) confirmed that bacteria, Lactobacillus acidophilus, in the yogurt expressed antibiotic activity. Over four decades later, Lilley and Stillwell (1965) were the first to define ‘probiotics’ as, ‘substances secreted by one organism that stimulates the growth of another.’ More recently, probiotics have been defined as, ‘microorganisms that beneficially affect the host animal by providing intestinal microbial balance’ (Fuller, 1989). The U.S. Office of Regulatory Affairs of the Food and Drug Administration (FDA, 1995) and the Association of American Feed Control Officials (AAFCO, 2007) have narrowed the definition of probiotics to “a source of live, naturally occurring microorganisms” (Yoon and Stern, 1995) and require feed manufacturers to use the term “direct-fed microbials” (DFM).

A review of the literature indicates an array of positive effects achieved through DFM application in other species. In ruminants, microbial cultures have been shown to decrease the incidence of ruminal acidosis (Ghorbani et al., 2002), improve feed efficiency and daily gain in beef cattle (Ware et al., 1988), potentially replace or reduce the use of antibiotics in neonatal and stressed calves (Abu-Tarboush et al., 1996), and enhance milk production in dairy cows (Komari et al., 1999). In poultry, DFM
supplementation improved egg production, feed consumption, feed conversion, eggshell thickness, and yolk color, in addition to decreased yolk cholesterol (Mohan et al., 1995; Li et al., 2006). Weaned pigs offered DFM showed reduced incidence, severity and duration of diarrhea and reduced fecal shedding of *Salmonella* spp. (Casey et al., 2007). Probiotics offered to humans reduced serum cholesterol level and colon cancer, and improved calcium absorption, vitamin synthesis, and lactose tolerance (Fuller, 1989), as well as reduced the incidence of diarrhea in children (Van Niel et al., 2002).

**Normal Flora**

Probiotics are commonly comprised of bacterial species isolated from the gastrointestinal (GI) tract of mammals. For that reason, it is important to understand the community of microorganisms that colonize the intestinal tract, referred to as the “normal flora” (Parker, 1974). The makeup of the normal flora of an individual depends upon the host species, age, sex, level of stress, and diet and consists of a vast variety of microorganisms, with bacteria comprising the largest component of the biomass (Todar, 2007). Due to a co-habitative relationship, the normal flora derives from the host a supply of nutrients, a stable environment, constant temperature, protection, and transportation whereas the host obtains from the normal flora certain nutritional metabolites, stimulation of the immune system, and exclusion of pathogens (Hungate, 1966; Todar, 2007). Within the intestinal lumen, different microenvironments exist; acidophiles will populate the proximal duodenum where acid secreted from the stomach is more persistent, while species less tolerant to low pH inhabit the cecum or distal colon. Some species thrive at the mucosal surface, while others are more stable in the crypts (Conway et al., 1995). However, our understanding of mammalian normal flora and its mode of action has been virtually unexplored. It has been estimated that only a fraction (<1%) of microbial species have been recovered through isolation and cultivation, suggesting that our understanding of the GI microbial ecosystem, based on the few strains we have identified, is likely misleading (Weese et al., 2004).

**Substrate Specificities**

Perhaps a better understanding of bacterial substrate specificities would provide some answers on how DFM can be more effectively supplemented in the equine diet. It has been suggested that probiotics are likely host species-specific and that it is not likely that all DFM preparations would exhibit benefits across-species (Gibson and Fuller, 2000). The bacterial species in the GI tract have specialized niches, varying in their substrate specificity. In many cases, the metabolic end-products excreted by one species can serve as a growth substrate for another. Consequently, the biomass of each species is directly correlated with the amount of substrate available (Gibson and Roberfroid, 1995).

**Amylolytic Bacteria.** Amylolytic bacteria, unique for their ability to secrete exogenous α-amylase, rapidly utilize starch. Some of the common amylolytic bacteria of the normal flora are *Ruminobacter, Prevotella, Streptococcus, Selenomonas, Butyrivibrio, Eubacterium, and Clostridium* spp. (Cotta, 1988). Many of the amylolytic species can produce lactic acid as one of the by-products of starch fermentation (Dunlop and Hammond, 1965) and are consequently referred to as lactic acid-producing bacteria. Goodson *et al.* (1988) demonstrated that an abrupt increase in starch increased amylolytic bacterial population from 85.2 to 92.2% of total cecal bacteria numbers of the pony.

**Lactic Acid-Producing Bacteria.** Lactic acid-producing bacteria (*Enterococcus, Lactobacillus, Bacillus*, and *Bifidobacteria* spp.) refers to a class of bacteria that are Gram-positive, non-motile, aerotolerant anaerobic rods and cocci that produce lactic acid as a major product of fermentation (Moat, 2002). LAB are the most commonly used bacteria in commercial DFM preparations intended for mammalian and avian supplementation because they are robust to feed manufacturing, processing and storage. LAB are typically co-classified as amylolytic species due to their starch-based substrate specificity. In fact, a common LAB found in the rumen and in the colon of horses, *Streptococcus bovis* (Alexander and Davies, 1963), has been implicated as a causative agent of lactic acidosis and laminitis in horses and feedlot cattle fed carbohydrate-rich diets (Owens *et al.*, 1998). Recently, the USDA published
an article (CAST, 2007) extending a precaution to the human nutrition industry that LAB-based probiotics consumed with carbohydrate-rich diets could potentially induce lactic acidosis in humans. Due to a high rate of direct-fed LAB supplemented in carbohydrate-rich diets intended for animals, a similar precaution should be heeded in the animal nutrition industry as well.

**Lactate-utilizing Bacteria.** While the volatile fatty acids, acetate and butyrate, are directly produced from the fermentation of plant polysaccharides by individual species of bacteria, propionate production requires interactions between species (Hobson and Stewart, 1997). For example, *Prevotella ruminicola* and *Ruminobacter amylophilus*, both amylolytic species, produce succinate as a by-product of starch fermentation, which is rapidly converted to propionate by other bacterial species (Hobson and Stewart, 1997). Similarly, lactate produced from starch fermentation can be converted to propionate by lactic acid-utilizing bacteria (LUB). Roxas (1980) demonstrated that feeding a carbohydrate-rich diet to sheep increased the populations of amylolytic and LUB in the rumen. Similarly, Julliand et al. (2001) demonstrated an increase in colonic LUB numbers and lactate production as the proportion of barley increased in the diet of horses. The predominant LUB species of the normal flora are *Selenomonas*, *Propionibacterium* and *Anaerovibrio* (Hobson and Stewart, 1997).

**Fibrolytic Bacteria.** Whereas mammals do not secrete endogenous cellulase and zylanase needed for the degradation of the fibrous constituents making up the cell walls of plants, bacteria in the cecum and colon do. The predominant cellulolytic bacteria found in the rumen and hindgut of most herbivores are *Fibrobacter succinogens*, *Ruminococcus albus* and *Ruminococcus flavefaciens* (Williams and Strachan, 1984). Cellulolytic bacteria populations grow in proportion to the plant cell walls they digest and are typically found bound to the fibrous digesta they are degrading. The cellulolytic bacteria common in the rumen are also present in the equine cecum (Julliand et al., 1999); however, where *R. flavefaciens* is the most predominant cellulolytic bacteria found in the rumen (of both ponies and sheep), *F. succinogens* is the main bacterial species of the rumen (Julliand et al., 1999). In monogastrics, amylolytic species are more common in the small intestine, whereas the cellulolytic species are in higher concentrations toward/in the cecum (Kern et al., 1974; Macy et al., 1982). Cellulolytic organisms are pH- and lactic acid-sensitive (Medina et al., 2002). Consequently, offering horses high-starch diets will depress the number of cellulolytic bacteria in the large intestine (Julliand et al., 2001; Medina et al., 2002).

**Lipolytic Bacteria.** Lipolytic bacteria express exogenous lipase that can rapidly hydrolyze the acyl ester bonds of dietary triglycerides, cleaving all fatty acids with little specificity (Garton et al., 1959, 1961). In ruminants, certain species of *Anaerovibrio*, *Butyrivibrio*, *Ruminococcus* and *Eubacterium* spp. have demonstrated lipase activity (Harfoot and Hazlewood, 1997). While there is a great deal of equine literature available on fat digestibility and palatability, there is a scarcity of research on bacterial lipase activity and manipulation of microbial lipid metabolism in equine. In contrast, extensive work has been published and reviewed in ruminants (Jenkins, 1993, 1994).

**Proteolytic Bacteria.** Proteolytic species generally refers to those species of bacteria that express proteases, peptidases, di- and tri-peptidases, and/or deaminase enzymes that break down protein. Kern et al. (1973, 1974) reported that 19.7% of the cecal bacteria in ponies are proteolytic in nature and are 30-times more prevalent in the ileum than in the cecum or colon. In comparison, Fulghum and Moore (1963) determined that up to half of all the bacteria in the rumen demonstrate some proteolytic activity. The major proteolytic species of the rumen and cecum are *Streptococcus bovis*, *Ruminobacter amylophilus*, *Butyrivibrio fibrisolvens*, and *Prevotella ruminicola* (Kern et al., 1973; Hobson and Stewart, 1997). While there is sparse equine literature in this genre, ruminant research largely revolves around improving production efficiency and reducing N excretion into the environment by balancing rumen degradable and non-degradable intake protein (Cole and Todd, 2007).

It is logical to assume that whether endogenous or exogenous populations of microbes are present or introduced in the equine hindgut, their growth and metabolic activity are substrate-dependent processes. Perhaps, because diet composition influences the type and population proportions of bacteria in
the GI tract (Hungate, 1966; Kern et al., 1973), manipulation of the microbial ecosystem by substrate alone (via the diet) may be more effective than offering DFM without consideration of the substrate available. The work done on prebiotics, ‘a non-digestible food ingredient (typically a carbohydrate that can be neither hydrolyzed or absorbed by the upper part of the digestive tract) that beneficially affects the host by stimulating growth and/or activity of certain bacterial components of the intestinal micro flora’ (Gibson and Roberfroid, 1995), may be a novel approach and have a competitive advantage to the live-fed probiotic bacteria in the GI tract. However, for the sake of brevity, prebiotics will not be further discussed here, so the reader is directed to recent reviews in equine literature (Weese, 2003; Julliand, 2006).

**Efficacy of Bacterial Cultures in Equine**

There is a paucity of literature published on DFM administration to horses, however, that which is available renders only disjointed data and raises more questions. Optimal dosages have not been determined for the respective DFM bacterial species intended for equine, consequently a variety of bacterial species have been administered at different dosage levels between studies, therefore making comparisons among studies difficult. Most of the equine research focuses primarily on the administration of DFM in an effort to reduce the incidence of diarrhea and colic and to inhibit pathogens; few report beneficial effects.

When DFM were administered to horses hospitalized for colic, Parraga et al. (1997) detected no effect on the reduction of *Salmonella* sp. shedding, incidence of diarrhea, duration of antibiotic treatment, or length of hospitalization. In a series of studies, Weese et al. (2003, 2004, and 2005) evaluated bacterial DFM intended for horses. First, Weese et al. (2003) administered a bacterial strain, extensively studied in humans for treatment of diarrhea, to mature horses and foals to determine if the bacteria demonstrated the ability to survive GI tract transit. The authors reported that the bacteria appeared to colonize the hindgut for 9 d following cessation of administration in foals, but only for 48 h in mature horses. The latter suggests that the bacteria recovered in the feces of mature horses were present due to mere transient passage and not of any colonization. However, it is worth mentioning, that colonization occurred in the foals only at a prohibitively high dose. Next, Weese et al. (2004) screened 47 bacterial species from the equine intestine to be used as a potential probiotic and isolated only one strain that demonstrated acid-and bile-tolerance, aerotolerance, and inhibition against more than one pathogen in vitro. Then, Weese and Rousseau (2005) administered that same strain to neonatal foals for the prevention of diarrhea, but instead found that it actually exacerbated diarrhea (in addition to increasing the incidence of depression, anorexia, and colic). These results raised the authors’ concerns about the number of untested probiotic products intended for horses that are available on the market. Similarly, Swanson et al. (2003) also found that supplementing DFM in the diet of foals exacerbated the incidence of diarrhea. Conversely, others (Yuyama et al., 2004) reported that a DFM preparation, consisting of 5 bacterial strains (also isolated from the equine GI tract), decreased the incidence of diarrhea and enhanced the weaning weights of foals. In general, these studies are difficult to compare because each used a different combination and source of bacterial species in the DFM supplements and the dosage levels offered to the horses varied. Unfortunately, echoing the comments made by Weese (2003), the current literature provides little to no guidance on how to successfully incorporate DFM supplements into the equine diet.

Most commercial DFM preparations contain combinations of bacterial species. This makes researching the effect of individual organisms difficult to isolate. Swyers et al., (2007a) evaluated the effects of administering direct-fed LAB to mature horses to determine if the bacteria could ameliorate the acidotic risks associated with feeding high-starch concentrates to horses. The study focused primarily on *L. acidophilus*; comparing the single strain DFM to a mixed DFM consisting of four LAB species (*L. acidophilus, L. casei, Bifidobacterium bifidium,* and *Enterococcus faecium*). The authors found a tendency for elevated fecal pH in horses offered only *L. acidophilus*, but otherwise the DFM had a limited effect on reducing the risk of acidosis. While it seems contrary that supplementing direct-fed LAB would reduce the incidence of acidosis (given that these bacteria inherently increase the concentration of organic
acids and decrease pH during starch fermentation), it has been suggested that providing an exogenous source of amylase could increase the digestion of starch in the small intestine (Kienzle, 1994) and reduce the amount of ‘escape’ starch able to reach the cecum (Potter et al., 1992). In some feedlot cattle studies, supplementing high-starch rations with direct-fed LAB decreased the duration and severity of acidosis (Huffman et al., 1992; Van Koeveling et al., 1994; Ghorbani et al., 2002). The discrepancy between expected effects and the actual outcomes from these studies make it difficult to elucidate clear conclusions.

**Fungal Cultures**

In addition to bacteria, certain fungal cultures, particularly yeast, have also been evaluated for their probiotic properties. Unlike bacteria, yeast are classified as eukaryotes cells, or fungi, that are considered facultative anaerobes; being able to exist with or without oxygen. Yeast culture (YC), typically available as *Saccharomyces* or *Aspergillus* spp., can be offered either as, ‘a dry product composed of yeast and the media on which it was grown and dried in such a manner as to preserve the fermenting capacity of the yeast’ (AAFCO, 2007), or as a DFM only if the YC contains ‘live (viable) yeast cells,’ (FDA, 1995). The former would better fit the category of a protein supplement or a prebiotic.

In a review of both monogastric and ruminant literature, the most consistent response to YC is an increase in cecal and rumen bacterial yield, specifically of the cellulolytic species, and the subsequent positive effects on cellulose degradation (Glade, 1991a, 1991b, 1992; Nagaraja et al., 1997; Medina et al., 2002). It is theorized that YC offered in the diet promotes a higher pH environment in the gut due to increased growth and fermentative capacity of cellulolytic species; this would also allow for increased fiber digestion (Morgan et al., 2007; Jouany et al., 2007).

A critical property for live YC, or for any probiotic, is to express successful survival of GI transit. Medina et al. (2002) showed that viable yeast cells were found in the large intestine of fistulated horses 4 h after feeding a *S. cerevisiae* preparation, which is in agreement with results also observed in the rumen (Durand-Chaucheyras et al., 1998; Feims et al., 1993) and ileum of sheep (Newbold et al., 1990). More recently, Desrochers et al. (2005) demonstrated that a different preparation of YC survived passage of the GI tract, but did not colonize it.

**Toward a Solution**

In summation, the equine literature suggests that there is a lack of efficacy or benefit rendered when DFM are applied in equine diets. It seems likely that our understanding of the equine normal flora and how it can be manipulated is still in its infancy. Explanations for a lack of effect of DFM from equine studies could include: 1) the use of microorganisms with no beneficial “probiotic” properties to equine, 2) inadequate dosing, or 3) poor quality control.

**Required Properties of a Probiotic.** The selection of microorganisms with desirable properties is critical for conducting accurate and consistent dosage and efficacy trials in equine. The following criteria, which has been previously outlined (Weese, 2001, 2002, 2003), must be met before microorganisms can be considered a probiotic:

1. Exhibit aerotolerance (robust to oxygen exposure) and viability (survivability) during commercial processing, transit, storage, and feeding.
2. Once ingested, DFM must survive transit through the acidic environment of the stomach, resist bile digestion, attach to intestinal tract epithelial cells, and colonize the GI tract mucosa.
3. Exert antimicrobial factors against one or more pathogens.
4. Be nonpathogenic and cause no harmful effects to the host animal, regardless of dose.
Dosage. The author is unaware of any DFM dose titration studies conducted with equine. In humans, a dose of $1 \times 10^8$ to $1 \times 10^{10}$ cfu/d has been used as a recommendation for a minimum therapeutic dose (Kailasapathy and Chin, 2000). Weese (2001) extrapolated from human dosages, that an average horse (~450 kg) would likely require $1 \times 10^9$ to $1 \times 10^{11}$ cfu/50 kg BW/d of a viable organism able to colonize the intestinal tract. While the digestive physiology of humans and horses are quite different, this is at least a reference point from which to start future dosage trials.

Quality Control. While DFM are generally regarded as safe (GRAS; FDA, 1995), reports of substandard quality control have given rise to concern for the integrity of commercial DFM preparations intended for animal use. Previous research indicates a significant percentage of probiotic preparations that either did not contain the organism(s) or guaranteed CFU stated on the label, or contained additional species (Hamilton-Miller et al., 1999; Hamilton-Miller and Shah, 2002; Weese and Arroyo, 2003). Swyers et al. (2007) reported that lactobacilli remained viable and incurred minimal CFU loss during feed processing, however found no difference in the number of naturally-occurring bacterial CFU in control diets compared to diets with $10^8$ cfu/g of treatment-type DFM added. In this particular study, no contamination of treatment-type organisms occurred, rather the naturally-occurring bacteria were able to grow anaerobically on MRS agar and appeared to be treatment-type bacteria until DNA identification was performed. The authors concluded that counting of naturally-occurring bacteria as treatment-type DFM in finished feed preparations represents a quality control problem and that current enumeration methods may need to be reconsidered. Further validation studies are needed to evaluate the accuracy of commercial enumeration and quality control methods.

Conclusions

Based on the benefits of DFM application in other species, it seems likely that DFM could offer solutions in equine nutrition and health. However, given the information available in current equine peer-reviewed articles, it is unclear what the solid recommendation for DFM supplementation in equine diets should be. A coordinated effort in future equine DFM application research would provide equine professionals with clearer suggestions for microbial species selection, viability, quality assurance, dosage, and expected efficacy for nutritional supplementing purposes. Because microbial growth, viability and metabolic activity are substrate-dependent processes, future advances in DFM application would benefit from substrate-based research.

References


Roxas, D.B. 1980. Effects of abrupt changes in the ration on rumen microflora of sheep. PhD dissertation, Ohio State University, Columbus, OH.


THE EFFICACY OF SOME COMMONLY SUPPLEMENTED HERBS
IN EQUINE NUTRITION

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Summary

A majority of the many herbs and other functional foods on the market today have not been scientifically tested; this is especially true in equine research. The following paper will review literature pertinent to herbal supplementation in horses as well as other species. Common equine supplements like, echinacea, garlic, ginger, ginseng, and yucca among many others, are not regulated and few studies have investigated a safe yet effective dose of these compounds. Ginseng is commonly studied and has been found to exert an inhibitory effect on IL-1β and IL-6 gene expression, decrease TNF-α production by macrophages, decrease cyclooxygenase-2 expression, and suppress histamine and leukotriene release. On-going studies in horses are testing the anti-inflammatory effects of a single dose of ginger, post-exercise. Echinacea, a common immunostimulant, or ‘cold fighter’, has been reported to have an anti-inflammatory and antioxidant properties. Equine studies found that garlic fed at > 0.2 g kg⁻¹ d⁻¹ developed Heinz body anaemia. Yucca contains steroid-like saponins, which produce an anti-inflammatory, antioxidant, and anti-spasmodic effect to reduce pain associated with arthritis. Herbs can have a drug-like action that can interact with other components in the horses’ diet. Some herbs contain prohibited substances like salicylates, digitalis, heroin, cocaine and marijuana. Drug-herb interactions are also common side effects and caution needs to be taken when determining which ‘natural product’ to use. Few herbs have had sufficient research to warrant concrete recommendations for efficacy and dosage in equines.

Introduction

Herbal medicine, also called “phytomedicine”, is the use of therapeutic plants, plant parts or plant derived substances to aid in fighting against infections, diseases or enhancing overall health (Jonas, 1997). In the United States, the herbal market exceeds $3.2 billion, where 32 to 37 % of Americans use herbal agents each year (Johnston, 1997). This number is thought to be much higher in Europe where herbal agents are more widely accepted by medical professionals. In 2005 this number for the herbal market was thought to be higher, with garlic and echinacea being the top two selling herbs, respectively (Blumenthal, 2005). The horse industry in the United States was surveyed in 1997 regarding supplement use and found that about 70 % of horse operations fed one type of supplement or another. Furthermore, nearly 5 % of those operations fed herbal supplements (USDA, 1998). Since then, it is thought that the herbal market targeting horses has grown exponentially; however no new statistical data is available on the subject.

Herbal supplements that affect the immune system can be classified as adaptogens, immunostimulants or both. Adaptogens increase resistance to stressors, physical, chemical or biological, where immunostimulants activate the nonspecific, or innate defense mechanisms against viral, bacterial or cellular infections. Most of the studies to date in laboratory animals, humans and other species have determined that the immunologic effect of herbal supplements does not enhance normal immune response but may help if the immune system is compromised.

This review will focus on specific herbs and other functional foods that are commonly used in the horse industry. Published literature however, is scarce on this front, so research in human and other species is included to better illustrate the benefit of the supplements.

Herbal Actions and Uses

Table 1 summarizes the active component, action, drug interactions, and equine research present for the major herbs described.
<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Active components</th>
<th>Actions</th>
<th>Potential toxicity or interaction</th>
<th>Equine research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bee pollen</td>
<td>Propolis</td>
<td>β-carotene, caffic acid, kaempferol, phenethyl caffeate, p-hydroxyacetophenone, benzylhydroxybenzoate, coumaric, cinnamic acid</td>
<td>antioxidant, antimicrobial, antifungal, anti-inflammatory, immunoregulatory</td>
<td>None reported</td>
<td>Y</td>
</tr>
<tr>
<td>Devil’s claw</td>
<td>Harpagophytum procumbens</td>
<td>Iridoid glycosides, acetylated phenolic glycosides, terpenoids</td>
<td>anti-inflammatory</td>
<td>May effect blood sugar, gastric ulcers, prolong bleeding time</td>
<td>Y</td>
</tr>
<tr>
<td>Echinacea</td>
<td>Echinacea purpurea, E. angustifolia, E. pallida</td>
<td>Polysaccharides, glycoproteins, alkamides, cichoric acid</td>
<td>anti-inflammatory, antioxidant</td>
<td>May interfere with drugs processed by liver enzymes, not for use with a depleted immune system, or during pregnancy</td>
<td>Y</td>
</tr>
<tr>
<td>Flaxseed</td>
<td>Linum usitatissimum</td>
<td>omega-3 fatty acids, phytoestrogens, flavonoids</td>
<td>antioxidant, anti-inflammatory, chemopreventive</td>
<td>May decrease or prolong absorption of other drugs, prolong bleeding time</td>
<td>Y</td>
</tr>
<tr>
<td>Garlic</td>
<td>Allium sativum</td>
<td>sulfoxides, gamma-glutamylcysteines</td>
<td>Anti-bacterial, anti-viral, anti-fungal, anti-parasitic</td>
<td>Heinz body anemia, uterine stimulant, prolong bleeding time, gastric ulcers</td>
<td>Y</td>
</tr>
<tr>
<td>Ginger</td>
<td>Zingiber officinale</td>
<td>paradol, gingerol, myoga</td>
<td>anti-inflammatory, anti-thrombitic, antioxidant, anti-bacterial</td>
<td>May effect blood sugar, prolong bleeding time, gastric ulcers</td>
<td>Y</td>
</tr>
<tr>
<td>Ginseng</td>
<td>Panax ginseng, Panax quinquefolius, Eleutherococcus senticosus</td>
<td>ginsenosides, essential oils, phytosterols</td>
<td>anti-inflammatory, antioxidant</td>
<td>May interfere with drugs processed by liver enzymes, potentate diuretics, decrease blood sugar, prolong bleeding time</td>
<td>N</td>
</tr>
<tr>
<td>Valerian</td>
<td>Valeriana fauriei, V. officinalis, V. edulis, V. wallichii</td>
<td>valerenic acid, iridoid glycosides</td>
<td>Sedative, anti-spasmodic</td>
<td>May enhance effect of tranquilizers and anesthetics, may be prohibited substance, cause diarrhea and colic</td>
<td>N</td>
</tr>
<tr>
<td>Yucca</td>
<td>Yucca schidigera</td>
<td>saponins, resveratrol, yuccaols A-E</td>
<td>anti-inflammatory, antioxidant, anti-spasmodic, anti-platelet</td>
<td>May potentate NSAID’s, cause diarrhea</td>
<td>N</td>
</tr>
</tbody>
</table>
**Bee Pollen and Propolis**

Bee pollen and propolis are similar resinous substances collected from various plant sources by honeybees. Propolis has been found to contain polyphenols, flavonoids, as well as several specific antioxidant compounds including beta-carotene, caffeic acid, kaempferol, and phenethyl caffeate, p-hydroxyacetophenone, benzylhydroxybenzoate, coumaric and cinnamic acid (Ahn et al., 2004; Gomez-Caravaca et al., 2006; Christov et al., 2006). The composition of propolis varies greatly as a result of collection from different geographic regions, the time of collection, and various species of vegetation from which the pollen is collected. Reported biological properties include antioxidant, antimicrobial, antifungal, anti-inflammatory, and immunoregulatory actions, as well as being a nearly nutritionally complete foodstuff (Liebelt and Calcagnoti, 1999).

Propolis with strong antioxidant activity, as determined by beta-carotene bleaching, 1,1-diphenyl-2-picrylhydrazyl free radical scavenging, and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assays, was also found to have high total polyphenol content (Ahn et al., 2004). Additionally, propolis and polyphenolic compounds derived from propolis were found to have immunomodulatory effects evidenced by decreased pulmonary tumor nodules in mice with experimentally transplanted tumors (Orsolic et al., 2004). Tichy and Novak (2000) found a mixture of antimicrobial compounds in ethanol extracts of propolis that were effective in inhibiting *viridans Streptococci*. Furthermore, several different propolis samples were found to exhibit significant antimicrobial activity against gram-positive bacteria and yeasts (Uzel et al., 2005). Anti-inflammatory effects of propolis were found in a mouse paw edema model in which nitric oxide inhibition occurred after propolis administration (Tan-No et al., 2006). Ethanol and water extracts of propolis were effective in reducing inflammation purportedly through the inhibition of prostaglandin E2 and nitric oxide levels in ICR and Wistar rats with induced edema, pleurisy, and acute lung damage. Additionally, these same rats with induced arthritis exhibited reduced interleukin (IL)-6 in inflamed tissues after administration of propolis extracts (Hu et al., 2005).

Little research has been done to evaluate the efficacy of bee pollen or propolis supplements in horses. There are numerous anecdotal reports of the benefits of supplemented bee pollen in horses including improved oxygen utilization, lower heart rates, and firmer muscle tone (Turner et al., 2006). A recent pilot study in horses examined the effects of bee-pollen based supplementation on physical fitness parameters, immunological status, and nutritional variables in Arabian horses in training. Results indicated that supplementation with a commercial 55 % bee-pollen supplement for 42 days did not alter physical fitness or immunological variables in the horses. However, supplementation did significantly increase feed intake and nutrient retention in the same horses (Turner et al., 2006). Similar results were seen in rats supplemented with propolis showing increased weight gains, improved utilization of iron, increased calcium and phosphorus absorption, and improved regeneration efficiency of hemoglobin (Haro et al., 2000).

**Devil’s Claw**

Devil’s claw (*Harpagophytum procumbens*) is reported to have an anti-inflammatory effect in humans and laboratory animals. On the animal health market devil’s claw is primarily used for its painkilling and anti-inflammatory properties, and has many testimonials claiming relief from rheumatism and other joint disorders (Anon, 2003). Its effectiveness could be dependent upon the route of administration and may not be effective in the form of an intra-peritoneal injection. The active ingredients are various iridoid glycosides, acetylated phenolic glycosides, and terpenoids. Studies have shown that extracts with > 50 mg of harpagoside (a glycoside) per day are helpful in alleviating lower back pain in humans (for review see Chrubasik et al., 2002). Most of the human clinical studies reported a decrease in pain intensity and an increase in flexibility after being supplemented with devil’s claw extract.

Studies in laboratory animals have shown that topical application of devil’s claw decreases the expression of cyclooxygenase (COX)-2, which is a rate-limiting enzyme involved in the inflammatory cascade (Kundu et al., 2005). However, it was not clear in this study as to which specific herbal components were contributing to this action. Harpagoside alone has been shown to
suppress COX-2 and inducible nitric oxide synthase (iNOS) at both the mRNA and protein level in vitro (Huang et al., 2006). However, it did not exhibit any inhibitory effect on COX-1 activity, thus the activity of harpagoside is not attributed to the antioxidant properties of devil’s claw. Its effectiveness in reducing pain and inflammation associated with rheumatoid and osteoarthritis can be explained by its ability to block the production of inflammatory mediators like prostaglandin E2 (PGE2; Chantre et al., 2000). A study in horses with naturally occurring osteoarthritis looked at the effect(s) of ‘Mobility’, a proprietary polyherbal composite joint supplement containing devil’s claw. An anti-inflammatory effect was observed in the horses due to a reduction in PGE2 synovial fluid content (Pearson et al., 1999).

**Echinacea**

Echinacea (Echinacea sp.), a common immunostimulant, or ‘cold fighter’, has been reported to have anti-inflammatory and antioxidant properties. The equine industry typically uses echinacea as an immune booster to complement a healthy immune system (Anon, 2003). It is recommended that the best way to use echinacea is to supplement at the first signs of illness or infection. If administered too late in the cycle the herb will be less effective.

In humans (Wagner & Jurcic, 1991) and mice (Roesler et al., 1991), echinacea extracts have been shown to stimulate phagocytosis. Other studies have demonstrated a stimulating effect on lymphocyte function and proliferation in normal and diseased human mononuclear cells (See et al., 1997).

Three main species include *Echinacea purpurea*, *E. angustifolia*, and *E. pallida*. These species have been studied for their medicinal properties and were found to have a wide range of benefits (for review see Block & Mead, 2003). Many research studies have looked at the biochemistry, immunopharmacology and clinical use of echinacea, however most of those papers are in German. The common active components of these various echinacea species include polysaccharides, glycoproteins, alkamides, and cichoric acid, which is a derivative of caffeic acid. However, it must be noted that depending on the species and commercial preparation of these products, the concentrations of these components will vary greatly. Some human studies have found that echinacea can enhance cytokine production, including tumor necrosis factor alpha (TNF-α), IL-1, IL-6, and IL-10 by macrophages (Burger et al., 1997).

One case study on two horses with strangles showed improved symptoms and a return to normal appetite after 24 hours of administration (unknown citation). Another study used eight horses that were supplemented echinacea for 42 days at a level equivalent to 1000 mg standardized extract (O’Neill et al., 2002b). They concluded that the horses treated with echinacea were immune stimulated, however results only showed increases in lymphocyte count and decreases in neutrophil count at day 35 of the 42 day supplementation period. Increases in red blood cell count and hemoglobin were also found over time.

**Flaxseed**

Flaxseed (*Linum usitatissimum*) contains high levels of omega-3 fatty acids, and is often reported to enhance a horses’ hair coat. In horses, this supplement is marketed for its high omega-3 fatty acid content and is used in coat, skin, and hoof conditioners. Flaxseed is one of the highest sources of α-linolenic acid, and also contains phytoestrogens, flavonoids, and various amino acids and minerals (Cunnane et al., 1993). Due to its soluble fiber content comparable to that of oat bran, flaxseed is used most often in humans as a laxative. Recently there have been an increased number of supplement companies incorporating flaxseed or linseed components into their products, however published research is limited.

One study tested flaxseed as a treatment for allergic skin diseases in horses and found a significant improvement in a skin test response to Culicoides or ‘sweet itch’ as compared to placebo treated horses (O’Neill et al., 2002a). Research studies in other species have reported antioxidant, anti-inflammatory, and chemopreventive properties of flaxseed and flaxseed oil. One study reported that in male Fischer rats with experimentally-induced carcinogenesis of the gastrointestinal tract, 15
Flaxseed diet supplementation significantly increased colon tissue and serum levels of omega-3 fatty acids, a decreased size and incidence of tumors, as well as decreased COX-1 and -2 levels (Bommareddy et al., 2006). Another study evaluated the antioxidant properties of flaxseed supplementation in albino rats challenged with CCI toxin. Results indicated a 1.2-fold increase in the lipid peroxidation value, increased restoration of catalase, superoxide dismutase, and peroxidase compared to CCI challenged control animals (Rajesha et al., 2006). Additionally work was done to evaluate nutrient utilization in dairy cows following flaxseed supplementation and found that total tract nutrient utilization was improved without adverse effects on ruminal fermentation (Gonthier et al., 2004).

There is some concern for cyanide poisoning in horses fed flaxseed, which is why it is commonly boiled to remove the potentially toxic cyanide components (Oomah et al., 1992). However, symptoms do not become evident for a long time and no reported symptoms were evident in the study by O’Neill. In theory, the lack of toxicity was attributed to the ability of the stomach acid to inactivate enzymes within the seeds, which are required to interact with the glycosides to form cyanide.

**Garlic**

Garlic (*Allium sativum*) has properties including anti-bacterial, anti-viral, anti-fungal, and anti-parasitic. Garlic’s active components include organosulphur compounds, which are responsible for the majority of garlic’s physiological effects. The intact bulb of the garlic plant contains a complex mixture of cysteine sulfoxides, and γ-glutamylcysteines. When the bulb is disrupted the sulfoxidas are cleaved to the active form of thiosulfinate allicin (Munday and Munday, 2001). Garlic is typically included in equine supplements for its expectorant action to help break up mucus. However, this action is secondary to the primary use of garlic in the horse industry – fly control. The sulphur content is also theorized to help cleanse the blood (Anon, 2003). In one study, the efficacy of ‘Breath’, a polyherbal composite supplement containing garlic, was evaluated in horses with naturally occurring, symptomatic chronic obstructive pulmonary disease (COPD). A significant decrease in respiratory rate was found with no deleterious effects detected in hematology and biochemistry screenings (Pearson, 2003).

Garlic extracts containing phytochemicals have been shown to have antioxidant properties in other species. The antioxidant characteristics of garlic extract manifest themselves in reactive oxygen specie (ROS) scavenging, enhancing cellular antioxidant enzyme status including superoxide dismutase, catalase and glutathione peroxidase. Garlic extract is further attributed to inhibiting lipid peroxidation, protecting DNA against free radical-mediated damage and mutations, inhibiting multi-step carcinogenesis, protecting against some forms of ultraviolet-induced immunosuppression, and preventing age related deterioration of brain function (in a senescence-accelerated mouse model) (reviewed in Borek, 2001; Thabrew et al., 2000).

Toxicity is a possibility with symptoms including gastric irritation, decreased sperm production, Heinz body anemia, and occupational asthma. In dogs, 5 g of fresh garlic kg⁻¹ increased oxidation of hemoglobin within red blood cells and decreased total hemoglobin concentration (Hu et al., 2002). Garlic consumption also led to oxidation of red blood cells in sheep (Stevens, 1984). Equine studies found that freeze dried garlic fed at > 0.4 g kg⁻¹ d⁻¹ resulted in symptoms indicative of Heinz body anaemia (Pearson et al., 2005). In this study 100 % of the horses (n = 2) fed garlic showed an increase in mean corpuscular volume and hemoglobin, Heinz body score, platelet count, serum-free and total billirubin concentration, and decreases in red blood cell count, blood and mean corpuscular hemoglobin concentration, and serum haptoglobin concentration.

**Ginger**

Ginger (*Zingiber officinale*) has been shown to have anti-thrombitic, antioxidant, anti-inflammatory, and anti-bacterial properties. In the 1970’s ginger was first found to have anti-inflammatory properties including inhibition of prostaglandin synthesis. After that time more research was completed and found that major constituents in ginger include paradol, gingerol, and myoga (for review see Grzanna et al., 2005).
[8]-Paradol, a natural constituent of ginger, has shown anti-inflammatory properties as a potent COX-1 inhibitor and anti-platelet aggregation in human whole blood. These properties make it a potential treatment for musculoskeletal disorders (Srivastava and Mustafa, 1992). Ginger has shown potential for use in cancer treatment. 6-gingerol, another natural constituent of ginger, protected human leukemic HL60 cells from oxidative stress and induced cell death in promyelocytic leukemia HL60 cells. It also caused DNA fragmentation and inhibited Bcl-2 expression. Another component of ginger, myoga (Zingiber mioga Roscoe), showed powerful cytotoxic effects on human T lymphoma Jurkat cells. Recently ginger has received attention due to anti-inflammatory properties extending beyond the inhibition of prostaglandins (for a review of this literature see Grzanna et al., 2005).

On-going studies in horses are testing a single dose of ginger on anti-inflammatory and cardiovascular effects post-exercise (Liburt, 2005). Intensely exercised horses, until the point of fatigue, administered with ginger extract one hour prior to exercise had a reduced recovery time in the fast phase of the VO2 recovery curve where the metabolic cost of exercise rapidly is replenished. On the other hand, ginger has a tendency to increase pro-inflammatory cytokines TNF-α and interferon (IFN)-γ. It was speculated that the caustic ginger extract solution irritated the gastrointestinal tract, which could be confounded by the increased creatine kinase levels seen after this administration as well (Liburt, 2005). Even though ginger has been theorized in horses and proven in humans to cause gastric ulcers, many ulcer relief herbal supplements for horses contain ginger as a major ingredient.

**Ginseng**

Ginseng (Panax sp.) is commonly studied in terms of its immunostimulating properties. It has been found to exert an inhibitory effect on IL-1β and IL-6 gene expression, decrease TNF-α production by macrophages, and decrease COX-2 expression, and suppress histamine and leukotriene release (for review see Radad et al., 2006). On the equine supplement market ginseng is marketed and sold for use in stimulating the immune system, decreasing stress, and increasing optimal performance, however no published research was found at this time.

Ginseng has three main species of interest, the Asian ginseng is Panax ginseng, the American ginseng is Panax quinquefolius, and the Siberian ginseng is correctly called “eleuthero” or Eleutherococcus senticosus (for review see Block & Mead, 2003). The main component of each of these species includes glycosidal saponins called ginsenosides. Other minor components include essential oils, phytosterols, amino acids, peptides, vitamins and minerals. Many of the ginsenosides have antioxidant properties that protect membranes of nerve and immune cells.

Studies using human immune cells have demonstrated a stimulating effect on lymphocyte function and proliferation in normal and diseased human mononuclear cells (See et al., 1997). The results from this study are consistent with other published research on its immune-stimulating properties in laboratory animals and humans.

**Valerian**

Valerian (Valeriana sp.) has tranquilizing and sedative properties due to its influences on neuromediators such as µ-aminobutyric acid (GABA; Peeters et al., 2004). There is strong scientific evidence that it decreases CNS activity in mice equal to that of Phenobarbital (Hendriks et al., 1985). Valerian is also effective in treating insomnia and other sleep disorders in humans. The mechanism of action starts with valerenic acid inhibiting the enzyme system that causes the breakdown of GABA in the brain. This respective increase of GABA is associated with sedation and a decrease in CNS activity (Riedel et al., 1982; Houghton, 1999).

The components of valerian include valerenic acids, such as monoterpenes and sesquiterpenes, and iridoid glycosides that give the root a sedative and anti-spasmodic activity. In the volatile oil component of valerian, sesquiterpenes, are responsible for its biological effect (Houghton, 1999). Valeriana fauriei, V. officinalis, V. edulis, and V. wallichii are more commonly studied species of valerian. The amount of active ingredient in each depends on the form and preparation of the product (e.g. capsule, tincture, tea, etc.). It has been determined that the highest concentration of
Valerenic acids were recovered in powder capsules, whereas the lowest amount was found in tinctures and teas (Lefebvre et al., 2004).

No known studies have been done in horses to date, but many ‘calming aids’ or ‘stress relief’ supplements include valerian as one of the major active ingredients (Anon, 2003). Caution needs to be taken when supplementing valerian however, as certain show organizations, such as the International Federation for Equestrian Sports (FEI), ban this product from use during competition.

One study evaluated the effectiveness of ‘Sedafit’, a commercial herb product containing Valeriana officinalis L. and passiflora incarnate L., in reducing the physiological response to stress in pigs undergoing transportation simulation (Peeters et al., 2004). Data showed a significantly reduced increase in cardiac response variables including heart rate, ventricular ectopic beats, and sinus tachycardia (ST) elevation. Additionally, the supplement did not affect intermediate metabolites (glucose, lactate, creatine kinase, and nonesterified fatty acids). Therefore it was suggested that the supplement is effective as a mild sedative with anti-anxiety properties (Peeters et al., 2004). Another study evaluated the tranquilizing effect of a 31.6 mg kg\(^{-1}\) dose of valeranone in rats administered an electric shock avoidance test. Results indicated a mild sedation, however not to the extent of a 10 mg kg\(^{-1}\) dose of chlorpromazine (Rucker et al., 1978).

**Yucca**

Yucca (Yucca schidigera) contains steroid-like saponins, which produce an anti-inflammatory, antioxidant, and anti-spasmodic effects to reduce pain associated with arthritis. Many equine joint supplements contain yucca among other anti-inflammatory agents. Yucca is theorized to decrease respiratory problems, such as COPD in horses. The saponins are natural detergents that form stable foams, which contain both fat- and water-soluble components. As much as 10 % of the yucca stem contains saponins making it one of the richest sources (Cheeke et al., 2006). Yucca also contains other active components including polyphenols like resveratrol and yuccaols A-E (Oleszek et al., 2001; Piacente et al., 2004). These phenols are exclusively found in the bark and are not present in the mechanical extraction of the yucca extract.

As of the late twentieth century, the only studies performed on the anti-arthritic effects of yucca were in the 1970’s by Bingham who reported that pain and swelling of human arthritic patients were relieved by yucca supplementation. The theory behind this efficacy was due to the saponins anti-protozal activity. More recently the potent antioxidant activity of the polyphenols is also thought to give yucca its anti-arthritic properties. It has been proven that yuccaols inhibit iNOS, an inflammatory agent that increases during inflammatory responses. Resveratrol along with the yucca phenols was also found to inhibit NF-\(\kappa\)B, a transcription factor that controls the expression of iNOS (Tsai et al., 1999; Marzocco et al., 2004).

Yucca has also been proven to have various anti-platelet effects. One study found resveratrol and other yucca phenolics to reduce the level of ROS in blood platelets, along with changes in the production of superoxide radicals, inhibition of free radicals activated by thrombin, and decreased lipid peroxidation (Olas et al., 2003).

At this time there are no equine studies showing the benefits of yucca supplementation, however studies have looked at ruminal fermentation and metabolism of yucca in sheep (Eryavuz & Dehority, 2004; Santoso et al., 2006) and cattle (Hristov et al., 2003). For a review on research in other animals (chickens, mice, pigs, sheep, cattle, rabbits and quail) and the biochemistry of yucca see Piacente et al., 2005.

**Other Herbs and Functional Foods**

Black tea, orange peel, and cranberry extracts have also been studied in intensely exercising horses (Liburt, 2005; Streszalova et al., 2006). Black tea contains aflavin, a polyphenol, which is a strong inhibitor of the gene expression for IL-8. Black tea extract administered prior to horses exercising until exhaustion on a treadmill, decreased mRNA expression of TNF-\(\alpha\) and IFN-\(\gamma\), but produced higher lactate levels throughout exercise (Streltsova et al., 2006). Orange peel extract
contains citrus-derived polymethoxylated flavones that have an inhibitory effect on TNF-α expression. The same study in horses found that orange peel extract decreased IFN-γ expression at fatigue, and appeared to decrease recovery time of cardiovascular parameters.

Cranberry (Vaccinium macrocarpon) polyphenols have been shown to protect endothelial cells against stress-induced up-regulation of oxidative and inflammatory mediators. Phenolics in cranberries, like quercetin and cyanidin, have highly effective radical scavenging structures. Cranberry appears to attenuate the TNF-α response, but not the appearance of IFN-γ (Liburt, 2005). This may prove useful in lessening delayed onset of muscle soreness (DOMS) following strenuous activity.

**Herb-Drug Interactions**

Many people believe that because herbs are ‘natural’ products that it also qualifies them as ‘safe’ however, evidence of various herb toxicities and negative side effects has shown this to be a dangerous misnomer. Herbs can have a drug-like action that can interact with other components in the horse’s diet. Some herbs contain prohibited substances like salicylates, digitalis, heroin, cocaine and marijuana. Drug-herb interactions can create various side effects ranging from mild to severe; thus caution needs to be taken when determining which ‘natural product’ to use.

A general review of various species and drug herb interactions can be found in Miller, 1998 and Izzo *et al*., 2005. Harman (2002) and Poppenga (2001) have written extensive reviews on the toxicology of herbs in equine medicine. Below is a list of a few known interactions or negative effects, which are also summarized in Table 1.

Valerian components have been found to prolong the action of barbiturates (Dunayev *et al*., 1987) and can interact with alcohol (Miller, 1998). It has also been shown to inhibit cytochrome P450, the body’s major detoxification enzyme, which can lead to multiple drug interactions if not used with caution (Lefebvre *et al*., 2004). Echinacea has shown that persistent use is related to hepatotoxic effects and should not be taken with other hepatotoxic drugs like steroids. Garlic, along with the potential to cause Heinz Body Anemia in horses as detailed above, was found to create gastrointestinal upset, allergic reactions and dermatitis in humans. Garlic also decreased systolic and diastolic blood pressure, however, there was insufficient evidence to recommend its use in clinical hypertension (Miller, 1998). Ginger has proven to inhibit thromboxane synthetase and increase bleeding time, which could be detrimental if used with anti-clotting drugs like warfarin. Some of ginseng’s adverse effect includes hypertension, insomnia, vomiting, headache, nervousness, sleeplessness, and epistaxis in humans. It is also recommended when utilizing ginseng, to discontinue use of warfarin, heparin, aspirin, and other NSAIDs (Miller, 1998; Poppenga, 2001).

**Summary**

In conclusion various herbs are being used in the equine industry, some of these include bee pollen, devil’s claw, echinacea, flaxseed, garlic, ginger, ginseng, valerian and yucca. Despite many anecdotal reports of efficacy, most of the herbal supplements have never been proven safe and effective in horses; therefore caution must be taken when selecting a supplement. Herb drug interactions are also a potential problem and if a horse is at risk of developing a potential toxicity or drug interaction, a veterinarian or nutritionist should be contacted.

**References**


Summary

The electrolytes sodium, chloride, and potassium are critical for the health of the horse. Deficiencies tend to be rare for most horses as access to common salt (NaCl) and a forage-based diet typically provide adequate amounts of these minerals. In circumstances where sweat production is great, inadequate salt consumption and diets low in forage may necessitate the supplementation of these electrolytes as horses can lose substantial amounts of the electrolytes through sustained sweating. When electrolytes are properly provided, they should encourage a horse to drink more water. When provided improperly, horses may not drink sufficiently and may remain dehydrated. If electrolytes are provided in the water, the supplemented water should be provided immediately after exercise before a horse has had time to cool out and the drive to drink is diminished. If a horse has cooled out and if electrolytes are provided in the water, the recommendation would be to also provide unsupplemented water in case the horse refuses to drink the water supplemented with electrolytes. Other approaches to supplement horses include adding the additional electrolytes in the concentrate portion of the diet or to provide them in an oral drench. If commercial preparations are given, it is important to look at how much they provide compared to how much the horse needs. Often, the actual concentration of electrolytes is quite low, thus, making them a poor option. A more cost effective approach is simply to provide a mixture of common salt (NaCl) and lite salt (KCl). Regardless of technique for supplementation, it is also critical to allow hot horses to drink water to encourage rapid rehydration. While some care should be taken to limit the rate at which a hot horse is allowed to drink, allowing the horse to cool down before drinking appears to decrease the total amount of water drunk.

Introduction

Thermoregulation is an important part of exercising. When glycogen and fat are utilized during exercise, a relatively small proportion goes toward the production of ATP while upwards of 80% of the energy is released in the form of heat. While a small increase in the temperature of muscle is advantageous in terms of increased enzyme activity, enhanced muscle performance, and enhanced oxygen delivery, too great of an increase is detrimental to performance, as well as possibly to the overall health of a horse. A primary way in which a horse dissipates extra heat is through evaporative cooling that is typically facilitated through sweating. As a horse sweats, the horse has the potential to lose substantial amounts of water and electrolytes (sodium, chloride, and potassium). Whereas the sweat of humans becomes more dilute with ongoing exercise (effectively conserving electrolytes), the composition of equine sweat remains relatively unchanged as exercise continues. Therefore, providing adequate amounts of electrolytes in the diet or through supplementation is critical. That being said, many individuals may supplement more than needed or, supplement without sufficient knowledge of the horse’s requirements and, thus, may pay a lot for supplements that provide only a tiny fraction of the amount needed by the horse. Additionally, because many people are afraid of having their horse colic or founder, they often fail to water hot horses adequately. Withholding water may result in horses failing to rehydrate properly and could limit upcoming performance.
Sodium

Sodium is the major extracellular cation and plays a major role in maintaining the osmotic regulation of body fluids (NRC, 2007). It is important in acid-base balance and is involved in the generation of action potentials in excitable tissues (Johnson, 1995). As a result, the normal function of the central nervous system is dependent upon it. It is found in greatest quantities in the skeleton, with large amounts also being found in the ingesta, blood, muscle, and skin (Meyer, 1987). If horses are chronically sodium depleted, they tend to decrease their water intake and eventually will quit eating (Meyer et al., 1984). Acute sodium deficiency results in a lack of muscle coordination. Fortunately, as long as plenty of water is available, there does not seem to be a problem with sodium toxicity and the maximum tolerable concentration of sodium chloride in the diet has been set at 6% of the dietary intake (NRC, 2005).

The absorption of sodium from the diet is quite high. Schryver et al. (1987) reported a range of absorption between 75 to 94% and Reynolds et al. (1998) reported an apparent absorption rate of almost 100%. Though the absorption rate is high, many natural equine feedstuffs are quite low in sodium. As a result, sodium chloride is often added to manufactured concentrates at rates from 0.5 to 1.0% (NRC, 2007). Additionally, most individuals provide some sort of salt free-choice – either in a block form or as a loose salt. Given that horses have an appetite for sodium, providing such access to it helps most horses meet their needs though it is not guaranteed that they will. Jansson and Dahlborn (1999) reported that consumption from a salt block was sometimes not adequate to meet even maintenance requirements and emphasized supplementation should be considered especially in exercising horses that have substantial sweat losses. Supplementation of exercising horses has also been promoted for the benefits in maintaining hydration (Sosa Leon et al., 1998; Butudom et al., 2002). However, before determining if supplementation is necessary, the first step is to compare the amount provided in the diet (not including intake from free-choice salt) to the amounts recommended. If the recommended amounts are being fed, there is no reason to supplement with additional sodium; however, providing access to free-choice salt would still be a relatively inexpensive practice that could help to eliminate any unanticipated deficiencies.

Dietary Sodium Recommendations

The 2007 NRC has provided recommendations for the minimum amount of sodium that horses should consume daily. Below are the equations used to predict the requirements of various categories of horses, as well as the assumptions that were used when deriving these equations such as the amount of sodium lost as endogenous losses, as well as the rate at which sodium is absorbed.

Daily Sodium Requirements and Recommendations:
Maintenance = 0.02 g x kg BW
(to meet endogenous losses of 18 mg Na/kg BW with a 90% absorption rate)

Growth = (0.02 g x kg BW) + (1.0 g x ADG in kg)
(to meet endogenous losses of 18 mg Na/kg BW with a 90% absorption rate and to meet growth requirements of 0.85 g Na/kg BW gain with an 80% absorption rate)

Pregnancy (months 9, 10, 11) = (0.02 g x kg BW) + (0.002 g x kg BW) = (0.022 g x kg BW)
(to meet endogenous losses of 18 mg Na/kg BW with a 90% absorption rate and to meet fetal deposition rate of 1.9 mg Na/kg BW [Drepper et al., 1982])

Lactation:
Foaling to 3 months = (0.02 g x kg BW) + (0.032 x kg BW x 0.17)
(to meet endogenous losses of 18 mg Na/kg BW with an absorption rate of 90% and to meet milk production needs estimated at 0.032 kg milk per kg BW containing 0.153 g Na/kg that is absorbed at a 90% rate)
4-5 months = (0.02 g x kg BW) + (0.026 x kg BW x 0.14)  
(to meet endogenous losses of 18 mg Na/kg BW with an absorption rate of 90 % and to meet milk production needs estimated at 0.026 kg milk per kg BW containing 0.4 g Na/kg that is absorbed at a 90 % rate)  

> 5 months = (0.02 g x kg BW) + (0.020 x kg BW x 0.14)  
(to meet endogenous losses of 18 mg Na/kg BW with an absorption rate of 90 % and to meet milk production needs estimated at 0.020 kg milk per kg BW containing 0.4 g Na/kg that is absorbed at a 90 % rate)  

Exercise = (0.02 g x kg BW) + (3.1 g x BW loss in kg during exercise)  
(to meet endogenous losses of 18 mg Na/kg BW with an absorption rate of 90 % and to meet additional requirement for work associated with sweat loss of 2.8 g of Na/kg of BW loss as an estimate of sweat loss during exercise with a 90 % absorption rate)  

Of these equations, several require knowing such things as the rate of gain of growing horses or the body weight loss during exercise. While not difficult to measure, the NRC committee recognized many individuals will not make those measurements and, thus, provided estimates of these variables to determine nutritional requirements in a computer program that is available for free at http://nrc88.nas.edu/nrh.

**Chlorine**  
In the diet, chlorine is normally found accompanying sodium as chloride. Chloride is an extracellular anion that is involved in acid-base balance and osmotic regulation. The 1989 NRC did not establish a chlorine requirement as it was recognized that a chlorine deficiency is unlikely to occur without a sodium deficiency (Lewis, 1995). However, a chlorine deficiency has been linked to metabolic alkalosis (Coenen, 1988, 1991). As with sodium, as long as adequate water is available, a chlorine toxicity would not be expected though, if developed, likely would result in issues with the central nervous system.  

Virtually all chloride consumed is absorbed (Schryer et al., 1987). Chloride concentrations in feedstuffs have been reported to vary from 0.05% for corn and soybean meal up to 3% for molasses (NRC, 1982). By comparison, common salt is 61% chloride and is typically used to meet chlorine needs. If electrolyte supplementation occurs, it is typically done with sodium chloride figuring as a major ingredient.

**Dietary Chlorine Recommendations**  
Though the chlorine requirements of the horse have not been as strongly established as many other minerals, there was sufficient evidence from which the NRC committee could develop the requirements for the various horse categories. Like with sodium, the equations for determining the minimal amount to provide daily in the diet are listed below, along with the assumptions and basis for the equations. Furthermore, estimates of growth and weight loss during exercise are provided by the 2007 NRC’s computer program for individuals who do not have those values.

**Daily Chlorine Requirements and Recommendations:**

**Maintenance** = 0.08 g x kg BW  
(to meet fecal, renal, and cutaneous endogenous losses, perspiration losses, and to prevent changes in acid-base balance and the development of hypochloremia)  

**Growth** (up to six months) = (0.08 g x kg BW) + (0.013 g x kg BW)  
(to meet maintenance requirements and to meet growth requirements; Coenen, 1999)
Growth (six to 12 months) = (0.08 g x kg BW) + (0.005 g x kg BW)  
(to meet maintenance requirements and to meet growth requirements; Coenen, 1999)

Growth (12 to 24 months) = (0.08 g x kg BW) + (0.0025 g x kg BW)  
(to meet maintenance requirements and to meet growth requirements; Coenen, 1999)

Pregnancy (months 9, 10, 11) = (0.08 g x kg BW) + (0.002 g x kg BW) = (0.082 g x kg BW)  
(to meet maintenance requirements and to meet additional pregnancy requirements; Coenen, 1999)

Lactation = (0.08 g x kg BW) + (0.011 g x kg BW) = (0.091 g x kg BW)  
(to meet maintenance requirements and to meet additional lactation requirements; Coenen, 1999)

Exercise = (0.08 g x kg BW) + (5.3 g x BW loss in kg during exercise)  
(to meet maintenance requirements and to meet additional requirement for work associated with sweat loss of 5.3 g of Cl/kg of BW loss as an estimate of sweat loss during exercise with a 100 % absorption rate)

Potassium

Potassium is the major intracellular cation and is involved in acid-base balance, osmotic pressure, and neuromuscular excitability (Kronfeld, 2001). Most potassium within the body is found in skeletal muscle (Johnson, 1995) with only a small amount being found in the blood (Meyer, 1987). A potassium deficiency has been shown to cause a lack of appetite, weight loss, and an unthrifty appearance (Stowe, 1971). Toxicities are rare as extra potassium is excreted readily through the urine when sufficient water is available. When water is restricted, horses are believed to quit eating if potassium concentrations are too high, negating the possibility of developing a potassium toxicity. Though the maximum tolerable concentration of potassium is listed as 1% of intake by the NRC (2005), many forages have a much greater concentration of potassium and are commonly fed to horses without issues.

The absorption of potassium is somewhat variable ranging from about 60% (Pagan, 1994) to nearly 100% (Reynolds et al., 1998). Likewise, there is a wide range in the concentrations in feedstuffs with cereal grains often containing 0.3 to 0.4% potassium and forages often containing between 1 and 2% potassium (NRC, 2007). As a result, most horses easily meet their potassium requirements through the forage they consume. Only in horses that sweat excessively, or those that consume little forage, would supplementation typically be warranted.

Dietary Potassium Recommendations

Much more work has been done to determine the potassium requirements of horses than has been done with chlorine. Again, as with sodium and chlorine, below are the equations derived by the NRC committee to establish the amount of potassium that should be provided daily to horses of various categories along with the factors that were used to establish those recommendations.

Daily Potassium Requirements and Recommendations:

Maintenance = 0.05 g x kg BW  
(to meet endogenous losses of 40 mg K/kg BW with an 80 % absorption rate)

Growth = (0.05 g x kg BW) + (3.0 g x ADG in kg)  
(to meet endogenous losses of 40 mg K/kg BW with an 80 % absorption rate and to meet growth requirements of 1.5 g K/kg BW gain with a 50 % absorption rate)
Pregnancy (months 9, 10, 11) = (0.05 g x kg BW) + (0.0017 g x kg BW) = (0.0517 g x kg BW)
(to meet endogenous losses of 40 mg K/kg BW with an 80 % absorption rate and to meet fetal deposition rate of 1.36 mg K/kg BW with an 80 % absorption rate)

Lactation:
Foaling to 3 months = (0.05 g x kg BW) + (0.032 x kg BW x 1.4)
(to meet endogenous losses of 40 mg K/kg BW with an absorption rate of 80 % and to meet milk production needs estimated at 0.032 kg milk per kg BW containing 0.7 g K/kg that is absorbed at a 50 % rate)

4-5 months = (0.05 g x kg BW) + (0.026 x kg BW x 0.8)
(to meet endogenous losses of 40 mg K/kg BW with an absorption rate of 80 % and to meet milk production needs estimated at 0.026 kg milk per kg BW containing 0.4 g K/kg that is absorbed at a 50 % rate)

> 5 months = (0.05 g x kg BW) + (0.020 x kg BW x 0.8)
(to meet endogenous losses of 40 mg K/kg BW with an absorption rate of 80 % and to meet milk production needs estimated at 0.020 kg milk per kg BW containing 0.4 g K/kg that is absorbed at a 50 % rate)

Exercise = (0.05 g x kg BW) + (2.8 g x BW loss in kg during exercise)
(to meet endogenous losses of 40 mg K/kg BW with an absorption rate of 80 % and to meet additional requirement for work associated with sweat loss of 1.4 g of K/kg of BW loss as an estimate of sweat loss during exercise with a 50 % absorption rate)

Water Requirements

Determining the specific requirements for water can be challenging given that the demands can vary greatly depending on environmental conditions. Horses in a hot climate likely require more than horses in a thermoneutral or cold environment. Likewise, other dietary constituents, such as salt and even just dry matter intake, can greatly alter water requirements (Jansson and Dahlborn, 1999). Furthermore, the amount of water the horse receives from its diet alters the amount of liquid water it needs. When lush pasture is available, horses can receive a large portion of their water requirement from the forage they consume. Despite these difficulties, the 2007 NRC estimated the maintenance requirement for water of a 500-kg horse kept at thermoneutral temperatures and receiving a hay diet at the rate of 1.5 kg/100 kg of BW to range from 21 to 29 L/day. Heavily exercising horses can be expected to have a two- to three-fold increase in water intake compared to maintenance to accommodate the sweating needed for heat dissipation. It is these heavily exercising horses that have lost a substantial amount of water (and electrolytes) through sweat, for which dehydration can cause problems because of the well-meaning intentions of individuals who fail to provide water and electrolytes properly.

Supplementation of Electrolytes to Maintain Hydration

For most horses, providing a common salt block and good quality forage should meet the requirements for sodium, chlorine, and potassium. In most cases, it is only the heavily exercising horse that will require supplementation. For those horses, there are several ways to provide extra electrolytes. They can be provided to horses in their drinking water, mixed with their grain, or provided as an oral drench. Both of the latter two techniques work pretty well in guaranteeing the horse receives the additional electrolytes. However, providing it in the water often will not provide adequate results. Unless supplemented water is provided while the horse is still hot and has not cooled down, the horse may not drink much of it. However, providing it while the horse is still hot and before it has been given unsupplemented water has been shown to actually increase the total amount of water a horse will drink.
(Butudom et al., 2002). By contrast, if a horse is supplied with electrolyte-supplemented water after the horse has cooled down, the salinity may discourage water consumption and the horse may stay dehydrated.

**Evaluating Supplements**

Another important point about electrolytes is that if one is using a commercially-prepared dose, such as what is often sold in tack or feed stores, it is highly suggested that one look at the ingredients to see the amount of electrolytes that are actually provided and compare them to what the horse requires or to what is lost during exercise. Dr. Hal Schott, of the Michigan State University College of Veterinary Medicine, provides a nice comparison when he shows that an endurance horse producing 25 L of sweat during a 45 km exercise test would lose about 175 g of NaCl and about 55 g of KCl. One commercially prepared electrolyte replacement provided 0.625 g NaCl, 0.5 g of a K-complex, and 7.5 g of glucose per a 34-g dose. To replace the amount lost in sweat, it would take 128 doses at a cost of $4.69 per dose for a total cost of over $600. A very inexpensive alternative would be a mixture of 1 ounce (28 g) of salt (NaCl) and 1 ounce (28 g) of lite salt (KCl) per hour of exercise. Simply put, even if a horse needs electrolyte supplementation, buying a commercially-prepared supplement might be an extremely costly way to provide the needed electrolytes. Even worse, if given at the normal dosing rate, the amount of electrolytes provided may simply be insufficient to actually compensate for the amount lost through sweating.

**Conclusions**

Fortunately, for most horses, the sodium and chlorine requirements are easily met by allowing the horse daily access to some form of common salt – either in a salt block or as loose salt. Furthermore, the forage consumed by most horses has sufficient potassium to far exceed the minimum requirements of most horses. However, horses that produce a lot of sweat, either because of hot environmental conditions or because of exercise, may certainly need supplemental electrolytes. Unfortunately, besides being expensive, the concentration of electrolytes in many commercially-prepared formulas do not add much to the overall intake of electrolytes so care should be taken in comparing the amount provided by a supplement to the amount needed by a horse. Furthermore, it should be emphasized that though electrolyte balance is important, something else that is commonly neglected is the importance of providing water to hot horses. While it might not be advisable to let a hot horse drink all it wants in one bout (though this is routinely done with endurance horses without any issues), allowing a horse to drink all it wants before it has cooled down is a very important management practice that can help avoid problems with dehydration.

**References**


CRITICAL REVIEW OF RESEARCH EVALUATING GLUCOSAMINE-BASED NUTRACEUTICALS FOR TREATMENT OF JOINT PAIN AND DEGENERATIVE JOINT DISEASE IN HORSES

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Summary

It was more than 25 years ago that two German researchers quietly associated supplementation of glucosamine to horses with improvement of joint disease. In the 25 years since, glucosamine and its related chemical chondroitin have become the most extensively used non-allopathic treatment for articular inflammation and arthritis in horses. While the practice of supplementing arthritic horses with these products has, in earlier times, been considered a complete unknown in terms of safety and efficacy, the story is quite different today. An expanding body of knowledge on glucosamine continues to raise scientific curiosity about the general principles and cellular basis of treating equine osteoarthritis and inflammation with glucosamine-based nutraceuticals. This review critically interprets the published in vivo studies designed to investigate glucosamine-based nutraceuticals for horses with joint pain and/or inflammation. These studies have contributed valuable preliminary information as to the possible usefulness of glucosamine-based nutraceuticals in horses, and demonstrate an encouraging trend to manufacturers of these products investing in research. Importantly, however, these studies have significant limitations that until now have not previously been addressed. For example, measures of bioavailability in horses have not acknowledged fundamental binding of glucosamine to serum proteins and as such likely provide gross underestimates. Other more general limitations include underpowered studies, unbalanced research design, non-existent or inappropriate controls, unclear or non-representative experimental conditions and uncharacterized or complex, heterogeneous experimental materials. Shortcomings such as these have heavily influenced interpretation, as well as misinterpretation, of results. Because of these substantial limitations, the existing data on glucosamine-based nutraceuticals for horses cannot be considered conclusive evidence supporting the efficacy of glucosamine-based nutraceutical products. Recommendations are made as to minimum scientific requirements for future research into glucosamine-based nutraceuticals for horses.

Introduction

Lameness is among the most important causes of poor performance in racing horses (Verheyen and Wood 2004). While non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids remain important therapeutic resources for treatment of overt clinical lameness, nutraceuticals are becoming widespread as therapeutic and prophylactic management strategies for horses with low-grade, sub-acute articular damage and for those at risk of developing articular problems (Trumble, 2005; Neil et al., 2005). It was more than 25 years ago that two German authors quietly associated supplementation of glucosamine to horses with improvement in clinical signs of joint disease (Jaeschke and Steinbach, 1982). In the 25 years since, glucosamine and its related chemical chondroitin have become the most extensively
used non-allopathic treatment for articular inflammation and arthritis in horses (Trumble, 2005). The contemporary scientific literature abounds with new papers almost daily on glucosamine sulphate (GS), glucosamine hydrochloride (GH) and chondroitin sulphate (CS) for treatment of cartilage inflammation using a wide variety of animals, including humans. With this expanding body of knowledge on glucosamine arises a new scientific curiosity about the general principle of, and cellular basis for, treating equine arthritis and inflammation with glucosamine-based nutraceuticals.

In an industry well-accustomed to extrapolating in vitro data and data generated from research in non-equine species, it is encouraging to see scientific research appearing that attempts to directly investigate anti-arthritic nutraceuticals in horses. Importantly, these publications illustrate an encouraging trend to equine supplement manufacturers investing in product research to demonstrate safety and/or efficacy. Often, however, there are methodological and/or analytical compromises made in these investigations which impact the interpretation of the data and limit the conclusions that can be drawn. The purposes of this paper are to provide a critical review of the in vivo equine literature published between 1994 and 2006 relevant to the use of glucosamine-based nutraceuticals, and to make recommendations for minimal essential criteria that should be adopted in future equine research in this area.

**In vivo Studies in Horses**

It is important that the experimental model chosen to test nutraceutical products provides a reasonable representation of the pathophysiological condition for which it is (to be) used as a prophylaxis or a treatment. For this reason, an early report of glucosamine-based nutraceuticals in horses (White et al., 1994) was not particularly useful for furthering our understanding of the effect of dietary glucosamine-based nutraceuticals for horses. However, White et al. (1994) made an important point with respect to the type of study designs that are not well suited to the assessment of nutraceutical products. The authors evaluated the effect of a dietary nutraceutical “Cosequin” (Nutramax; 9g twice daily for 30 days) on inflammation induced by intra-articular injection of Freund’s Complete Adjuvant (FCA). Cosequin is a dietary product formulated for performance horses, and is composed of glucosamine hydrochloride (GH; 60%), chondroitin sulphate (CS; 20%), manganese (0.5%), ascorbate (3.5%) and undisclosed filler (16%). The authors concluded that Cosequin had no inflammatory effect in an equine model of inflammation induced by intra-articular FCA. FCA is a concentrated formulation of mycobacteria (usually *Mycobacterium tuberculosis*) in mineral oil. Injection of FCA results in a large increase in tumour necrosis factor-alpha (TNF-α) (Geboes et al., 2007), pronounced effusion and neutrophilia (Levy et al., 2000) and marked and sustained lameness (grade 4, AAEP scale; Toutain and Cester, 2004; White et al., 1996). It is highly likely that this very rapid onset and severe model of inflammation overwhelmed any inflammatory pathways that had the potential to be inhibited by dietary Cosequin. This model of inflammation did not emulate the condition for which the product was intended; powerful drugs are typically used to treat acute, severe inflammation. In contrast, dietary nutraceuticals are generally applied as a preventative and/or to treat low-grade, chronic lameness (Trumble, 2005). If a horse turned up lame in a stable to a similar magnitude as that induced by intra-articular FCA, it is extremely unlikely that horse would be treated with a dietary nutraceutical. Thus a negative result in this model does not further our understanding of the effectiveness of the product within a context of intended use. A second important limitation of this study is that, while the composition of the experimental product was provided, there was no confirmatory analysis of product label composition conducted by the investigators. This is not to suggest that the label information for the product was inaccurate; rather it is to demonstrate independently that the information is not inaccurate. A well-known characteristic of the nutraceuticals industry is one of inconsistent quality control (Oke et al., 2006), and confirmatory analysis of experimental products should become standard due diligence on the part of investigators.

In contrast to the White study, authors of a subsequent in vivo study of Cosequin concluded that the product was beneficial in arthritic horses (Hanson et al., 1997). Twenty-five horses with degenerative joint disease received 9 g (for horses < 500 kg) or 12 g (for horses > 500kg) of Cosequin twice daily for 6
weeks, and the authors reported improvements in lameness grade and stride length. The most important limitation to this study is that there was no control group maintained under similar conditions in the absence of supplementation with Cosequin. Thus it is not possible to determine whether the improved outcomes were due to normal healing as a function of time, reduced activity, supplementation with Cosequin, or to other confounding factors. Indeed, many cases of lameness in performance horses improve over time without any treatment at all (Ross et al., 1999), which suggests that the null hypothesis tested in this study (i.e. “baseline measurement and the following repeat examinations are the same”) had a good chance of being rejected even in the absence of any treatment. Some important discussion points missing from this study include the duration and aetiology of lameness of the experimental horses, records of exercise and/or rest after inclusion of horses in the study, and potential influences of differing diets or management strategies for the horses over the course of the study. Without at least this information the experimental data provide little useful information on the efficacy of the supplement. Unilateral study designs (without appropriate control groups) such as this can provide some useful information. But importantly, balanced interpretation is critical in that researchers need to acknowledge potential confounding factors to the reader in order to support the interpretations and conclusions made. So, like the White et al. (1994) study, the experimental design of this study prohibits a conclusion addressing whether Cosequin actually does work in horses or not.

Caron et al. (2002) reported the in vivo effect of GH on serum markers of bone [osteocalcin (OC) and pyridinoline crosslinks of type-I collagen (PYD)] and cartilage [keratan sulphate (KS)] metabolism. Sixteen juvenile Standardbreds in early race training completed the 48-week study. Nine treatment horses received 4g GH twice per day and 7 control horses received 4 g glucose twice per day. GH had no effect on serum concentrations of OC, PYD or KS and, as such, the authors concluded that they were not able to obtain evidence that GH influences metabolism of bone or cartilage in growing and exercising horses. A number of factors influence the interpretation of these data. The choice of glucose as a placebo may be questioned because glucose can influence type I collagen expression in vitro (Zhang et al., 2007). Although the small amount of glucose fed probably did not have a marked effect in the Caron et al. (2002) study; however there are no data in the literature to confirm this. The control group in this study should have demonstrated a significant decline in PYD over the time course of the protocol, an effect previously demonstrated in young exercising horses (Brama et al., 2000) and growing chickens (Pedrini-Mille et al., 1988). The fact that this decline was not observed in either the control or treatment group suggests that either the study was underpowered or that both the treatment (glucosamine) and the control (glucose) influenced this particular dependent variable. This raises a major concern regarding the absence of control groups for stage of growth or activity level of the experimental animals. The authors acknowledge that they did not use a sedentary and/or mature control group for this study, but they inappropriately dismiss this as unimportant to interpreting outcomes of the study. In fact, having no such controls leaves the authors and readers with no way in which to tease out effects of growth and/or exercise from effects of glucosamine (or placebo) on the serum levels of these biomarkers. The authors chose a model (exercise training) expected to significantly elevate OC (Smith et al., 2007) and keratan sulphate (Yoon and Halper, 2005); such an elevation has the potential to mask any stimulatory effects of glucosamine on these biomarkers. An experiment quantifying the effect of glucosamine on steady-state PYD, KS and OC, even in vitro, would be a useful prefix or adjunct to this study, and would improve the interpretations that can be made from it.

In an 8-year study of 10 show hunter-jumpers (2 years prior to treatment + 6 years of dietary treatment of 10 g/day of an undisclosed glucosamine-based nutraceutical product), Rodgers (2006) reported a significant reduction in the frequency of intra-articular injection of hyaluronan and steroid from an average of 1.7 to 0.85 injections per year. Also, the mean duration between injections increased from 6.8 to 10.0 months after 2 or more years of supplementation, after which there was no additional improvement. Rodgers (2006) concludes that the dietary glucosamine-based nutraceutical decreases the need for distal tarsal joint injections to maintain soundness. This study did very well in considering a number of confounding factors, appeared to have good control over lameness evaluations, and was long
term. But the study included only a small number of horses, and lacked any parallel control group. As such, it cannot be considered “proof” that the benefits noted over time accrued primarily as a result of supplementation with the glucosamine–based nutraceutical.

Forsyth et al. (2006) presents the most recent efficacy report of a glucosamine–based nutraceutical “Synoquin” for improving articular function in horses. In this study, 20 mixed breed, mixed age elderly geldings and mares were randomly allocated to treatment (n=15) or control (n=5) diet for 12 weeks. Treatment and control diets contained Synoquin (VetPlus) or an equal amount of ‘filler’ – the composition of which is not disclosed in the paper. Synoquin is composed of chondroitin sulphate (CS — 19% w/w), GH (50%), N-acetyl-D-glucosamine (5%) and filler (25%). The label composition was not confirmed by the investigators. Given the heterogeneous profile of the horses and the unbalanced allocation of horses to treatment and control groups, use of random assignment of horses to the groups is a limitation of this study. Allocation of horses to the control group should have been stratified to be representative of horses in the treatment group; this is particularly important because the control group (n=5) was substantially smaller than the treatment group (n=15). Each horse in the control group should have represented characteristics of 3 horses in the treatment group. Matching of control horses by sex, age, breed, and/or body mass with treatment horses would have improved the validity of the of the data obtained. Furthermore, the baseline characteristics of horses allocated to each group should have been provided, particularly as the data were only reported as mean change from baseline. Without any information on what the baseline data were for each group it is not possible to determine whether the results indeed reflect an improvement due to treatment, as concluded by the authors, or if the differences were simply artefacts of differing baselines. The authors do report that the data were of equal variance, but presumably it was the transformed data (i.e. ‘change from baseline’) that were of equal variance, and not the actual baseline means from each group. This is a major limitation which substantially limits the conclusions that can be drawn from the data.

Bioavailability and Pharmacokinetics

Bioavailability may be defined as the total amount of ingested substance that is absorbed across the gastro-intestinal tract into the blood for distribution to tissues. Bioavailability of glucosamine is inherently difficult to measure accurately and, in general, these difficulties often result in underestimates of bioavailability. Important considerations when evaluating bioavailability of glucosamine in any species include: (1) there is substantial first pass removal of glucosamine by intestinal epithelial cells and the liver in all mammals which has been quantified (humans, dogs and rats; reviewed by Setnikar and Rovati, 2001, Anderson et al. 2005); (2) the parent compound or its metabolites may be metabolized by cells of the blood, gastrointestinal tract and/or the liver; (3) glucosamine is very rapidly bound to plasma globulins, such that less than 1% of glucosamine remain free in plasma. Therefore when plasma proteins are precipitated prior to analysis of the supernatant for glucosamine, the glucosamine may effectively be precipitated along with the protein resulting in net analysis of only free glucosamine. Thus, it may be erroneously concluded that the bioavailability of glucosamine in horses is very low (2.5 or 5.9%; Du et al., 2004 and Laverty et al., 2005, respectively). In this regard it is important to note that even though glucosamine is transported within the circulation bound to globulins, it does appear to remain in dynamic equilibrium with free plasma glucosamine and hence is capable of being rapidly extracted by virtually all cells receiving nutritive blood flow. In humans, dogs and rats the absolute oral bioavailability of glucosamine, based on the globulin-incorporated radioactivity of $^{14}$C-labelled glucosamine, is 40-45%, with g.i. absorption as high at 88% of the administered dose (Setnikar and Rovati, 2001).

There are other inherent difficulties in studying the bioavailability and kinetics of glucosamine and glucosamine-based nutraceuticals. Glucosamine is an endogenous substance that can be formed within cells using glucose as a precursor. Due to its structural similarity to glucose, it is a competitive inhibitor of glucose transport, probably via GLUT2 in hepatocytes (Uldry et al., 2002) or GLUT3 in chondrocytes (Windhaber et al., 2003). Glucosamine is taken up by virtually all cell-types in the body,
leading to apparent volume of distribution that exceeds total body water (Setnikar and Rovati, 2001; Anderson et al., 2005). Rapid tissue extraction of glucosamine could in and of itself contribute to the very low plasma concentrations measured by Du et al. (2004) and Laverty et al. (2005). But because ~99% of circulating glucosamine is bound to globulin, and lost from the plasma phase with current deproteinizing methods, highly sensitive analytical techniques are necessary to track free plasma glucosamine kinetics after oral dosing; techniques that have only recently become available.

The first report of GH and CS bioavailability in horses (Du et al., 2004) was composed of 2 parts. Initially 10 horses received i.v. or oral doses of GH + low molecular weight CS (LMWCS; 8kDa; 3 + 9 g) or GH + high molecular weight CS (HMWCS; 16kDa; 3 + 9 g). Not surprisingly, given the rapidity and magnitude of glucosamine binding to plasma globulins, the authors were not able to detect GH in plasma with this protocol.

In their second experiment, 2 horses received i.v. or oral GH (125mg/kg; approx. 62.5 g per horse). An apparent bioavailability of GH based on free serum glucosamine (~2.5%) was significantly lower than both CS products. With respect to experiment 2, it is unfortunate that data from only 2 horses were used to estimate bioavailability of GH, because the two horses showed markedly different absorption profiles after oral administration of GH. The maximum plasma concentration ($C_{\text{max}}$) of GH in Horse 1 (~5 μg/mL) was achieved about 2.5 h after dosing, whereas Horse 2 reached a maximum of ~15 μg/mL in approximately 1.5 hours. Similarly, the Area Under the Curve (AUC) for GH was varied widely between the two horses (33.2 ± 23.8 μg/mL h).

Limitations of the Du et al. (2004) studies include the failure to account for the majority of glucosamine in plasma, the probable interaction between CS and GH because CS was administered in the presence of GH in the first experiment, the dose of GH administered to horses in the 2nd study (125 mg/kg) was far in excess of what would normally be given to horses prophylactically or therapeutically (usually ~20 mg/kg), and the small number of horses used in the 2nd experiment.

The second equine glucosamine / chondroitin bioavailability and pharmacokinetics study (Laverty et al., 2005) addressed some of the limitations noted in the former study. In this study 8 horses received i.v. or oral doses of GH at 20mg/kg – a dose typical of what might be used in the field – after an overnight fast. The pharmacokinetic behaviour of a single oral dose was determined by collecting jugular venous blood over a 12 hour period. Synovial fluid was also obtained from each horse at 0, 1 and 12 hours after dosing in order to quantify hypothesized increases in synovial fluid GH. As with Du et al. (2004), only free glucosamine was determined, and the fraction bound to plasma globulins ignored. The mean apparent bioavailability of GH (5.9%) was similar to that reported by Du et al. (2004), and confirmed the high variability between horses with respect to magnitude ($C_{\text{max}}$) and time course of pharmacokinetic behaviour. Synovial fluid GH also increased, albeit to a negligible degree (>9-fold less than that seen in serum); a small increase in synovial fluid GH persisted beyond the time at which GH was no longer detectable in serum. The authors concluded that the appearance of ingested glucosamine in synovial fluid could not be expected to exert any substantial modification to chondrocyte metabolism, and thus in vivo bioactivity must result from a non-physiological, i.e. pharmacological, effect of GH on tissues other than cartilage.

The apparent disparity between serum and synovial fluid concentrations of post-dosing glucosamine in the Laverty et al. (2006) study is challenged by others, who report human synovial fluid concentrations of glucosamine only about 25% lower than that of blood after dosing (Persiani et al., 2007). It is unlikely that this simply reflects a species difference. Perhaps most important is that synovial fluid glucosamine was determined after 2 weeks of oral dosing with GS (Persiani et al., 2007) whereas Laverty et al. (2006) described accumulation 12 hours after a single oral dose. While there was no attempt at time-course assessment of glucosamine in human synovial fluid, Persiani et al. (2007) were likely detecting an accumulation of glucosamine over time, consistent with the reported accumulation of chondroitin sulphate in canine synovial fluid a period of repeated dosing (Adebowale et al., 2002).
Another important consideration is that the increase in synovial fluid GH represents only the net effect of free GH transport into synovial fluid. This includes uptake of glucosamine by cells within the joint, binding to other molecules, and incorporation into joint structures. Indeed, in a review of their detailed pharmacokinetic studies in mammals, Setnikar and Rovati (2001) noted that 2 h after administration of $^{14}$C-labelled glucosamine to rats and dogs, the radioactivity accumulated in the knee cartilage of the treated animals to values 13-fold greater than in plasma. Therefore, it is unlikely that the discrepancy is just a species difference. It is not clear why the researchers performing the glucosamine bioavailability studies in horses did not quantify the total plasma glucosamine concentrations. They were seemingly aware of this literature, yet neglected to even mention its importance in determining bioavailability and discuss the impact on tissue distribution.

While a definitive conclusion as to the bioavailability and fate of oral glucosamine in horses has not been provided by these two equine studies, they do support at least 2 important hypotheses:

1) glucosamine and chondroitin are absorbed from the intestinal tract of horses
2) glucosamine does appear in the synovial fluid of horses

**General Recommendations for Future Research and Conclusions**

Further studies, accounting for total plasma glucosamine, and preferably using larger numbers of horses to account for the wide variability in individual horse responses to dietary glucosamine, are needed to more clearly define the differential bioavailability and post-absorptive behaviour of GH, GS, and CS in horses. It might also be interesting to look at the post-dosing intracellular glucosamine or glucosamine metabolite content of equine erythrocytes as this may be a significant sequestration compartment for this species. Glucosamine is often administered to performance horses in order to pre-empt or treat joint disease (Trumble, 2005). It is possible that glucosamine is actively transported into erythrocytes and because equine hematocrit can increase to more than 60% of total blood volume during high intensity exercise (Catalani et al., 2007), erythrocyte distribution and fate may be an important consideration in this species. Furthermore, glucosamine-based nutraceuticals are administered to horses almost exclusively as part of the diet. Thus, bioavailability and pharmacokinetics of glucosamine in the presence of a normal meal would provide meaningful information on what can be expected of the supplement under normal conditions of use.

Species-specific research into glucosamine-based nutraceuticals for horses has, as yet, failed to produce substantial evidence for the functionality of these products in this species. Conclusions of both ‘effective’ and ‘not effective’ in the peer reviewed equine literature have been compromised to varying degrees by numerous experimental factors including underpowered studies, unbalanced research design, non-existent or inappropriate controls, unclear or non-representative experimental conditions (i.e. models) and uncharacterized or complex, heterogeneous experimental materials. Future studies on glucosamine-based nutraceuticals in horses need to embrace fundamental experimental paradigms in design and interpretation of data. Use of appropriate statistical methods for determining sample size will reduce the problem of underpowered studies. Studies should have balanced treatment and control groups, and the experimental material should be independently characterized by the investigators – i.e. investigators should perform confirmatory analysis on label composition and purity. Experimental research designs should reflect the expected conditions of use, and placebos should be chosen such that they have confirmed inactivity in the experimental model or condition. The improved application of sound scientific principles to the assessment of glucosamine and its related nutraceutical products will lead to a better understanding of the role that this class of products can play in supporting joint health in horses.
References


CURRENT RESEARCH IN EQUINE JOINT HEALTH:
INFLAMMATORY RESPONSES TO THREE MODES OF INTENSE EXERCISE IN
STANDARDBRED MARES – A PILOT STUDY

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Summary

The objective of this study was to compare exercise-induced inflammatory responses in horses undergoing three different treadmill exercise tests based on cytokine and nitric oxide (NO) responses as well as differences in markers of exercise intensity. It was hypothesized that a higher intensity exercise test would increase peripheral (in circulation) and local (within joint fluid) cytokine and NO responses as well as markers of exercise intensity to a greater degree than less intense exercise tests. Four unfit Standardbred mares completed each of three exercise tests including a graded exercise test (GXT), an interval exercise test (IET), and a repeated sprint exercise test (RSET). Blood and synovial fluid samples were taken 24 h before (PRE) exercise and at three time points after exercise (20-30 min [END], 2 h, and 24 h REC). Blood was analyzed for total protein, haematocrit, total nitrite concentration and cytokine mRNA transcript (IL-1β, TNFα, IFNγ, IL-6, and IL-10) which was quantified using quantitative real-time polymerase chain reaction (qRT-PCR). Synovial fluid (SF) was also analyzed for total nitrite concentration. Results showed significantly higher plasma total nitrite concentrations (P < 0.05) for the RSET across all sampling times and up-regulation of pro-inflammatory cytokines (IL-1β, TNFα, IFNγ, IL-6; P < 0.05) following exercise for all exercise tests. There was significantly (P < 0.0001) higher synovial fluid total nitrite concentration in hock joints compared to carpus joints regardless of exercise test or sampling time. Mares spent more time at greater than 90 % of their HRmax during the RSET, which was twice the total duration of the GXT, then they did for either the GXT or the IET. Therefore, the RSET was found to be the most rigorous exercise test. These data may be useful in validating an exercise-induced inflammation model in horses that could further be used to elucidate inflammatory pathways associated with systemic and local joint inflammation.

Introduction

Local and systemic inflammation is an immune response to the presence of microorganisms or to injury which allows repair of damaged or infected tissue and a return to homeostatic conditions through a balance of local innate immune mechanisms and brain-derived immunoregulatory output via the autonomic nervous system (Higgins and Lees, 1984). Inflammation has been implicated in the pathogenesis of many debilitating chronic diseases such as arthritis, inflammatory bowel diseases, respiratory diseases, heart disease, and autoimmune diseases, just to mention a few (Han and Ulevitch, 2005). Hormone-like proteins called cytokines mediate inflammatory responses by autocrine, paracrine, and endocrine effects and can be considered markers of inflammation (Cannon, 2000). Strenuous exercise, energy crisis, stress hormones and oxidative stress are examples of physiological stimuli that modulate cytokine production (Cannon, 2000). Pro-inflammatory cytokines and inflammatory response cytokines including tumor necrosis factor alpha, interleukin-1 beta, interleukin-6, and interferon gamma (TNFα, IL-1β, IL-6, and IFNγ, respectively) as well as anti-inflammatory cytokines including interleukin-
IL-10 are responsible for profound physiological changes both locally and systemically. Dysregulation of the inflammatory response resulting in excessive production of pro-inflammatory cytokines or their production in the wrong biological context may lead to chronic inflammation which is detrimental to a horse's welfare or even life-threatening (Han and Ulevitch, 2005). Equine athletes, like their human counterparts, suffer from challenges to the immune system and inflammation related to exercise. Post-exercise problems associated with increased expression of inflammatory markers can range from the mild symptoms of delayed-onset muscle soreness to debilitating problems related to soft tissue, joint and bone damage (Auer et al., 1989). More specifically, exercise-induced inflammation is problematic in the equine athlete resulting in impaired health, lost training time and millions of dollars in veterinary expenses.

Nitric oxide (NO) is a physiologic mediator of inflammation and its specific role in the joint needs further investigation. Nitric oxide is a highly reactive, cytotoxic free radical and a by-product of the oxidation of L-arginine to citrulline, catalyzed by nitric oxide synthase (NOS). Nitric oxide plays a critical role in vasodilation, synaptic transmission of the central nervous system (Moncada and Higgs, 1993), and immune function (Sureda et al., 2006). It catalyses the IL-1-induced inhibition of proteoglycan synthesis (Bird et al., 1997), plays a role in chondrocyte apoptosis (Kim et al., 2003) and was demonstrated to activate matrix metalloproteinases (Murrell et al., 1995). It has been reported that in clinical cases of osteoarthritis, synovial fluid concentrations of nitric oxide were elevated (Farrell et al., 1992; Johnston and Fox, 1997) and that nitric oxide is quantifiable in synovial fluid during an inflammatory response (Dimock et al., 2000; van den Boom et al., 2005). Furthermore, concurrent induction of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase by cytokines may occur in inflamed tissue where NO may stimulate COX-2 to produce more prostaglandins (Johnston and Fox, 1997). Although the specific role of NO in the pathogenesis of degenerative joint disease has not been elucidated, production by synovial fibroblasts and chondrocytes has been documented (Johnston and Fox, 1997).

The objective of this study was to compare exercise-induced inflammatory responses in horses undergoing three different treadmill exercise tests varying in intensity, based on cytokine response in peripheral blood, plasma and synovial fluid nitric oxide concentrations, and differences in markers of exercise intensity. It was hypothesized that a higher intensity exercise test would increase peripheral (in circulation) and local (within joint fluid) cytokine and NO responses as well as markers of exercise intensity to a greater degree than less intense exercise tests.

Materials and Methods

Four healthy, unfit Standardbred mares aged 8.5 ± 2.0 yrs, weighing 472.3 ± 15.7 kg were used in this study. Mares were selected based on similar body condition (Henneke et al., 1983), percent body fat (Westervelt et al., 1976), and similar heart rate responses to a graded exercise test performed one month prior to the study. Mares were housed on 2-acre dry lots and were offered free choice water, salt, moderate-quality mixed grass hay and 2 kg of a 12% crude protein pellet to meet daily maintenance needs. Mares were habituated to the treadmill laboratory and to running on the treadmill prior to the initial GXT, and were not exercised in between the GXT and the start of the trial. The Rutgers University Institutional Animal Care and Use Review Board approved all methods and procedures used in this experiment.

Each mare completed three different treadmill exercise tests in a completely randomized order with 1 wk in between each test. During each exercise test, the mares ran on a high speed equine treadmill (Sato I-Equine Dynamics, Yellow Springs, OH) that was fixed at a 6 % grade. Immediately before exercise each mare was fitted with a heart rate monitor (Polar Equine Heart Rate Monitor; FitMed Inc., Mill Valley, CA) and resting heart rate as well as heart rate during exercise were recorded during the last 15 s of each step, and was monitored after exercise until recovery. Maximal heart rate (HR\text{max}) was used as a determinant of fitness to standardize the exercise protocols, since the treadmill speed at which HR\text{max}
is achieved during a stepwise test is correlated with maximal oxygen consumption \((V_{\text{O2max}})\). In each case horses ran to fatigue or until the test was completed. At time of fatigue or end of the test, horses completed 1 min of walking at 1.5 m/s.

The GXT lasted an average of 9.6 min and was previously described (McKeever and Malinowski, 1997). The IET lasted an average of 18.3 min and began with a warm up of 2 min walking at 2 m/s and 8 min trotting at 4 m/s. The warm up was followed by 2 intervals each consisting of 2 min at 100% HR\(_{\text{max}}\) (8-11 m/s) and 4 min at 4 m/s (Williams and Carlucci, 2006). The RSET lasted an average of 21 min and began with 2.5 min of walking at 2 m/s followed by 4 min of trotting at 3.2 m/s. Following warm up, mares completed 2 min sprints at 7, 8, 9 and 10 m/s with 2 min at 1.5 m/s in between each sprint (Graham-Thiers et al., 2003; Wilson et al., 1998).

Blood samples (20 mL) were taken via jugular venipuncture 24 h prior to the exercise test (PRE), 15-30 min (END), 2 h (2 h REC), and 24 h post-exercise (24 h REC). Samples were placed in pre-chilled EDTA tubes (Vacutainer, Becton Dickson, Inc., Franklin Lakes, NJ), immediately placed on ice and analyzed for packed cell volume or haematocrit using microhaematocrit technique (Spiracrit, Oxford Labware-Division of Sherwood Medical, St. Louis, MO). Samples were then centrifuged at 1500 g for 5 min and analyzed for plasma total protein (TP) via refractometry (Refractometer, Leica Microsystems, Buffalo, NY). The plasma supernatant was collected and frozen at -80˚C for later analysis of plasma total nitrite as an indicator of NO concentration. Rectal temperature (RT) was also taken at PRE and END sampling times.

Synovial fluid samples were collected bilaterally via aseptic arthrocentesis from both radiocarpal joints and both tibiotarsal joints at the same sampling times mentioned above. Samples from the same joint spaces were pooled form the right and left legs into pre-chilled EDTA vacutainer collection tubes and immediately placed on ice. Each pooled sample was centrifuged for 20 minutes at 1500 g and the supernatant was aliquoted and stored at -80˚C for NO analysis.

Peripheral blood (2.5 mL) was also collected via jugular venipuncture into PAXgene® blood RNA collection tubes (Qiagen/Becton Dickenson, Valencia, CA) at the sample times. Total RNA was isolated from the tubes according to manufacturer’s instructions and the RNA was quantified using a spectrophotometer (Biophotometer, Eppendorf, Westbury, NY). The optical density (OD) ratios (260:280) in general for the collective samples, were consistently greater than 1.9 µg mL\(^{-1}\) and the RNA yields were ~ 50 µg mL\(^{-1}\). A detailed description of RNA preparation, reverse transcription, and amplification was described previously (Streltsova et al., 2006). Relative quantification (2\(^{-\Delta\DeltaCT}\) method) was used to analyze the changes in gene expression (Ainsworth et al., 2003). Levels of cytokine (target) gene expression were normalized to that of the endogenous control gene (β-GUS) and fold changes in target gene expression were calculated relative to the calibrator sample, which was a resting sample in each case.

Statistical significance was set at \(P < 0.05\). Cytokine data are presented as relative mRNA transcript (RMT) or the mean fold changes in target gene expression in response to intense exercise ± standard error. Data were analyzed using a general linear model (GLM) ANOVA using SAS 9.1. The model used exercise test and sampling time as main effects, horse was nested within exercise test, and interactions between exercise tests and time were tested. Values that were determined to be 2.0 standard deviations or more above the mean were considered outliers and were excluded from the analysis. Post-hoc analysis of all significant main effects was performed using the Ryan, Einot, Gabrielle, Welsch Multiple Range test.

**Results**

During the GXT mares spent 8 out of 9.6 min total test length at greater than 60% of their HR\(_{\text{max}}\), 5 min of which were spent at greater than 90% of their HR\(_{\text{max}}\). During the IET mares spent 17 min out of 18.3 min total test length at greater than 60% of their HR\(_{\text{max}}\), 4 min of which were spent at greater than 90% of their HR\(_{\text{max}}\).
% of their HR\textsubscript{max}. Lastly, mares spent 17 min out of 21 min total test length at greater than 60% of their HR\textsubscript{max}, 8 min of which were spent at greater than 90% of their HR\textsubscript{max}.

Rectal temperature was higher at the END sample for all three protocols (39.6 ± 0.47 °C) when compared to the PRE sample (37.3 ± 0.03 °C). Haematoctrit was significantly (P < 0.0001; Fig. 1) greater (45.8 ± 0.4%) at the END sample for all exercise tests when compared to PRE (37.0 ± 0.0%; P < 0.05), 2 h REC (39.3 ± 1.1%; P < 0.05) and 24 h REC (37.5 ± 0.05%; P < 0.05) samples. Similarly, total plasma protein (TP) was significantly higher (P = 0.002; Fig. 2) at the END sample (6.7 g dL\textsuperscript{-1} ± 0.2) compared to PRE (6.3 g dL\textsuperscript{-1} ± 0.1; P < 0.05), 2 h REC (6.5 g dL\textsuperscript{-1} ± 0.05; P < 0.05) and 24 h REC (6.5 g dL\textsuperscript{-1} ± 0.02; P < 0.05) samples for all exercise tests.

There was no main effect of protocol on any cytokine transcript data however, for TNF\textalpha, IFN\gamma, IL-6, and IL-1\beta there was a main effect of sampling time (P = 0.0014; P = 0.012; P = 0.0060; P < 0.0001, respectively). Gene expression for TNF\textalpha (1.02 ± 0.01 RMT; P < 0.05) at the PRE sample was significantly lower compared to all other sampling times (END 1.67 ± 0.01; 2h REC 2.05 ± 0.3; 24h REC 2.02 ± 0.04, P < 0.05; Fig. 3). Interferon-\gamma peaked at the END sample (7.4 ± 1.5 RMT; Fig. 4) where it was significantly (P < 0.05) higher when compared to all other sampling times across all exercise tests. Interleukin-6 showed similar results as IFN\gamma, and was highest at the END sample (1.72 ± 0.4 RMT; Fig. 5) when compared to all other sample times. Interleukin-1\beta transcript was significantly higher (P < 0.05) at the 2 h REC (4.97 ± 0.6; Fig. 6) sample compared to the other sample times across all exercise tests.
There was a main effect of protocol on NO concentration (P < 0.0001). Significantly higher concentrations of NO in circulation were associated with the RSET (0.085 ± 0.007 mg dL⁻¹; P < 0.05) when compared to the IET (0.067 ± 0.008 mg dL⁻¹) and GXT (0.053 ± 0.005 mg dL⁻¹; Fig. 7) at all sampling time points. Carpal and hock SF NO concentrations showed no main effects of protocol or sampling time, however, there was significantly (P < 0.0001) more NO in the hocks (0.083 ± 0.005 mg dL⁻¹) compared to the carpal joints (0.053 ± 0.002 mg dL⁻¹; Fig. 8).

Discussion and Conclusion

Despite numerous studies evaluating cytokine changes in response to different modes and intensities of exercise in humans, there has been limited work of this nature done in horses, and to the best of our knowledge this is the first study to investigate plasma cytokine response to three different modes of intense exercise in horses (Ainsworth et al., 2003; Colahan et al., 2002). The RSET was found to be the most rigorous protocol (4 bouts of near maximal sprints), closely followed by the IET (2 maximal bouts), indicated by up-regulation of pro-inflammatory cytokines, higher total plasma NO, and other markers of performance.

Plasma cytokine response was consistent with increases in TP and RT after exercise. The increase in plasma TP immediately post exercise may be a reflection of decreased plasma volume due to a fluid shift during intense exercise that is
affected by the intensity and duration of the exercise (McKeever et al., 1993). There was a 7.2% increase in RT during the IET and RSET and only 4.5% increase during the GXT. Furthermore, maximal heart rates were achieved, sometimes multiple times throughout the duration of a test, an indication of the exercise intensity and physical effort of each horse. Mares spent more time at greater than 90% of their HRmax during the RSET, which was twice the duration of the GXT, then they did for either the GXT or the IET. A normal increase in haematocrit due to splenic contraction during exercise was observed from PRE to END sample times for each protocol.

Tumor necrosis factor-α was a target inflammatory mediator in this study due to its central role in the initiation of the immune response to injury or infection. Its ability to augment its own production as well as that of IL-1β, eicosanoids, reactive oxygen species and nitrogen intermediates, makes it an obvious therapeutic target in efforts made to prevent or control inflammatory responses (Pavlov and Tracey, 2004). In this case TNFα increased after exercise and remained elevated until 24h REC, unlike the other inflammatory cytokines which had returned to baseline values by 24h REC, suggesting an extended vs. a transient pro-inflammatory response to intense acute exercise. Interleukin-1β up-regulates a variety of genes including those that up-regulate its own expression and that of IL-6, and also induces enzymes necessary for the synthesis of leukotrienes, prostaglandins and NO (Moldoveanu et al., 2001). Prostaglandins and IL-6 up-regulate the production and secretion of IL-10 which in turn inhibits TNFα, IL-1β, and IFNγ production. Therefore, IL-6 can be considered an inflammatory response cytokine since it does not induce an inflammatory response (Petersen and Pedersen, 2005). The up-regulation of IL-6 in this study may have served to keep an exercise related inflammatory response in check, thereby preventing a chronic condition (Petersen and Pedersen, 2005). The post exercise increase in IFNγ in this study was an exception to that reported in human literature, and may be due to an increase in NK cells during short intense bouts of exercise (Suzuki et al., 2002).

The higher plasma NO concentration relative to the RSET remained constant over each sampling time, indicating no effect of the onset of exercise. This elevated concentration may be a reflection of environmental influence(s) that were not able to be controlled for during the study. The higher concentration of NO in the hock joints compared to the carpal joints may be attributed to conformational and biomechanical features unique to Standardbreds. A study by Grondahl and Dolvik reported that 14.3% of 753 young Standardbred trotters were diagnosed with osteochondrosis in the tibiotarsal joint (Grondahl and Dolvik, 1993). The role of NO in joint inflammation and pathogenesis of joint disease in horses has not fully been characterized warranting further investigation.
In conclusion, the RSET protocol was found to be the most rigorous when compared to the GXT and IET. The data presented mirror those reported in humans (Brenner et al., 1999) by demonstrating an up-regulated pro-inflammatory cytokine response to intense exercise in horses accompanied by supporting performance data indicative of exercise intensity. Unfortunately the NO data obtained from this study was inconclusive, and warrants further investigation. Future studies including more frequent and/or additional sample times past 24 h REC may help to more thoroughly characterize the NO response to intense exercise in horses.

By investigating physiological changes in relationship to pro-inflammatory cytokine status and other markers of inflammation, an imbalance or dysregulation of the physiological response can be recognized providing early detection of injury or chronic inflammatory state, often caused by overtraining in elite athletes. These data may be useful in validating an exercise-induced inflammation model in horses providing a valuable tool for investigating environmental and physiological factors that are integral in inducing or inhibiting inflammatory processes in the equine athlete. This 'natural' model may be a better alternative to pharmaceutical or surgical intervention to study inflammation in horses. Once a greater understanding of inflammation and its role in chronic disease is further elucidated, the efficacy of novel therapies, such as oral supplements and new management strategies, can be evaluated for anti-inflammatory or preventative measures that could potentially extend the athletic career of a horse or provide an economical and effective means to manage an existing condition.

Future Direction

Research in this area is ongoing. Anti-inflammatory and antioxidant properties of an oral antioxidant supplement are being evaluated in Standardbred mares having undergone intense acute bouts of exercise. The RSET was utilized as an exercise-induced inflammation model for this study. In this random crossover design, mares were placed into two treatment groups, one received a placebo and the other received superoxide dismutase (SOD) powder for 6 weeks. During this time, each mare underwent a RSET at week 4 and 6, after which they went through a 6 week wash out followed by the second half of the study in which mares were crossed over and completed the two RSET, identical to the first half of the study. Peripheral blood and synovial fluid samples were collected both before, during, and after each exercise test out to 36 h REC. They will be analyzed for markers of inflammation including cytokine transcripts (TNFα, IFNγ, IL-1β, IL-6, IL-10), nitric oxide, and prostaglandin E2, and C-reactive protein, as well as markers of oxidative stress and antioxidant status including 8-isoprostane, superoxide dismutase, creatine kinase, total glutathione, glutathione peroxidase, vitamin E, and β-carotene among others. Identification of an oral supplement with antioxidant and anti-inflammatory properties would provide one alternative to (invasive) pharmaceutical therapy and a more affordable way to prevent and/or treat chronic inflammatory disorders in horses.

References


FEEDING MANAGEMENT PRACTICES AND SUPPLEMENT USE IN TOP LEVEL EVENT HORSES

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Introduction

The main goal of feeding a top level Three-Day Event horse is to deliver nutrients in optimal amounts using sound feeding management practices that allow the horse to maximize its performance. The 2007 edition of the National Research Council’s (NRC) Nutrient Requirements of Horses provides nutritional recommendations for all classes of horses, including those trained and competed at high levels of competition. The NRC identifies four levels of activity; light (recreational riding or occasional showing), moderate (school horses or frequent showing), heavy (polo, ranch work, or low level eventing), and very heavy (racing, endurance, or elite level eventing). Horses in the ‘very heavy’ activity level, like Three-Day Event horses, often have energy, protein, vitamin, and mineral requirements 1.5–2.0 times their requirement for maintenance (NRC, 2007).

The main component of any horse’s diet should be forage, but Three-Day Event horses require supplementation to the diet to meet increased nutrient demands due to exercise training and competition. The basic principle of supplementation is to give a horse one or more dietary ingredients above what is normally required to meet nutrient requirements for maintenance. However, supplements are also given with the goal of improving performance, preventing a problem from occurring, and to combat or manage a problem after it arises. Supplementation usually occurs in the form of feeding concentrate, dietary ingredients including bran and oats, or nutrients including vitamins, minerals, or fat. Many commercial supplement products contain ingredients that provide one or more vitamins, minerals, amino acids (protein), fuel sources (carbohydrates and fats), herbs, and direct-fed microbials (bacteria and yeast). In 1998, it was estimated that 94.4% of horse operations surveyed in the United States fed a grain or concentrate to their horses and that 69.8% of those operations also fed supplements (USDA, 1998).

The objective of this research was to characterize the nutrition and feeding management practices, including concentrate and supplement use, of Three-Day Event horses prior to and during a competition in order to compare nutrient intakes to oxidative stress and inflammation experience before and after the Cross-Country phase of the event. This paper presents the portion of study data collected on feeding management practices only.
Materials and Methods

Three-Day Event

Horses participating in the study were competing in one of two divisions at the Jersey Fresh Three-Day Event in Allentown, NJ in 2006 and/or 2007. The event was recognized by the Federation Equestre Internationale as a Concours Complet d’Equitation International offering Two Star (CCI**) and Three Star divisions (CCI***). Levels of events are noted by stars, ranging from One Star to Four Star, with Four Star requiring the highest level of training and experience (FEI, 2006). The Three-Day Event in 2006 and 2007 consisted of three phases; Dressage, Cross-Country and Stadium Jumping. The competition followed a standard format consisting of a veterinary inspection after arrival (d 1), Dressage (d 2 or 3), Cross-Country (d 4), a second veterinary inspection followed by Stadium Jumping (d 5). The Cross-Country course was a modified short format (FEI, 2006).

Subjects

Each rider participating in the study signed a waiver allowing their horse(s) to participate in the study and they agreed to complete survey questions related to the feeding management practices of their competition horse(s).

Survey

A survey was developed that asked competition riders about the nutritional management of their competition horse(s). Rider information included contact information and where they sought out nutritional information. Horse information included age, sex, breed, # of competitions and years at that level, ongoing performance problems, and distance traveled to the event. Nutritional information included feeding times, type of pasture and hours spent turned out on pasture, type and amount of hay, concentrate, and supplements, and feeding practices associated with transport and Cross-Country. Surveys were executed by study investigators on d 1-4 by asking the questions directly to the riders and then recording results immediately. This provided an opportunity to ask the riders to clarify their answers thus providing more accurate and informative data. In addition, hay and concentrate fed at the competition were weighed as part of the 2007 survey. Feeds or feedstuffs that were considered concentrates included commercial feed products, cereal grains and by-products, sugar beet pulp, and wheat or rice bran.

Body Weight and Body Condition Score

Body weight (BW) was determined using an electronic scale, and was recorded before the start of Dressage, immediately following Cross-Country, and 18 to 24 hours after Cross-Country, but before Stadium Jumping. Body condition score (BCS; Henneke et al., 1983) was assessed by one of the study investigators trained in the procedure, and was taken at the same intervals as BW. Whole blood was also collected during these time points as part of separate companion studies, not reported herein, on the effects of oxidant status (2006; Williams and Burk, 2007) and inflammatory status (2007; Williams et al, 2008) on exercise performance in top level event horses.

Statistical Analysis

Data are presented as mean ± standard error. Two sample t-tests were used to compare data between divisions within each year of the study.

Results

Subjects

Demographic information, initial BW, and initial BCS of horses participating in the study are shown in Table 1. There were no differences in age, initial BW, and initial BCS for horses in the CCI**
and CCI*** horses in 2006 and in 2007. For both years of the study, the majority of horses were of the male gender and Thoroughbred breed. The average age for all horses across both years was $11.1 \pm 0.3$ yrs. Horses competing in the 2006 CCI** division had been competing at that level for 0-1 yr (50.0%), 1-2 yr (30.0%), or 3-5 yr (20.0%), whereas horses in the 2007 CCI*** division had been competing at that level for 0-1 yr (52.2%), 1-2 yr (26.1%), or 3-5 yr (21.7%). In 2007, more specific information was obtained for the number of years the horses had been competing at that level with CCI** averaging $1.6 \pm 0.2$ yr and CCI*** horses averaging $2.3 \pm 0.4$ yrs.

Table 1. Subject information, average initial body weight (BW), and initial body condition score (BCS) for horses competing in the CCI** and CCI*** divisions of the 2006 and 2007 Jersey Fresh Three-Day Event.

<table>
<thead>
<tr>
<th>Item</th>
<th>2006 CCI**</th>
<th>2007 CCI***</th>
<th>2006 CCI**</th>
<th>2007 CCI***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject participation</td>
<td>58% (19 out of 33)</td>
<td>56% (23 out 41)</td>
<td>19.5% (10 out 51)</td>
<td>50% (25 out of 50)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>11.3 ± 0.7</td>
<td>11.2 ± 0.5</td>
<td>11.5 ± 1.1</td>
<td>10.8 ± 0.5</td>
</tr>
<tr>
<td>Sex, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>78.9</td>
<td>91.3</td>
<td>100.0</td>
<td>88.0</td>
</tr>
<tr>
<td>Female</td>
<td>21.1</td>
<td>8.7</td>
<td>0.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Breed, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoroughbred</td>
<td>78.9</td>
<td>59.1</td>
<td>50.0</td>
<td>72.0</td>
</tr>
<tr>
<td>Thoroughbred cross</td>
<td>10.5</td>
<td>22.7</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Warmblood</td>
<td>5.3</td>
<td>9.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Other</td>
<td>5.3</td>
<td>9.1</td>
<td>30.0</td>
<td>8.0</td>
</tr>
<tr>
<td>BW, kg</td>
<td>529.2 ± 7.7</td>
<td>529.7 ± 7.1</td>
<td>521.7 ± 17.9</td>
<td>535.8 ± 8.5</td>
</tr>
<tr>
<td>BCS</td>
<td>5.2 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>5.0 ± 0.1</td>
</tr>
</tbody>
</table>

Placings. Twelve subjects completed the 2006 CCI** event (63.2%) with seven placing in the top 10 (1st, 2nd, 3rd, 6th, 8th, 9th, 10th). Of the non-finishers, three withdrew before Cross-Country, one withdrew during Cross-Country, and one did not pass the final veterinary inspection. In the 2006 CCI***, 13 subjects completed the event (56.5%) with four placing in the top 10 (3rd, 6th, 9th, and 10th). Non-finishers included one that withdrew before Cross-Country, five that withdrew on Cross-Country, one that was eliminated on Cross-Country, and three that either withdrew after Cross-Country for unknown reasons or did not pass the final veterinary inspection. Seven subjects completed the 2007 CCI** event (70%) with one placing in the top 10 (6th place). Of the non-finishers, one withdrew before Cross-Country, one was eliminated during Cross-Country, and one was eliminated at the final veterinary inspection. In the 2007 CCI***, 20 subjects completed the event (80%) with six placing in the top 10 (3rd, 5th, 6th, 8th, 9th, and 10th). Non-finishers included one that withdrew during Cross-Country, three that withdrew after Cross-Country, and one that did not pass the final veterinary inspection.

Nutritional Management

The majority of riders in both years of the study indicated that they sought out nutritional advice from their trainers first and feed dealers second (Table 2). The majority of subjects were allowed access to pasture, and fed hay and/or concentrate 2 or 3 x/d (Table 3).
Table 2. Sources sought out for nutritional information by riders competing in the CCI** and CCI*** divisions of the 2006 and 2007 Jersey Fresh Three-Day Event.

<table>
<thead>
<tr>
<th>Source</th>
<th>2006</th>
<th>2007</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCI** (n = 18)</td>
<td>CCI*** (n = 22)</td>
<td>CCI** (n = 10)</td>
<td>CCI*** (n = 23)</td>
</tr>
<tr>
<td>Barn Manager</td>
<td>5.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Books/Articles/Magazines</td>
<td>33.3</td>
<td>13.6</td>
<td>0.0</td>
<td>26.1</td>
</tr>
<tr>
<td>Feed Dealer</td>
<td>38.9</td>
<td>31.8</td>
<td>30.0</td>
<td>34.7</td>
</tr>
<tr>
<td>Internet</td>
<td>11.1</td>
<td>9.0</td>
<td>10.0</td>
<td>21.7</td>
</tr>
<tr>
<td>Nutritionist</td>
<td>22.2</td>
<td>13.6</td>
<td>20.0</td>
<td>30.4</td>
</tr>
<tr>
<td>Own Experiences</td>
<td>0.0</td>
<td>13.6</td>
<td>0.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Trainer</td>
<td>55.5</td>
<td>36.4</td>
<td>70.0</td>
<td>30.4</td>
</tr>
<tr>
<td>University Faculty</td>
<td>27.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Veterinarian</td>
<td>0.0</td>
<td>27.3</td>
<td>20.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

*Data expressed as % of subjects

Table 3. Feeding management practices of horses competing in the CCI** and CCI*** divisions of the 2006 and 2007 Jersey Fresh Three-Day Event.

<table>
<thead>
<tr>
<th>Item</th>
<th>2006</th>
<th>2007</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCI**</td>
<td>CCI***</td>
<td>CCI**</td>
<td>CCI***</td>
</tr>
<tr>
<td>Feeding Frequency*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once/d</td>
<td>5.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Twice/d</td>
<td>47.3</td>
<td>39.1</td>
<td>60.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Thrice/d</td>
<td>42.1</td>
<td>52.2</td>
<td>20.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Four Times/d</td>
<td>5.3</td>
<td>8.7</td>
<td>20.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Concentrate Type Fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial Feed Product</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>96.0</td>
</tr>
<tr>
<td>Cereal Grain</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Beet Pulp</td>
<td>26.3</td>
<td>17.4</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Wheat Bran</td>
<td>21.0</td>
<td>8.7</td>
<td>10.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Rice Bran</td>
<td>21.1</td>
<td>26.1</td>
<td>10.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Pasture Access, hr/d</td>
<td>12.3 ± 1.3</td>
<td>9.1 ± 1.3</td>
<td>13.3 ± 2.2</td>
<td>11.5 ± 1.5</td>
</tr>
<tr>
<td>Pasture Type Fed</td>
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<tr>
<td>Grass mix</td>
<td>41.2</td>
<td>68.4</td>
<td>40.0</td>
<td>40.9</td>
</tr>
<tr>
<td>Grass/clover mix</td>
<td>58.8</td>
<td>31.6</td>
<td>60.0</td>
<td>59.1</td>
</tr>
<tr>
<td>Hay Species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orchardgrass</td>
<td>0.0</td>
<td>0.0</td>
<td>30.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Timothy</td>
<td>44.4</td>
<td>34.8</td>
<td>10.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Coastal Bermudagrass</td>
<td>0.0</td>
<td>0.0</td>
<td>20.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Mixed grass</td>
<td>16.7</td>
<td>34.8</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>11.1</td>
<td>0.0</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Mixed grass/alfalfa</td>
<td>27.8</td>
<td>30.4</td>
<td>10.0</td>
<td>52.0</td>
</tr>
</tbody>
</table>

*Data expressed as % of subjects unless otherwise specified

*Based on feeding frequency at competition

Forage. Three CCI*** horses in both 2006 (13.0%) and 2007 (12.0%) were not given access to pasture as a part of their daily ration prior to the competition. Average hours of pasture access for those horses allowed access is summarized in Table 3. There were no differences between CCI** and CCI*** horses in their hours of pasture access in both years of the study. In 2006, all subjects averaged 10.6 ± 1.1 h/d of pasture turnout whereas 2007 subjects averaged 12.1 ± 1.3 h/d. The data recorded in 2006 was
insufficient to estimate pasture intake. In 2007, pasture intake was estimated to be 3.3 ± 0.5 kg/d for CCI** horses and 3.0 ± 0.4 kg/d for CCI*** horses. Pasture intake was calculated by taking into account the initial BW of the horse, hours of pasture access, and total percentage of body weight needed to be consumed to meet DE requirements (2.5%; NRC, 2007). The species of hay offered to horses at the competition is denoted in Table 3. Amount of hay fed to subjects each day was not calculated in 2006 due to insufficient data. In 2007, the daily amount of hay fed at the competition was 9.3 ± 1.1 kg/d for CCI** and 8.6 ± 0.7 kg/d for CCI*** horses.

Concentrate. The type of concentrate offered to horses in both divisions and years of the study is detailed in Table 3. The percentages of riders that fed a commercial feed product in addition to one or more concentrates was 57.9, 52.2, 60.0, and 76.0 for 2006 CCI**, 2006 CCCI***, 2007 CCI**, and 2007 CCI***, respectively. The amount of concentrate fed to subjects competing in 2006 was reported in quarts, scoops, or lbs and was therefore insufficient to accurately calculate daily intake for those horses. Ranges for concentrate reported was 2 to 18 qts, 2 to 6 scoops, and 2 to 11 lbs for CCI** horses and 2 to 14 qts, 2.25 to 6 scoops, and 3 to 16 lbs of concentrate/d for CCCI*** horses. In 2007, the total daily intake of concentrate for CCI** and CCCI*** horses was estimated to be 6.2 ± 1.3 kg/d (range 0.6 to 12.0 kg/d) and 5.2 ± 0.6 kg/d (range 1.4 to 16.5 kg/d), respectively. As a percentage of BW, concentrate offered was 1.2 ± 0.2 % (range 0.1 to 2.0 %) and 1.0 ± 0.1 % (0.2 to 2.9 %) for 2007 CCI** and CCCI*** horses, respectively. Sixty percent of CCI** and 40% of CCCI*** horses were being fed more than 0.5% BW of concentrate in one meal in 2007.

Total feed intake. Total intake (as fed basis) of hay and concentrate offered to horses at the 2007 CCI** and CCCI*** Three-Day Event was 15.5 ± 1.9 kg/d and 13.7 ± 0.9 kg/d, respectively. When intake is expressed as a percentage of BW, horses competing in the CCI** and CCCI*** were offered 2.9 ± 0.3 and 2.6 ± 0.6 % of their BW, respectively. Ratio of hay to concentrate fed to horses was 60:40 in the 2007 CCI** competition and 62:38 in the 2007 CCCI*** division.

Supplements. Oral supplements administered regularly to horses leading up to or at the competition are reported in Table 4. In both years of the study, the most administered type of oral supplement was electrolytes followed by salt and oral joint supplements. The average number of supplements fed on a regular basis to horses competing in the 2006 CCI**, 2006 CCCI***, 2007 CCI**, and 2007 CCCI*** were 4.2 ± 0.4, 4.2 ± 0.3, 4.2 ± 0.5, and 4.3 ± 0.7, respectively. Whether the horses received an intra-muscular, intra-venous or intra-articular injection along with the oral joint supplement was not determined in 2006. In 2007, the majority of horses competing in both divisions received one or more injectable joint products (100 % for CCI** and 80.0 % for CCCI***). A total of 70.0 and 44.0 % of the CCI** and CCCI*** horses, respectively, received both an oral and injectable joint products prior to the competition.

Feeding Associated with Transport. The majority of horses in the 2006 CCI** (78.9%) did not have any change in their forage and concentrate feeding prior to being transported to the show facility. Of those horses that had changes in their feed regimen, 5.3% were fed less concentrate prior to transport, 5.3% had reduced access to pasture prior to transport, and 10.5% were given a bran mash after transport. Two horses (10.5 %) were given a digestive health supplement prior to travel. Digestive health supplements included products aimed at preventing or treating ulcers and probiotics. In the 2006 CCI**, 60.9% of the horses had no change in concentrate or forage feeding prior to or after transport. The other horses were offered a bran mash either before or after transport (34.7 %) or decreased concentrate ration prior to transport (4.3%). Oral supplement use associated with transport included electrolytes (21.7 %), digestive health (4.3%), or mineral oil (4.3%). One horse (4.3%) was administered intra-venous fluids prior to transport.
Table 4. Percentage of oral supplement use for horses competing in the CCI** and CCI*** divisions of the 2006 and 2007 Jersey Fresh Three-Day Eventa,b.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All-in-onec</td>
<td>5.3</td>
<td>8.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Amino acids</td>
<td>10.5</td>
<td>4.3</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Anhydrosis</td>
<td>5.3</td>
<td>4.3</td>
<td>20.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>42.1</td>
<td>8.7</td>
<td>10.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Daily de-wormer</td>
<td>5.3</td>
<td>8.7</td>
<td>20.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Digestive healthd</td>
<td>21.1</td>
<td>30.4</td>
<td>40.0</td>
<td>48.0</td>
</tr>
<tr>
<td>Electrolyte</td>
<td>84.2</td>
<td>69.6</td>
<td>80.0</td>
<td>92.0</td>
</tr>
<tr>
<td>Hair coat quality</td>
<td>5.3</td>
<td>8.7</td>
<td>10.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Herbal</td>
<td>5.3</td>
<td>17.4</td>
<td>10.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Hoof</td>
<td>21.0</td>
<td>21.7</td>
<td>20.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Joint</td>
<td>47.4</td>
<td>43.5</td>
<td>70.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Multi-vitamin &amp; mineral</td>
<td>21.0</td>
<td>21.7</td>
<td>10.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Fat</td>
<td>31.6</td>
<td>39.1</td>
<td>20.0</td>
<td>48.0</td>
</tr>
<tr>
<td>Salt</td>
<td>57.9</td>
<td>65.2</td>
<td>50.0</td>
<td>76.0</td>
</tr>
<tr>
<td>Block, ad libitum</td>
<td>26.3</td>
<td>47.8</td>
<td>20.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Loose, topdressed</td>
<td>31.6</td>
<td>8.7</td>
<td>30.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Both of the above</td>
<td>0.0</td>
<td>8.7</td>
<td>0.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Weight Gain</td>
<td>5.3</td>
<td>4.3</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Otherg</td>
<td>26.3</td>
<td>21.7</td>
<td>40.0</td>
<td>28.0</td>
</tr>
</tbody>
</table>

Data shown as % of subjects
Includes supplements regularly fed leading up to and at the competition
Combination of ingredients including vitamins, minerals, herbs, joint health compounds, etc.
Includes supplements used for the prevention and treatment of ulcers and probiotics products
Included oils (vegetable, corn, rice bran, fish, etc)
Block included mineral and iodized (white) salt blocks; loose salt included table, kosher, and lite salts.
Included less frequently administered supplements including immune stimulants, fly control, calming aids, thyroid stimulant, apple cider vinegar, and dried molasses.

In 2007, 80% of CCI** horses did not have their concentrate or forage feeding regimen changed associated with transport. Of those horse’s that were fed differently associated with transport, 10% had concentrate decreased prior to transport, and 10% had their concentrate and forage withheld until 4-5 hours after transport. In the 2007 CCI***, 84% of horses did not have any changes in their concentrate and forage feeding associated with transport. Changes to the diet of the remaining horses included feeding a bran mash either before or after (12.0%), and a decreased concentrate ration (4.0 %). Oral supplement use associated with transport included administration of a digestive health supplement (32.0%) and/or electrolytes (16.0%).

Feeding Associated with Cross-Country. Feeding management practices and supplement administration before and after Cross-Country in both years of the study is shown in Table 5. In 2007, riders were asked how long after Cross-Country they fed their horses. In the 2007 CCI**, horses were fed either 1-2 hr (50%), 2-3 hr (10%), 3-4 hr (20%), or > 4 hr (10.0%) after Cross-Country. In the 2007 CCI***, horses were fed either 1-2 hr (56.0%), 2-3 hr (36.0%), 3-4 hr (4.0%), or > 4 hr (4.0%) after Cross-Country.
Table 5. Percentage of horses that had feeding management changes in relation to the Cross-County phase of the CCI** and CCI*** divisions of the 2006 and 2007 Jersey Fresh Three-Day Event.

<table>
<thead>
<tr>
<th>Item</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCI**</td>
<td>CCI***</td>
</tr>
<tr>
<td>Before Cross-Country</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No dietary change</td>
<td>31.6</td>
<td>56.5</td>
</tr>
<tr>
<td>Reduced hay</td>
<td>15.8</td>
<td>21.7</td>
</tr>
<tr>
<td>Reduced concentrate</td>
<td>31.6</td>
<td>8.7</td>
</tr>
<tr>
<td>Reduced hay and concentrate</td>
<td>21.1</td>
<td>13.0</td>
</tr>
<tr>
<td>Electrolyte supplementationb</td>
<td>26.3</td>
<td>4.3</td>
</tr>
<tr>
<td>After Cross-Country</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No dietary change</td>
<td>84.2</td>
<td>87.0</td>
</tr>
<tr>
<td>Reduced hay</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Reduced concentrate</td>
<td>10.5</td>
<td>8.7</td>
</tr>
<tr>
<td>Same amount, increased meals</td>
<td>5.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Reduced hay and concentrate</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Increased concentrate</td>
<td>5.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Electrolyte supplementationb</td>
<td>15.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Bran mash supplementation</td>
<td>10.5</td>
<td>4.3</td>
</tr>
</tbody>
</table>

aData shown as % of subjects
bDoes not include horses already receiving electrolytes on a regular basis

Intravenous Fluid Use. Intravenous fluid administration was not recorded in the first year of the study. In 2007, 30% of CCI** horses were administered IV fluids after Cross-Country (20% administered 4 L, 10% administered > 4 L). In contrast, 64% of 2007 CCI*** horses were administered IV fluids after Cross-Country (48% administered 4 L, 4% administered < 4 L, 12% administered > 4 L). For both divisions, horses received either Normasol-R or Lactated Ringer’s Solution depending on the administering veterinarian.

Discussion

Subject participation in 2007 for the CCI** riders dropped considerably compared to the participation in 2006. A common reason cited by riders for not wanting to participate in the study was that they were new to that competition level and they perceived the study as a possible distraction. Horse’s age averaged between 10.8 and 11.5 yrs, and the overwhelming majority of them were Thoroughbred geldings. Wardrope (2004) found that of the 75 horses competing at the 2004 Olympic Games in Eventing, only 11 did not have a Thoroughbred ancestor within the first two generations. This study confirms what is generally believed in the industry that the Thoroughbred is the chosen breed for upper level Three-Day Eventing. Body condition score of horses in this study averaged between 4.9 and 5.2, which is within the recommended range for performance horses (Geor, 2008). Body condition scoring is a useful tool to visually estimate fat cover, and thus energy status in horses (Henneke et al., 1983). Studies in endurance (Lawrence et al., 1992), race (Kearns et al., 2002) and lesson horses (Garlinghouse et al., 1999) have all observed a negative relationship between the higher BCS horses and the variables of performance being investigated.

Data from feeding management surveys are useful when comparing what and how horses are fed in the industry to research driven nutritional requirements and recommendations. Data collected in our survey study was also used to calculate nutrient intakes in order to relate them to antioxidant status.
(Williams and Burk, 2007) and inflammation (Williams et al., 2008) in the horses during competition. To our knowledge, this is the first feeding management survey conducted on horses competing in high level Three-Day Eventing competition in the United States; although others have conducted similar surveys on racehorses (Winter and Hintz, 1981; Schils and Jordan, 1989; Southwood et al., 1993; Williamson et al., 2007) and endurance horses (Ralston, 1988).

The horses participating in this study were considered to be in “very heavy” work leading up to and during the competition under NRC (2007) guidelines. In order to meet nutritional requirements, the majority of horses were fed a daily ration consisting of limited grass pasture, grass hay or a mixed grass/alfalfa hay, a commercial feed product, and supplementation with salt and/or one or more commercial supplement products. Whether all horses were fed to meet their nutrient requirements was not determined in this study, however, it was determined that total daily intake of forage and concentrate fed at the 2007 CCI** and CCI*** competition was 21 % and 3 % above NRC recommendations, respectively (NRC, 2007). Since their trainer and feed dealer were cited by most riders as their top source for nutritional information, those sources likely influenced feeding management practices observed in this study. It is likely that feeding management practices were also influenced by the level of training the horse required, where and how the horses were kept, length of transportation to and from the competition venue, time of year, individual needs of the horse, and individual feeding goals of the owner and/or rider. In contrast to what was found in this study, veterinarians and farriers were considered very important sources of nutritional information when horse operations were surveyed (USDA, 1998). Knowing whom horse owners go to for nutritional information is useful when planning outreach efforts that aim to improve nutritional management of horse, because it helps define the audience that should be targeted.

A large percentage of the horses had their daily ration divided into smaller meals fed two or more times per day. The majority of horses in this study were meeting minimum forage intake recommendations of 1.0% BW (Geor, 2008). Timothy hay is a popular hay crop in the Mid-Atlantic area and is thus fed by a lot of riders, as was the case in this study. Riders who fed a mixed grass alfalfa hay were likely adding 0.1-0.2 Mcal DE/kg more to the diet, which is a useful way to increase the amount of DE and nutrients fed while lowering the amount of concentrate needed. Nearly all of the horses were fed a commercial concentrate, indicating that riders have a high level of confidence in the ability of commercial feed companies to formulate concentrates that will help meet the needs of their horses. It has been advised that horses not be fed more than 0.4% of their BW in concentrate per meal to avoid risks associated with digestive upsets (Potter et al., 1992; Clarke et al., 1990) and metabolic disorders such as exertional rhabdomyolysis (McKenzie et al., 2003). Despite that recommendation, we observed a large number of horses fed more than the recommended amount of concentrate in one meal feeding. It is apparent that at this level of competition, horses are receiving large concentrate meals that should be divided into three or more meals per day.

There are a large number of commercially available oral supplements that are widely marketed to horse owners in stores, catalogs and on the internet. Horse owners often feed supplements without knowledge of their horse’s nutrient requirement needs or the potential impact of the nutrient(s) in the supplement on their horses overall nutrient intake. As a result, over-supplementation of certain nutrients as well as nutrient interactions and interferences may occur. On average, riders in our study fed four oral supplement products or feed ingredients in addition to the horse’s daily hay and concentrate ration. Riders appeared most concerned about their horse’s electrolyte balance as well as preventing or combating problems associated with joints. Supplementation with electrolytes to elite equine athletes is recommended to help replace that lost in sweat during acute exercise. Many of the riders surveyed fed electrolytes on a daily basis rather than in response to exercise level or ambient temperature. In addition to the daily electrolytes, many riders also fed salt granules or blocks that added additional sodium and chloride to the horse’s diet. Daily electrolyte supplementation may create nutrient excesses that may ultimately negatively impact the horse, and nutrient excesses lost in the urine and feces will negatively impact the environment.
The third most offered oral product was a joint supplement. Along with the oral forms, many study horses were medically treated with injectable chondroprotectants (including hyluronanic acid and polysulphated glycosaminoglycans). Since only two riders cited their horses as being diagnosed with joint problems, it is likely that owners/riders were supplementing and/or injecting their horses as a preventative measure. Oral joint products are theorized to provide extra building blocks for joint components and to reduce inflammation; however research in horses is scant and inconclusive (reviewed in Goodrich and Nixon, 2006). Horse owners should be aware of a recent study that found that 84% of joint supplement products did not contain what the labels stipulated, with products having between 0 to 115% of the named compounds (Adedowale et al., 2000). Compounds that are fed to horses to either treat or prevent joint disorders should be shown effective in clinical trials and the manufacturer that markets the compounds as products should have a demonstrated record of good quality control.

Of all of the horses participating in this study, only thirteen had been diagnosed with gastric ulcers. Given the high prevalence of gastric ulcers in performance horses (McClure et al., 1999), it is likely that the digestive aids were given more as a preventative measure rather than a treatment. The authors questioned whether horses receiving regular digestive health supplements and/or ulcer medications would be those that had a limited amount of daily turnout; however this was not the case. Of the 50% (5) of 2007 CCI*** horses that were offered a daily digestive health supplement, only 40% (2) were receiving less than 10 hr/d turnout (pasture or drylot). Of the 48% (12) of the 2007 CCI*** horses that were offered a daily digestive health supplement, 50% (6) were on less than 10 hr/d turnout, and only 25% (3) were on less than 5 hr/d turnout.

Other supplements including those high in fat were also popular among this group of Three-Day Event riders. The feeding of supplemental fat to horses has been a common recommendation in the horse industry given the many demonstrated and theorized performance advantages (Kronfeld et al., 1994).

Transport has been shown to be a significant stressor on horses (Friend, 2000), including increasing the risk of colic (Tinker et al., 1997; Hillyer et al., 2002) and gastric ulcers (Buchanan and Andrews, 2003). Despite that fact, the majority of the Three-Day Event horses were not fed differently in association with transport. Feeding a bran mash either before or after transport was the most popular practice in this study. Bran mashes can increase water intake and provide dietary nutrients for the horse, but are cautioned against when fed regularly because of the low calcium:phosphorous ratio. Electrolytes and digestive health supplements were commonly administered prior to transport. Electrolyte administration prior to transport may combat some modest changes in electrolyte balance that have been shown to occur during transport (Van den Berg et al., 1998), but Friend (2000) suggested that pre-transport electrolytes might further exacerbate water loss during transport. Only a very small percentage of study horses were offered less grain prior to transport, even though it is a common recommendation to do so to prevent colic during shipping (Rietveld and Wright, 2003).

Feeding management of horses was quite varied prior to the start of the Cross-Country phase. While the majority of horses had no change in their diet, many had a reduction in hay, grain or both prior to Cross-Country. A reduction or elimination of forage from the diet 3-5 hrs prior to an exercise bout may be an advantage due to a reduction in weight in the bowel (Meyer, 1995) and in cardiovascular load during exercise (Duren et al., 1992). However, forage also increases water consumption allowing for the digestive tract to serve as a reservoir for water and electrolytes that may help reduce dehydration and electrolyte imbalances. Several studies found that feeding small amounts of hay prior to exercise was not associated with poor performance, but that concentrate should be reduced (Pagan and Harris, 1999; Duren et al., 1999). A reduction in concentrate is often recommended 3-5 hours out from an acute exercise bout to allow metabolites to return to resting values following meal consumption (Duren et al., 1995; Stull and Rodiek, 1995). In our study, the CCI** horses were first to compete in the day beginning between 0800 and 0900, and were therefore more likely to have their pre-concentrate meal removed from the daily ration compared to a CCI*** horse.
The goal of post Cross Country feeding should be to replenish nutrients and energy reserves. The majority of horses in our study were fed their normal ration between 1 and 4 hrs after Cross-Country. Given that the feeding of hay and concentrate rather than hay alone after intense exercise was associated with higher muscle glycogen stores (Lacombe et al., 2004), a reduction in hay, concentrate or both after Cross-Country would not be recommended. There were some horses that received electrolytes and/or a bran mash following Cross-Country. The addition of a bran mash after Cross-Country was likely a technique to help with hydration, but the rationale behind that practice is not known nor has it been researched.

The Cross-Country phase of Three-Day Eventing is associated with significant losses in total body water that may persist overnight if rehydration strategies are not used (Ecker and Lindinger, 1995). In addition, horses competed in high ambient temperatures and relative humidity have a higher sweating rate (McCutcheon et al., 1995). However, research has shown that horses recover faster from endurance type exercise when rehydrated with saline or glucose-electrolyte solutions (Hyypa et al., 1996; Nyman et al., 1996; White, 1998). In 2007, the majority of horses were administered intravenous fluids by a veterinarian after Cross-Country, with a higher percentage of horses administered fluids that were competing in the CCI*** division. The fact that the CCI*** horses were ridden over the Cross-Country course in the morning when temperature and relative humidity were lower likely contributed to the lower administration of intravenous fluids in the CCI** horses compared to the CCI*** horses. The range for the temperature and relative humidity during the Cross-Country phase in 2007 was 25 to 32 °C and 55 to 93%, respectively.

**Conclusion**

This study presents the feeding management practices and supplement use in Three-Day Event horses that are deemed important by the riders to their competition success. While the majority of feeding management practices followed research driven recommendations, others did not. Horses in this study were more than likely being fed to meet their nutritional requirements given the amount of feed offered, the high rate of horses successfully completing the competition, and the optimal BCS at which the horses were maintained. However, the relatively high average supplement use of four supplements per horse raises the question about over-supplementation in these horses. Lastly, this study demonstrates the nutritional challenges faced by Three-Day Event riders given the variety of factors associated with Three-Day events including transport, housing, exercise level, and general stress associated with competition.

**Acknowledgements**

The authors would like to thank the Jersey Fresh CCI**/CCI*** event organizers and competitors who participated in this study along with the FEI veterinarians on staff at the event who helped support the study and encourage participation. The study also would have not been able to be completed without the Jersey Fresh Research Team, consisting of Rutgers University and University of Maryland staff, graduate and undergraduate students.

**References**


WHAT DOES IT ALL MEAN? ADDING SUPPLEMENTS TO YOUR HORSE’S FEED RATION

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Summary

The practice of supplementation to the diet of many different products has become a standard in
the equine industry. Manufacturers are generous with their descriptions of the benefits of using their
products to attract buyers. The attraction of being able to treat a horse with a ‘food’ instead of medicine is
as appealing to horse owners as it is to those looking at human supplements. Offerings of supplements
that are supposed to help every problem imaginable in the horse are available for a price. Proof of
effectiveness is still not a requirement for the supplements offered to animals. There are many reasons to
supplement, some of them sounder than others. Supplementation can be vital to the health and well-being
of the horse in cases where the basic diet is lacking in nutrients. Some supplements target specific
problems that can occur in horses and offer nutrients in normal or therapeutic amounts to alleviate or
prevent onset of the issues. Occasionally, supplements are given just in case there is something missing
from the diet. Pitfalls with supplementation are having unrealistic expectations of the effects or the time it
takes to have an effect. Further, without careful scrutiny of the supplements the diet can be
unintentionally unbalanced. There is a cost to trying to improve the health and well-being of the horse
which needs to be realized before starting one of the supplements that will require long term usage.
Government regulation of equine supplements to guarantee that consumers are getting what they are
paying for is sparse. In response to the lack of controls, manufacturers in the industry have developed an
association (NASC) that helps to police the manufacturing practices of the members in return for a seal of
approval that can be displayed on the supplement. The system is helping to rebuild consumer confidence
in products that have in recent history not always been living up to expectations.

Introduction

With many reasons for wanting to supplement a horse’s diet there are just as many considerations
to look at to make sure you are supplementing responsibly. Supplement manufacturers are very good at
describing what their products might do to improve the health and well being of a horse but they cannot
tell you how their product might affect the overall balance of the diet, since the diet of every horse is
different. Indiscriminate combinations of supplements can sometimes be more detrimental than helpful.
With the deluge of often unsubstantiated information it is not hard to imagine that supplements are
frequently fed for the wrong reasons or with unrealistic expectations. Further, horse owners are not
getting any help from the FDA on insuring that manufacturers are including the products claimed or the
guaranteed amounts on the labels. The purpose of this article is to point out a few things to think about
when trying to decide if adding the supplement will be worth the money.

Reasons for Supplementing

There are many reasons for adding supplements to a horse’s diet – some more sound than others. However, supplements cannot make up for a lack of proper basics in the care of a horse. Basics are plenty
of regular exercise and conditioning and a diet based on quality forage and concentrate. Some of the major reasons for supplementing are:

**Healing with food:** Hippocrates told us to ‘let food be your medicine’. As in human medicine there is an attraction to the concept of being able to heal a horse with food or natural substances instead of medication. Human nutritionists keep everyone abreast of the latest ‘supplement’ that will prevent numerous ailments that are affecting our population. We eat oatmeal and take fish oil to protect our heart. It is only natural to want to translate the same concepts to horse nutrition. Advertising plays heavily on this desire with the broad promises of what some supplements will do.

**Balance diet:** Sometimes it is impossible to get all of the nutrients required or the proper balance in a diet without adding some type of supplement. Many concentrates are formulated for higher intakes than some horses need and feeding below will result in less than desirable intakes of some of the vitamins and minerals. Topping off the feed with a vitamin and mineral supplement may be the best method of balancing out this type of diet. Another example would be trying to target a nutrient that is low or missing in the diet and supplement that one in particular. For instance, a horse on a grass hay diet may benefit from supplemental Vitamin E to balance the loss of vitamin E that occurs with the making and storage of the hay.

**Aid or prevent a problem:** Supplementing to compensate for losses of specific nutrients occurring in the horse may be done to target or prevent a particular problem. Replenishing electrolyte losses to a heavily sweating horse with an electrolyte supplement during a workout like an endurance ride may prevent metabolic problems associated with depletion. Sometimes therapeutic (well above the requirement) amounts of nutrients are fed to improve or ameliorate a problem with a horse. Glucosamine may provide the building blocks for arthritis prone joints. Biotin has been found to improve the quality of new hoof wall tissue when fed in therapeutic amounts. The majority of supplements on the market target these concepts.

**Improve digestion:** Under most normal circumstances, a horse does an adequate job of digesting its feed without help. However, if a horse has lost some of the ability to properly digest its food then addition of digestive aids to the diet may help the horse get more nutrients out of its diet. There are different digestive aid supplements that target the various parts of the digestive tract. Other supplements target the environment of the gut trying to rebalance so that the normal microbes or enzymes can do their best job of digesting.

**Improve overall health:** Like a security blanket, things get added into a horse’s diet ‘just in case’ it may be missing. Like the concept of the human ‘multi-vitamin’. Also, some supplements are thought to be good for the horse without targeting a specific problem, like feeding flax for the omega 3 fatty acids just because it is supposed to be good for the horse.

**Possible Problems with Supplementation**

As with anything, there are problems associated with the inclusion of additional nutrients to a normal diet. These are some of the pitfalls that can be associated with supplementation.

**Looking for a quick fix:** There are no short cuts to a healthy, sound horse and no miracles in a bottle. Expecting instantaneous results from some supplements can be unrealistic and end in disappointment. Outward signs of deficiency of a nutrient are often vague or not easily identifiable to begin with, so identifying whether a supplement has fixed the problem is difficult. Being objective is also difficult since after spending the money the consumer will be expecting results and can often impose a perceived improvement in the horse that is difficult to quantify. For example, the effects of a hoof supplement are only on the new growth of the hoof tissue, so the true benefit of supplementation will not be seen until sufficient amounts of hoof wall have grown out. However, if the consumer does not understand how the
supplement works and is expecting to have quicker results, they may be disappointed at the lack of improvement in the old hoof wall or think that they are observing improvement that may not really be there.

**Unbalancing not balancing:** A balanced diet is one that within the combination of the differing feedstuffs is providing all the required nutrients in the appropriate amounts and in proper proportions with one another to support normal health. Indiscriminate combinations of supplements may have detrimental effects on the balance. For example, not only is calcium in the diet required at 35 g per day for a 500 kg horse in moderate exercise but it should also be supplied in greater amounts than the phosphorus by a ratio of calcium to phosphorus between 1:1 and 6:1. When a diet consists of grass hay and a normal commercial concentrate the balance of calcium in relation to phosphorus should be fine. If a supplement high in phosphorus (like bran) is added in large enough amounts to imbalance the calcium:phosphorus ratio the horse may start to experience the effects without the owner knowing that they caused the problem. The major ratios important in the diet are listed in table 1.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Ideal</th>
<th>Tolerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium:Phosphorus</td>
<td>2:1</td>
<td>1:1 – 6:1 (adults)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:1 – 3:1 (growing)</td>
</tr>
<tr>
<td>Calcium:Magnesium</td>
<td>3:1</td>
<td>3:1 – 6:1</td>
</tr>
<tr>
<td>Zinc:Copper</td>
<td>3:1</td>
<td>3:1 – 5:1</td>
</tr>
</tbody>
</table>

Table 1: Ideal and tolerated ratios of certain basic minerals in the equine diet.

Another effect of unbalancing may be from the combination of various supplements that contain the same nutrient, bringing intake of that nutrient up to excessive levels. For example, a diet may consist of 6 kg of hay that was grown in a part of the country where there is adequate selenium in the soil providing 2 mg of selenium (meeting the requirement) and 4 kg of a concentrate providing an additional 2 mg of selenium. Then add to that a multivitamin supplement with 2 mg of selenium, a hoof supplement with 1 mg of selenium and a vitamin E & selenium supplement with another 1 mg of selenium. Inadvertently, the horse would be receiving an additional 6 mg of selenium on top of the 2 mg from the hay, which is well above the selenium requirement of 1 mg per day for a 500 kg horse in light exercise needed by the horse. Even without combinations of supplements, a toxicity situation can occur with a single supplement when the ingredient is high in some specific nutrient. The supplementation of kelp or seaweed can inadvertently result in iodine toxicity when fed in large amounts because of the high concentration of iodine in the product. A good supplementation program should be minimally disruptive to the balance of the diet. In table 2 are listed some of the nutrients with the lowest tolerance of excessive amounts in the diet.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Min</th>
<th>Max</th>
<th>Toxic</th>
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</thead>
<tbody>
<tr>
<td>Selenium</td>
<td>1 mg</td>
<td>3 mg</td>
<td>20 mg</td>
</tr>
<tr>
<td>Iodine</td>
<td>3.5 mg</td>
<td>4.5 mg</td>
<td>40 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>400 mg</td>
<td>500 mg</td>
<td>5000 mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>15,000 IU</td>
<td>30,000 IU</td>
<td>160,000 IU</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>3300 IU</td>
<td>6100 IU</td>
<td>22,000 IU</td>
</tr>
</tbody>
</table>

Table 2. Amount of nutrients in the diet with the lowest tolerance of over-supplementation for a 500 kg horse.
**Emptying the wallet effect:** Supplementation can be costly, often several dollars per day per horse, depending on how many supplements and what type. It is easy to be lured by the claims of different manufacturers into filling the feed room with expectations of improving the health and well being of the horse. Some may truly help, while others have not proven to be effective and sit cluttering up the shelves. Once the bucket is opened the money is spent whether the product is used or not, without knowing if it is helpful. Even careful selection of only proven products can put a dent in the pocketbook if the supplementation program is long term. Decide whether the conviction to help the horse for the long term is within the budget because some of the supplements only have an effect when they are being given consistently and progress will be lost if supplementation ceases.

**Efficacy research to back the supplement:** There are very few equine supplements that have been fully researched and tested for efficacy and safety in horses. Many supplements are formulated on theory that the ingredients may help. If there has been research, most often it has been done in other species or in vitro. The cost of doing research on horses can be expensive and could conceivably add significantly to the price of the product. Further, if a product can sell without researching it then it is difficult to convince a manufacturer to invest money in testing. There are a limited number of supplements on the market that are research based. If a company has developed a product through research they will ordinarily be more than willing to share the results. The consumer should not be shy about requesting evidence of research on the product from a manufacturer and scrutinizing the work that is presented for evidence that it really pertains to the product being sold.

**Is There Government Regulation of Equine Supplements?**

With human foods, there is a level of expectation by the consumer that when a manufacturer puts the ingredient list and nutrient information on the label that it is accurate. Further, that the best manufacturing practices are being followed by the manufacturer to ensure nothing happens to the consumer after using the product. These expectations are regulated by the Food and Drug Administration (FDA). Animal supplements do not fall under the same regulation as human foods or supplements and have been in regulation limbo. Essentially animal supplements fall under FDA regulation of the but the FDA is overtaxed with administration of human products and will only intervene when there is a report of adverse affects with an animal supplement.

This lack of controls over the manufacture of animal supplements allowed for a free-for-all in the market place when use of supplements was increasing exponentially. Some unscrupulous manufacturers made unsubstantiated promises of effectiveness of their supplements and did not include ingredients or levels of ingredients promised on their labels. As studies and complaints started to reveal the discrepancies in labeling, a group of reputable supplement manufacturers worked together to develop a system for some type of internal controls for the industry. They formed the National Animal Supplement Council (NASC) as a voluntary membership regulatory body which can offer a seal of approval to those manufacturers that follow their stringent controls on manufacturing of animal supplements. Pressure from distributors who sell a wide variety of brands to only sell NASC approved supplements is helping to encourage more supplement manufacturers to join. The effort may make the control of animal supplements better regulated and controlled than those on the human side.

The following description of the standards the NASC can be found on their website (www.nasc.cc).

Members must agree to adhere to NASC’s quality standards, part of which includes submitting to an independent audit to ensure conformance with quality system requirements.
NASC member companies are required to demonstrate compliance with the following criteria before they are granted permission to display the Seal on their products.

1. The company must have a **Quality Manual** in place that provides written Standard Operating Procedures for production process control.

2. The company must have an **Adverse Event Reporting/Complaint System** in place to continually monitor and evaluate products.

3. They must follow proper **label guidelines** for all products.

4. The company must include any specific warning and cautionary statements recommended by the Food & Drug Administration’s Center for Veterinary Medicine and the NASC Scientific Advisory Committee.

The system is still not perfect since it is a voluntary commitment for supplement manufacturers. Because membership into NASC is expensive, it is difficult for the smaller supplement manufacturers to justify the expense without pricing themselves out of the market. Further, NASC does not control how much of each ingredient the manufacturer is including into the product, only that the purchase and care of the ingredients is handled under their guidelines. Still it is a step in the right direction for the consumer.

**Conclusion**

Supplementation can be beneficial to the equine but there are things to consider before deciding whether to add anything to the diet. Having high expectations for the results of the supplement are not realistic unless the basic diet and exercise regime of the horse are sound. Finding the right supplement(s) to complement the basic diet will either be needed to balance out what’s missing in the diet or to address a particular issue with the body of the horse. Problems with supplementation can occur if the expectation is for immediate results, if it ends up unbalancing the diet instead, or if it ends up costing too much for too little results. Being assured of getting a quality product is becoming easier than in years past, particularly with the NASC seal appearing on more and more of the equine products.
Student Abstracts
DIET-INDUCED OBESITY IN HORSES IS ASSOCIATED WITH DECREASED INSULIN SENSITIVITY AND A COMPENSATORY INCREASE IN INSULIN SECRETORY RESPONSE

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ABSTRACT: Obesity is reportedly a growing problem in companion equid populations. Obesity has been associated with insulin resistance (IR) in horses and ponies, and both obesity and IR have been associated with increased risk of laminitis, particularly the pasture-associated form of this disease. However, it has not been demonstrated that an increase in adiposity over time with the induction of obesity is associated with disturbances in insulin sensitivity or glucose metabolism. This study investigated whether diet-induced obesity (body condition score ≥ 7.5, scale 1 – 9) is associated with changes in insulin sensitivity or basal concentrations of leptin, insulin and glucose. To induce weight gain, 13 Arabian geldings were fed 200% of their digestible energy requirements for 4 months. Before and after weight gain, adiposity was measured and frequently sampled intravenous glucose tolerance tests were performed and assessed by the minimal model of glucose and insulin dynamics. Data were analyzed by the Mann-Whitney U test and are reported as median values (interquartile range). At the end of the trial, body weight was increased ($P < 0.001$) by 20% from 426 (408 – 456) to 520 (489 – 534) kg, body condition score increased ($P = 0.001$) from 6.1 (5.5 – 6.8) to 7.7 (7.5 – 8.2), percent body fat estimated from subcutaneous fat thickness (ultrasonic assessment) increased ($P = 0.001$) from 14 (12 – 16) to 23 (19 – 25) %, and plasma leptin concentration increased ($P < 0.001$) from 2.5 (2.2 – 4.3) to 9.3 (6.9 – 12.4) ng/mL. Collectively, these data are representative of an increase in adiposity. Resting insulin concentration was increased ($P < 0.001$) from 4.1 (3.5 – 6.0) to 28 (21 – 39) mU/L, however glucose concentration (94 [92 – 98] mg/dL) remained unchanged ($P = 0.21$). Insulin sensitivity decreased ($P < 0.001$) by fivefold from 1.48 (1.02 – 1.85) to 0.36 (0.14 – 0.51) $\times 10^4$ L·min$^{-1}$·mU$^{-1}$, accompanied by an almost fivefold increase ($P < 0.001$) in the acute insulin response to glucose from 182 (167 – 237) to 936 (715 – 1141) mU/L·min$^{-1}$. These changes resulted in a similar ($P = 0.94$) disposition index (325 [143 – 505] $\times 10^4$), indicating that the decrease in insulin sensitivity was effectively compensated for by an increase in insulin secretory response. The results of this study demonstrate that diet-induced obesity in horses is associated with decreased insulin sensitivity and a compensatory increase in insulin secretory response. Avoiding obesity is important to minimize insulin resistance in horses. This avoidance may then reduce risk for laminitis and help maintain a healthy metabolic state.
ABOMASAL INFUSION OF BUTTERFAT INCREASES MILK FAT IN LACTATING DAIRY COWS

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ABSTRACT: The introduction of multiple component milk pricing system by the Federal Milk Marketing Administration in 2000 provides a powerful economic incentive for dairy producers to produce high value milk components, namely fat and protein. Milk fat is the milk component that is most easily manipulated by the diet. Attempts to increase milk fat using long chain fatty acids have produced variable or inconsistent results. The objective of this study was to compare the effects of abomasal infusion of butterfat containing all fatty acids (FA) present in milk, including the short and medium chain FA, with infusion of only the long chain FA (LCFA) present in milk, on the FA composition, milk fat yield and mammary lipogenic gene expression in lactating dairy cows. Eight rumen fistulated Holstein cows, in early lactation (49±20 DIM) were used in a replicated 4x4 Latin square design. Treatments were abomasal infusion of: 1) no infusion (Control); 2) 400 g/d butterfat (Butterfat); 3) 245 g/d LCFA using a blend of 59% cocoa butter, 36% olive oil, and 5% palm oil that provided equivalent amounts of LCFA found in 400 g of butterfat (LCFA); and 4) 100 g/d conjugated linoleic acid (CLA, negative control), providing 10 g of t10c12 CLA. Fat supplements were infused in equal portions 3 times daily at 0800, 1400, and 1800 h during the last 2 wk of each 3 wk experimental period. Daily dry matter intake (DMI) and milk production were unaffected by the infusion treatments. Butterfat infusion increased milk fat percentage by 14% (P < 0.03) to 4.26% and milk fat yield by 21% (P < 0.02) to 1421 g/day compared with Controls (3.74 % and 1178 g/day). Milk fat percentage and fat yield were decreased by 43% (P < 0.001) by CLA. The mammary lipogenic gene expression profiling showed increased expression of genes involved in the uptake and synthesis of fatty acids and triacylglycerol formation with the butterfat infusion, whereas LCFA showed opposite effects. While LCFA had no effect on fat synthesis, abomasal infusion of butterfat increased milk fat percentage and yield suggesting that the availability of short and medium chain FA may be a limiting factor for milk fat synthesis.
EFFECTS OF GINGER AND CRANBERRY EXTRACTS ON MARKERS OF INFLAMMATION & PERFORMANCE FOLLOWING INTENSE EXERCISE IN HORSES

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ABSTRACT: This study hypothesized that ginger (Zingiber officinale) and cranberry (Vaccinium macrocarpon) extracts would alter markers of performance, thermoregulation, muscle damage and mRNA expression for the inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-6 (IL-6) after an exhaustive bout of exercise in horses. Nine unfit Standardbred mares (age 10 ± 4 yrs, ~450 kg) completed 3 graded exercise tests (GXTs) in a randomized crossover design. The GXTs were conducted between 0700 and 1200 no less than 7 d apart. Mares received a dose of either water (2 L), cranberry (~30 g in 2 L water) or ginger (~30g in 2 L water) extract 1 h prior to testing. Blood samples were taken prior to dosing (pre-ex), at the end of each step on the treadmill, end of exercise, 2, 5 and 30 min, 1, 2, 4 and 24 h post-GXT. Plasma total protein concentration (TP) and hematocrits (HCT) were analyzed immediately following the exercise test. No effect of treatment (p>0.05) was seen on VO_{2max}, run-time to fatigue, TP or HCT. Analysis of creatine kinase (CK) and aspartate aminotransferase (AST) were done commercially. Samples were analyzed via RT-PCR for mRNA expression of TNF-α, IFN-γ and IL-6. A slight increase (p<0.05) in CK was seen in all groups at 2 h post-GXT. CK was substantially elevated (p<0.05) in the ginger group at 4 h post-GXT. All CK levels returned to baseline 24 h post-GXT. No change (p>0.05) was noted in AST. The cranberry group had significantly lower TNF-α mRNA expression compared to control and ginger. Ginger appeared to influence the upregulation and expression of IFN-γ mRNA at 30 min-post GXT, but, more strikingly, significantly decreased cardiovascular recovery time (ginger =101 ± 3 s, water =130 ± 14 s, cranberry = 131 ± 16 s). No effect of treatment or exercise (p>0.05) was seen on IL-6 mRNA expression. Results suggest that cranberry extract blunts the upregulation and expression of TNF-α mRNA, while ginger extract reduces cardiovascular recovery time in horses completing a short, exhaustive bout of exercise.
THE BIOAVAILABILITY OF A COMMERCIALY AVAILABLE CRANBERRY POWDER IN AN EQUINE MODEL

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ABSTRACT: The purpose of this study was to test the hypothesis that the metabolites from a commercially available 90mx cranberry powder could be detected and identified in horse plasma, urine and muscle after dosing via nasogastric tube. Three healthy mature Standardbred mares were used in a random cross-over design, were they received either 200g of extract dissolved in 2L of water (LD = low dose), 400g of extract dissolved in 2L of water (HD = high dose) or 2L of water (CON). Blood, urine and muscle samples were collected pre-dosing and at 0.5, 1, 2, 4, 8, 16 and 24 h post-dosing. HPLC analysis of the samples showed trace amounts of flavonols in some plasma samples, but overall quantitatively there were no significant increases in the horse’s urine or plasma. HPLC analysis of the muscle samples did show an increase over the control for the following flavonols and flavonol glycosides; quercetin, Q-3 arabinopyranoside, M-3 galactoside, Q-3 rhamnoside, Q-3 arabinofuranoside and Q-3 galatoside. These data show for the first time the uptake of an ingested flavonol into horse muscle. This lays the foundation for further investigation into the effects of flavonols on horse muscle which has been physiologically stressed by exercise or other variables.
CONCURRENT USE OF VETERINARY DRUGS AND HERBAL MEDICINES IN RACING STANDARDBREDS

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ABSTRACT: The use of herbal medicines is an increasing trend in racing Standardbreds owing in part to increasing evidence of efficacy in horses, and to growing concerns over adverse effects of allopathic medicines. However, herbal medicine use in racehorses remains largely unreported by trainers to veterinarians and their use concurrent with allopathic medicines presents a substantive risk for herb/drug interactions. The objectives of this study were: 1) to determine the frequency of herbal medicine use in Standardbred racehorses stabled on-track or at off-track training facilities; 2) record the most commonly used allopathic medicines used in Standardbred racehorses on-track and off-track and 3) determine the frequency of concurrent herbal medicine and allopathic medicine use on-track and off-track. Thirty-five on-track trainers (n=122 horses) and twenty-five trainers from seven off-track training facilities (n=110 horses) described the categories of allopathic drugs and herbal medicines used on their actively racing horses over the previous ten days. Frequency data for on-track and off-track horses were compared using McNemar’s Chi-square test, with the Yates Correction for Continuity. Frequency of use was considered statistically different between on-track and off-track horses when \[ P \leq 0.05 \]. The four most common categories of drugs used for both on-track and off-track training horses, in order of frequency of use, were: diuretics (primarily lasix) > anti-inflammatory drugs > bronchodilators > herbal medicines. 13.8% of horses were administered herbal medicines in the previous 10 days, of these 67% and 58% of on-track and off-track horses, respectively, received herbal medicine concurrent with at least one allopathic medicine. These data demonstrate that concurrent use of herbs and allopathic medicines presents significant risk of herb/drug interactions in racing Standardbreds and further research is needed in order to identify the specific types of herbal medicines being administered and the clinical implications of interactions with other medicines.
DIFFERENT FORMS OF VITAMIN E SUPPLEMENTATION
AND THEIR ABSORPTION IN HORSES

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ABSTRACT: Vitamin E is an integral part of the diets of many different species. This vitamin has been found to have antioxidant properties and is especially important in preventing lipid peroxidation of the cellular membranes, which can result from stress in the body due to exercise, aging and various diseases. Vitamin E is a fat-soluble vitamin which has eight different natural diastereomer forms; four tocopherols and four tocotrienols. Additionally, there are also eight acetate forms that correspond to the alcohol forms, each having different activity levels; α-tocopherol (TOC) being the most active. Due to its high level of activity, TOC is most commonly supplemented in the diet. The objective of this study was to measure vitamin E absorption in stalled horses fed different forms of the vitamin. It was hypothesized that the natural alcohol micellized form would show the greatest absorption in the studied horses. For a 14 d period, 20 Standardbred mares were kept in stalls, with 4 hr/d spent outside in a dry lot. They were fed free-choice grass hay and 2 kg of a non-fortified sweet feed divided into two meals. At 0800 horses were supplemented with 4000 IU of five different forms of vitamin E (Stuart Products, Inc.) top dressed on their morning sweet feed. TRT 1 was given 10 g of 400 IU/g natural acetate powder, TRT 2 was given 6.66 g of 600 IU/g natural acetate powder, TRT 3 was given 6.66 g of 600 IU/g natural alcohol powder, TRT 4 was given 20 g of 200 IU/g micellized alcohol powder and TRT 5 was given 8 ml of 500 IU/ml micellized alcohol liquid. Blood samples were collected into Sodium Heparin Vacutainer tubes before supplementation (d 0; after 1 week of stall and dry lot adaptation), and after d 7 and d 14 of supplementation. Blood was centrifuged and separated into plasma aliquots, and frozen at -80°C until analysis for TOC. Main effects of sample was significant ($P < 0.0001$), but there was only a trend for the effect of treatment ($P = 0.077$) and their interaction ($P = 0.077$). Treatments 1, 2, and 3 increased from d 0 to 7 ($P < 0.05$), but remained similar at d 14. Treatments 4 and 5 also increased ($P = 0.004$, and $P < 0.0001$, respectively) from d 0 to 7 but were significantly higher than TRT 1, 2, and 3 at d 7 ($P < 0.05$). Most treatments peaked at d 7 (TRT 1 = 3.64 ± 0.3; TRT 2 = 3.25 ± 0.3; TRT 3 = 3.34 ± 0.4; TRT 4 = 4.85 ± 0.6; TRT 5 = 6.55 ± 1.6 μg/mL) and either remained similar or decreased (TRT 5 = 4.31 ± 0.6 μg/mL, $P = 0.004$) at d 14. According to the data, the micellized alcohol liquid showed the greatest increase in TOC level in the blood, but decreased after two weeks of supplementation, unlike the similar form only in a powder, which remained similar to the peak concentration. This proves the hypothesis that the micellized alcohol would have the highest absorption in the body, which could be due to the ability of the micellized form to travel more efficiently through the digestive system and into the blood. Vitamin E helps protect vital membranes of the body’s cells and is necessary in the diet, so care should be taken to ensure that the body is receiving the most active form of the vitamin to maximize these protective benefits.
THE EFFECTS OF SUPPLEMENTAL SUPEROXIDE DISMUTASE ON PERFORMANCE AND OXIDATIVE STRESS IN EXERCISING HORSES

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ABSTRACT: Oxidative stress can be defined as an imbalance between the production of harmful free radicals or reactive oxygen species and the body's defense system via antioxidants. The body produces enough antioxidants to prevent any oxidative damage, but when an animal exercises, physiological conditions change so that free radicals are created at a faster rate, thus potentially disrupting the oxidant/antioxidant balance and damaging the homeostatic environment of the cell. This stress can be combated by supplementing antioxidants such as the enzyme superoxide dismutase (SOD), which catalyzes the breakdown of harmful superoxide radical into hydrogen peroxide and water. The objective of this study was to analyze the effects of an oral SOD supplement on performance parameters during and after intense exercise in horses. The study was a double-blind, placebo controlled, crossover design using 12 unfit Standardbred mares randomly placed into two groups and supplemented either 3g (3000 IU) of SOD (TRT) or 3 g of placebo (CON). After 4 and 6 wks of supplementation, each horse ran a repeated sprint exercise test (RSET) on an equine treadmill. The horses underwent a 6-wk washout period, then crossed over treatments and repeated the study, so each horse served as its own control. Blood samples were taken before (PRE), at fatigue, 20 min, 2 h, 4 h, 24 h, and 36 h of recovery (REC). Blood samples were analyzed for total plasma protein (TP) and hematocrit (Hct). Heart rate (HR) was taken throughout the RSETs during the last 15 s of each exercise step. There was no treatment effect for average HR (TRT = 158.3 ± 2.0, CON = 158.6 ± 1.9 bpm) or HR peak (TRT = 217.4 ± 2.4, CON = 218.5 ± 2.2 bpm) across all RSETs. The average length of exercise test was 18.3 ± 0.3 min and was not different for treatment, nor was percent of time spent at or above 90% HR peak during each test (36.2 ± 0.2 %). There was a significant effect of sample and exercise test for TP (P < 0.0001), and Hct (P < 0.05), with the peak occurring at the FATIGUE sample (7.6 ± 0.04 g/dl, 54.8 ± 4.8 %, respectively). The average TP was higher for RSET 1 and 2 vs. RSET 3 and 4 (P < 0.0001). These differences indicate that there is possibly an effect due to training as the study progressed. Also the environmental conditions, including ambient temperature may have confounded these results (RSETs 1 and 2 performed in July and August, RSETs 3 and 4 performed in Sept. and October). There were no significant differences between treatment groups for HR, TP, and Hct indicating that the SOD supplement may not have a direct effect on performance parameters. The future direction of this study is to analyze antioxidant variables and muscle membrane permeability which might reveal that SOD had a physiological effect on these mechanisms. Further analysis of circulating antioxidants (glutathione and glutathione peroxidase) and muscle enzymes (creatine kinase) will expand our knowledge as SOD’s potential benefits.
MORNING VERSUS AFTERNOON GLYCEMIC AND INSULINEMIC RESPONSES IN THOROUGHBRED BROODMARES

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ABSTRACT: Glycemic and insulinemic responses to meals influence equine health and performance through the impact they have on hormonal patterns and metabolism. Examples include insulin resistance, obesity, laminitis and developmental orthopedic disease. No other studies in the horse have been conducted to assess the difference in glycemic and insulinemic response to morning versus afternoon meals. The objective of this study was to quantify the morning and afternoon glycemic and insulinemic responses to a meal. Differences were considered significant at a $P < 0.05$. This study was conducted on 4 days over a 2 week period in July using 8 Thoroughbred mares. The study was a duplicated Latin square with four feeds formulated with 0, 200, 400 and 800 lbs/ton of added rice bran product. All diets were isocaloric and isonitrogenous. While the effects of diets were examined as part of the analysis, that data is not presented here and does not impact these findings. On each of the study days, blood plasma samples were taken via a jugular vein catheter at 0, 15, 45, 75, 105, 135, 165, 225, 285, 345, 405, 435, 465, 495, 525, 585, 645, 705, and 765 minutes. A grain meal of 1.5 kilograms was fed immediately following the 15 (AM (0800)) and 405 (PM (1400)) min samples. Glucose (mg/dl) and insulin (mIU/L) were measured in blood plasma with a glucose oxidase and chemiluminescent assay, respectively. Dependent variables were peak values for glucose and insulin, area under the curve for glucose and insulin, and the time to peak, which represents the time from feeding the meal to when glucose or insulin reached peak values. There was no detectable difference in the starch intake of the AM diets 323 ± 133 g in comparison to the PM 330 ± 127 g, or the time taken to eat in the AM 22 ± 23 min versus that in the PM 24 ± 21 min. Baseline (mean of samples 0 and 15) glucose and insulin were 94 ± 5.3 mg/dl and 6.1 ± 2.9 mIU/L, respectively. The AM peak values for glucose and insulin (143 ± 17 mg/dl, 57± 31 mIU/L) were higher than the PM peak values (123 ± 8.6 mg/dl, 37 ± 20 mIU/L). No difference was detected in the time to peak values for AM versus PM for glucose (127 ± 49, 118 ± 51 min) and insulin (137.4 ± 55.7, 118.9 ± 43.5 min). The glucose and insulin area under the curve in the AM (8103 ± 2860 min*mg*dl$^{-1}$, 8468 ± 5050 min*mIU*L$^{-1}$) were higher than the PM values (4170 ± 1744 min*mg*dl$^{-1}$, 4358 ± 2091 min*mIU*L$^{-1}$). Like other species, the horse shows a higher glycemic and insulinemic response to a similar meal in the morning when compared to the afternoon. The specific mechanism may relate to circadian patterns of other metabolic regulatory hormones like melatonin or may simply be due to the fact that glucose and insulin values have only just returned to baseline when the afternoon meal is fed. Broadly, time of day is a factor when evaluating glycemic and insulinemic response.
THE EFFECT OF SUPPLEMENTAL SUPEROXIDE DISMUTASE ON PLASMA NITRIC OXIDE IN EXERCISING HORSES

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ABSTRACT: One of the most important aspects of maintaining the integrity of the nation’s equine industry is keeping our equine athletes healthy and fit. However, during exercise, horses are exposed to an enormous number of mechanical and metabolic stressors that threaten to hinder their performance ability. One such stressor is oxidative stress, a byproduct of cellular respiration. Oxidative stress can lead to molecular, cellular and even tissue damage, and plays a vital role in inflammatory responses to exercise. The goal of our study was to determine what effects dietary supplementation with superoxide dismutase (SOD), an antioxidant enzyme, had on exercise-induced inflammation in Standardbred mares. Nitric Oxide (NO), the body’s potent vasodilator, was used as an inflammatory marker. This study used a double blinded, placebo controlled, cross-over design and involved 12 unfit, healthy mares between 3 and 15 years of age. The horses were randomly divided into two groups, with the first being supplemented daily with 3g (3000 IU) of SOD (TRT) and the latter being given 3g of a placebo (CON). The horses were exercised on an equine treadmill at d 28 and 42 of supplementation using a repeated sprints exercise test (RSET). Blood samples were collected at times PRE, fatigue, 20 min, 2 h, 4 h, 24 h and 36 h recovery (REC). Plasma was later analyzed for NO using the previously validated Quantichrom™ Nitric Oxide Assay Kit, which measured total nitrite concentration through colorimetric determination. Data were analyzed using a mixed model ANOVA with repeated measures in SAS (9.1). Main effects for exercise test \( (P < 0.0001) \), sample \( (P < 0.0001) \) and treatment \( (P = 0.005) \) were significant, along with a sample by test interaction \( (P = 0.0003) \). The TRT group \( (0.035 ± 0.002 \text{ mg/dL}) \) was significantly higher \( (P = 0.005) \) than the CON group \( (0.031 ± 0.002 \text{ mg/dL}) \) overall. The NO concentration of RSET 1 \( (0.040 ± 0.002 \text{ mg/dL}) \), and 2 \( (0.041 ± 0.002 \text{ mg/dL}) \), were significantly higher \( (P < 0.0001) \) than RSET 3 \( (0.026 ± 0.001 \text{ mg/dL}) \) and 4 \( (0.025 ± 0.001 \text{ mg/dL}) \). Samples PRE though 24 h REC showed a decrease in NO over time, however 36 h REC was significantly higher \( (P < 0.02) \) than other samples. The elevated plasma concentration of NO in the TRT group adds support to the claim that the superoxide anion is the major limiting factor in NO bioavailability. The SOD acts in the catalytic breakdown of the superoxide anion, which otherwise would be free to react with NO to form peroxynitrite, a cytotoxic agent. Some potential explanations for the differences between the RSETs include environmental and training effects, as well as changes in reproductive season. The elevated NO concentrations seen at sample 36 h REC could suggest a delayed inflammatory response needed to facilitate muscle repair correspondent with delayed onset muscle soreness (DOMS).
SUPPLEMENT USE AND NUTRIENT INTAKE IN ELITE THREE-DAY EVENT HORSES

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ABSTRACT: Supplementation in intensely exercised horses is needed to replenish the nutrients consumed. Supplementation is adding one or more dietary nutrients to the horse’s daily intake that is missing from feeding forage alone. This occurs to sustain or enhance performance. However, over supplementation can lead to toxic levels of nutrients, which can cause harm to the horse as well as the environment. Our objective of this study is to analyze the daily intake of commonly supplemented vitamins and minerals in horses competing in the Jersey Fresh CCI** (n=10) and CCI*** (n=25) three-day-event. The riders were asked to sign a release waiver form allowing their horses to participate in the study along with a nutritional management survey. To obtain precise measurements all grain, hay and bran was weighed as part of the study. Before competition each horse’s weight and body condition score were taken. The total intake of nutrients was calculated by using the manufacture or NRC (2007) nutrient content. Recommended daily intakes were calculated for each horse under going very heavy exercise using the NRC (2007) equations. The nutrients studied were vitamin E, potassium, phosphorus, calcium, sodium, and magnesium. The average daily intake of vitamin E from hay and grain alone was 1029.4 ± 174 IU, with the addition of supplements intake was 1667.2 ± 282 IU; the recommended intake was 1065.3 ± 92.8 IU/d. The average daily intake of potassium without supplements was 242.7 ± 15.8 g and with supplements was 248.4 ± 15.7 g with a recommended intake of 56.4 ± 4.9 g, leading to an excess intake of 4.3 and 4.4 times, respectively. Phosphorus total dietary intake without supplements was 57.2 ± 4.2 g and with supplements 57.9 ± 4.2 g. The recommended intake was 31.0 ± 2.7 g, creating an excess intake of 1.8 and 1.9, respectively. Calcium intake without supplements was 108.7 ± 7.2 g and with supplements was 110.5 ± 42.4 g with a recommended intake of 42.5 ± 3.7g, creating a 2.6 times excess. Sodium intake was 18.4 ± 10.1 g without supplements and 43.5 ± 28.7 g including supplements (not including salt blocks) with a recommended intake of 43.6 ± 3.8 g. Magnesium intake without supplements was 34.2 ± 1.9 g and with supplements was 35.5 ± 2.0 g; the recommended intake was 15.9 ± 1.4 g, creating an excess of 2.2 times. While vitamin E, calcium, and magnesium are in excess of the daily recommended amounts, they have not reached toxic levels. Potassium has exceeded the toxic level, which could cause cardiac arrhythmias and muscle tremors in intensely exercising horses. The excess phosphorus is of no harm to the horse if it is within the calcium:phosphorus ratio of 2:1. However, excess phosphorus excreted by the horse into the environment further increases the daily intake by depositing an increased amount of phosphorus into the pastures and hay fields, which could potentially reverse the calcium phosphorus balance in forages. While most of the vitamins and minerals studied were in excess but not toxic range, the concern comes economically. The excess feeding of supplements above the daily recommendations causes an increased excretion by the body leading to wasted nutrients.
GINGER AND CRANBERRY AND THE INSULIN RESPONSE TO INTENSE EXERCISE IN HORSES


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ABSTRACT: Previous studies have demonstrated that ginger (Zingiber officinale) and cranberry (Vaccinium macrocarpon) extracts alter markers of performance and mRNA expression for inflammatory cytokines after an exhaustive bout of exercise in horses. Those cytokines may alter the insulin response to acute intense exercise. Therefore, nine unfit Standardbred mares (age 10 ± 4 yrs, ~450 kg) were used to test the hypothesis that ginger and cranberry would alter the insulin response after intense exertion. Each mare completed 3 graded exercise tests (GXTs) in a randomized crossover design with the investigators blind to the treatment. The tests were conducted between 0700 and 1200 no less than 7 d apart. Mares received either water (2 L), cranberry (~30 g in 2 L water), or ginger (~30g in 2 L water) extract 1 h prior to testing. Blood samples were taken prior to dosing (about -1 hr to exercise), immediately after exercise (0 hr) and at 0.5, 1, 2, 4 and 24 h post-GXT. Plasma insulin concentrations were measured in duplicate using a RIA kit previously validated for use in horses. Data were analyzed using a three-way ANOVA with the null hypothesis rejected when P<0.05. There was a significant effect of time on plasma insulin concentration with a suppression immediately following GXT followed by a substantial (P<0.05) increase and a return to baseline (P>0.05) between 2 and 4 hrs post-GXT. There was a significant effect of treatment on the insulin response to exercise with lower concentrations at 0.5, 1 and 2 hrs. These data suggest that food extracts may modulate the insulin response to exertion.
THE EFFECTS OF INTENSE EXERCISE ON PLASMA NITRIC OXIDE IN HORSES PERFORMING IN A CCI** AND CCI*** THREE-DAY EVENT

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ABSTRACT: With increasing popularity of three-day eventing worldwide there is also increasing concern over the health and welfare of the equine athletes participating in the most physically demanding phase of the three-day event, the cross-country. There is also developing interest in the recently discovered free radical diffusible gas, nitric oxide (NO) as a systemically ubiquitous intracellular communicator in the inflammatory and immune responses. Increased levels of NO have been theorized to increase cellular permeability as a result of oxidative stress through the development of reactive nitrogen species. The goal of this study is to further understand the relationship between NO and muscle membrane permeability in horses performing in the cross-country phase of a CCI** and CCI*** three day event. Each rider participating in the study signed a waiver allowing their horse(s) to participate in the study. Thirty five horses competing in the 2007 Jersey Fresh CCI**/CCI*** three-day event were sampled via jugular venous puncture before the start of the competition (PRE), 20 to 30 min after the cross country phase (XC) and during the 18 to 24 h recovery period, but before stadium jumping (POST). The blood samples were evaluated for NO using an assay previously validated for horse plasma. To determine muscle membrane permeably the blood was analyzed for the muscle cell enzymes creatine kinase (CK) and aspartate aminotransferase (AST). The data was analyzed using a mixed model ANOVA with repeated measures in SAS (9.1). The main effect of sample was significantly different for NO ($P = 0.013$), CK ($P < 0.0001$), and AST ($P = 0.0007$). Nitric oxide decreased significantly ($P = 0.003$) between the PRE and POST (0.072 ± 0.007 and 0.046 ± 0.008, respectively), yet the XC sample was not different. However, the CK concentration increased between the PRE and XC samples ($P < 0.0001$), but remained similar to XC at the POST sample. The AST concentration decreased from PRE to XC ($P = 0.032$), and was higher than both PRE and XC at POST ($P = 0.059$, and $P = 0.0002$, respectively). There was also a negative correlation with NO and body weight ($r = -0.32, P = 0.002$). These results indicate that there is increased muscle membrane permeability as a result of exercise but decreased plasma concentrations of NO. This could represent that plasma NO is absorbed into the endothelium of the blood vessels after bouts of intense exercise or is used as a post-exercise inflammatory mediator in damaged joints or muscles. This was the first study to document plasma NO status in eventing horses. Using these results may aid in further study NO in other systems during exercise or the effects of different forms of exercise on NO availability. Once normal horse NO concentrations are documented we may be able to determine if eventing horses have greater NO plasma availability during exercise.

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POULTRY ROUNDTABLE DISCUSSION:
ENZYME USE FOR IMPROVED POULTRY PERFORMANCE

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Summary

The use of enzymes as a tool in feed manufacturing continues to gain acceptance in the poultry industry in the US and world-wide. Since the first phytase enzymes were approved, poultry nutritionists and feed mill managers have become more accustomed to incorporating enzymes in feeds as a routine practice, and have gained a better understanding of their modes of action, the technology required for efficient application to feed, and the economic benefits of their incorporation in rations. However, there are still myriad unknowns in enzyme technology as applied to poultry nutrition and feed manufacturing. In this roundtable exchange, we will discuss the current state of practical enzyme use in the industry and explore possibilities for the future. As the choice of available enzymes expands and application technology improves, the poultry industry’s interest in enzymes other than phytase continues to increase, particularly as nutritionists face major shifts in ingredient availability and cost. Growth areas are carbohydrolases, enzymes that degrade starch or non-starch polysaccharides, and proteases. As the technology expands, a wider variety of products with enhanced capabilities is certain to become available. In deciding which products to use and how to incorporate them into feeding programs, much remains to be learned about: evaluating the efficacy and stability of enzyme use in given feeding programs; matching available enzyme products with appropriate feed ingredients; deciding on the use of single enzymes, enzyme cocktails or enzyme combinations produced by single organisms; assigning appropriate nutrient benefits of enzyme products for feed formulation purposes to name a few. Please come share your thoughts on the enzyme needs for the future and ways to enhance current technology for the poultry industry. Proceedings of the discussion will be recorded and published to the Conference website.