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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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We thank the program participants for their cooperation in providing the material in this document.

SAVE THE DATES: MARCH 28-29, 2012

10TH MID-ATLANTIC NUTRITION CONFERENCE
AB Vista Technical Symposium

Wednesday, March 23rd

The Technical Symposium will be held at 2:00pm

2:00pm  Welcome
          Joe Slattery, AB Vista

2:10pm  Corn Quality and Impact on Nutrient Digestibility
          Bill Dozier, Auburn University

2:30pm  Differentiation of Xylanases and Potential Benefits of a
          Xylanase-Phytase Combination
          Tara York, AB Vista

3:00pm  Impact of Calcium on Phytase Efficacy in Poultry
          Roselina Angel, University of Maryland

3:25pm  Calcium Solubility and Digestibility: The Influence of
          Calcium Source and Phytase
          Carrie Walk, AB Vista

4:00pm  Exploiting Calcium-Specific Appetite in Broiler
          Nutrition
          Aaron Cowieson, University of Sydney, AU

4:50pm  Wrap Up and Final Questions

5:00pm  RECEPTION, Sponsored By AB Vista
PHOSPHORUS AVAILABILITY - WHEN WILL WE RUN OUT OF PHOSPHORUS?
Steve Auman
PotashCorp
1101 Skokie Blvd., Suite 400
Northbrook, IL  60062

Overview
A global shortage of phosphate is not imminent. Some researchers have estimated peak production by 2040 followed by a rapid decline (Cordell, The Story of P, 2008). The public has been alarmed by sensationalist news articles which reveal that a dwindling supply of phosphate will have grave consequences for food security. The truth is that World phosphate reserves are not shrinking rapidly, and there is a sustainable approach to managing phosphate rock extraction and utilization as nutrients for food production. Current projections show that there will be enough phosphate fertilizer to meet demand at least for the next 300 to 400 years, depending on which numbers you use to estimate reserves and consumption.

Phosphorus is an essential nutrient for growing food and is a key ingredient in certain industrial chemical products as well. It is obtained primarily from mined rock or sedimentary deposits of enriched ore found in many countries around the world.

Long term population and economic growth is driving the demand for food in developing countries. 90% of phosphorus is used for food production - manufacture of N-P-K blend fertilizers for crop production and manufacture of animal feeds. The FAO estimates that food production will have to nearly double by 2050 in order to feed the world's growing population.

The fertilizer industry recognizes that the quality of reserves is declining and the cost of extraction and processing the ore is rising as the highest quality and most easily accessed reserves are depleted first. Historically, supply has kept up with demand as technology contributes to improved efficiencies for production and use. This sustainable approach to phosphorus extraction and use will help avoid potential future phosphate scarcity. Future phosphate demand is projected to increase by 3% per year based on estimates of rising food demand to 2050. Fertilizer producers fully expect to meet that demand.

World Phosphate Rock Reserves
The current publicly available reference on world phosphate reserves is the United States Geological Survey (USGS). These estimates have not been updated for several years. British Sulphur, (April, 2009), estimated current world phosphate rock reserves at about 90 billion tonnes, down from a 1999 estimate of 128 billion tonnes and a 2006 estimate of 122 billion tonnes. The differences in annual estimates of phosphate rock reserves is due to interpretation of mining costs by various consultants and improved reporting and accuracy of reserve status by individual countries.
Morocco holds most of the world's economically viable reserves of phosphate rock - with the extraction cost for approximately 62% of its reserves estimated at less than $40/tonne.

The International Center for Soil Fertility and Agricultural Development (IFDC) has announced a multi-year "Phosphate Resources and Research Initiative" with the objective
of reassessing world phosphate reserves. The International Fertilizer Association (IFA), The World Phosphate Institute (Imphos) and the International Plant Nutrition Institute (IPNI) have agreed to participate. The first phase is a review of existing, publicly available information on reserves and an assessment of the existing methodologies for estimating phosphate rock resources. A paper is due out in 2010. The next phase would assess the technical and economic aspects of phosphate supply.

**Phosphate Rock Supply/Demand**

2010 Phosphate rock consumption is estimated at 175 million tonnes per year. Based on the latest rock reserve estimate of 90 billion tonnes, current reserve life is estimated to be 450 years. However, this estimate may be revised based on the new Phosphate Resource study estimates.

Phosphate Rock production is concentrated in a small number of countries. 28 countries mined phosphate rock in 2009 and the top five accounted for 80% of world production. China is the largest producer and consumes virtually all of its rock in domestic production facilities. The U.S. is the second largest producer, although its production has declined by approximately 15 million tonnes or 42 percent since 2000. About 5 million tonnes of this was due to the recent (August 20, 2010) shut down of Mosaic’s South Ft. Meade Phosphate Mine due to environmental permit issues.

While the global supply of phosphate rock is adequate, high-quality ore bodies are limited in number and construction of processing facilities carries high capital costs. Access to low-cost, long-term rock reserves is a key to success in the phosphate industry. The Vale Bayovar mine in Peru has estimated reserves of 100 million tonnes of 65-
67BPL rock, one of the largest rock deposits in South America. It is scheduled to come on stream in late 2010. Mosaic has a 35% interest in the mine and Mitsui another 25% interest. There are several factors that impact phosphate rock expansion and supply:

Investment costs to develop a new rock mine are substantial and risky due to the cyclical nature of the business. For example, over $1 billion has been invested in the new Bayovar mine in Peru. Total investments proposed by the fertilizer industry for currently planned capacity expansion to 2013 is close to US$90 billion.

Growing concerns about the impact of phosphate mining on ground water and air quality require environmental impact studies prior to mine development. These studies are expensive and time consuming.

The growing uncertainty about fossil fuel scarcity will impact energy cost which in turn will influence production and transportation costs.

Five countries account for approximately 78% of world phosphate rock production and exports. Approximately 17% of global production is exported with Morocco alone accounting for approximately 42% of world exports.

The International Fertilizer Association projects world phosphate rock capacity to grow to 248 Million Tonnes by 2013. About 15 million tonnes is earmarked for exports. In the
unlikely case that all expansion projects proceed as scheduled, a potential surplus may develop in the export market in the near term. However, rock markets are expected to remain tight to balanced in the medium term due to anticipated growth in global phosphate demand for phosphate fertilizers.

Economic factors would alter supply/demand volumes and change the resulting phosphate rock reserve life estimate. For example, easily accessible low cost high/grade phosphate ore reserves may be depleted within 50 years - sooner in areas such as Florida. Minning less accessible and lower grade phosphate rock would lead to higher fertilizer prices, more efficient phosphate rock mining, processing and use, and phosphate recovery methods.

The global phosphate market began to tighten in 2007 and tightened further in the first half of 2008 because of strong demand for phosphate fertilizer and limited previous economic justification for capacity building. Prices were further driven by global economic concerns about food scarcity and supply. The rumors failed to take hold, demand softened and phosphate rock prices fell back.

The bench-mark Moroccan Phosphate rock export pricing reflects the historical movement of prices and the impact of global economic conditions. Phosphate rock prices have stabilized since Q3 2009 to approximately $110/tonne. The market is expected to remain firm going forward as demand for phosphate fertilizer increases. This supply/demand outlook requires favorable prices to support further investment in capacity improvements and expansion.
Phosphoric Acid

Fertilizer accounts for 90% of phosphate rock use and 85% is converted to phosphoric acid. Non-fertilizer use includes animal feed, food and industrial applications. Since fertilizer is the biggest consumer of phosphoric acid, its market drivers have significant influence over the direction of the phosphoric acid market.
Phosphate rock is processed to upgrade the ore. It can be thermally reduced to remove the phosphorus or chemically reacted with acids to form phosphoric acid. Most phosphoric acid processes use sulfuric acid to acidulate the ground phosphate rock, creating phosphoric acid and by-product gypsum. Phosphoric acid is a convenient raw material for manufacturing phosphate fertilizers, mineral supplements for animals, and technical and food grade phosphate products. Sulfur cost has a major impact on phosphoric acid production cost.
Global Phosphoric Acid Capacity vs Demand
The top five phosphoric producing countries account for 80% of world exports, while Morocco alone accounts for approximately 41%. Approximately 9% of global production is traded.

Most phosphoric acid expansions in the next few years are expected to be in China, associated with new granulated fertilizer phosphate capacity. OCP in Morocco plans to increase phosphoric acid capacity in 2010, providing opportunity to utilize its excess granulation capacity. The construction schedule for Saudi Arabia’s M’aden project calls for process testing to commence by the end of 2010 but industry consultants expect full start-up to be delayed, awaiting completion of the railway from the mine at the north end of the country to the plant on the Arabian gulf.

![New Global Phosphoric Acid Capacity vs Demand](image)

World Phosphoric Acid Market Structure
India is the largest importer of phosphoric acid with approximately 60% of world imports. Phosphoric acid prices declined in 2009 due to decreased demand from the fertilizer and other industries.

US phosphate production is rebounding in 2010, driven by low producer inventory levels and improved demand prospects. US export sales could also increase slightly based on improved demand from Latin America and India. US Domestic sales are expected to increase following a sharp reduction in 2008/2009 fertilizer application and significant destocking of distributor inventories.
Global Phosphoric Acid Capacity vs Production

Phosphoric acid markets are expected to tighten slightly in the next couple of years as growth in demand is expected to exceed the addition of new capacity. Operating rates are expected to increase until 2011. Saudi Arabia's Ma'aden project is expected to begin commercial production that year, at which time the industry operating rate is expected to decline. Market conditions will be affected by the balance between the rate at which the new capacity is brought on stream and the rate at which non-integrated producers permanently cease production.

Global phosphoric acid capacity is forecast to be about 50 Million tonnes P205 in 2011. About 80% of this expansion will occur in China, Saudi Arabia and Morocco.
World Phosphate Rock Outlook Summary

Supply

Major future high analysis phosphate fertilizer supply growth is expected to take place in China, Morocco, and Saudi Arabia.

China's supply growth may displace a small amount of low analysis phosphate production, replace phosphate fertilizer imports, maintain a substantial level of exports, and satisfy increased domestic demand.
Trade
South America and Asia will lead the growth in phosphate fertilizer import demand.
Asian growth will be dominated by India.
Chinese imports of phosphate fertilizer are expected to continue to decline
The US will have almost no involvement in the increases.

Conclusion
Supply/Demand Balance
Solid market conditions are expected in 2010
Markets may remain slightly tight in the near and mid-term to 2013. During this period, as the MA'ADAN project comes on stream, adjustments will be required in trade flows.
Some phosphate fertilizer producers that are purchasing phosphate rock rather than having their own rock production are expected to exit the business.
LIST OF REFERENCES


5. Company Private Market Research Files, Potash Corporation, Al Mulhall Sr. Director Market Research, Saskatoon, Saskatchewan, Canada

Summary

Phosphorus is an environmental issue for water quality. Recycling this nutrient from poultry and livestock waste streams as a feed phosphate reduces the amount applied to crops as fertilizer phosphates and harvests the potential energy trapped in this resource. Economically these phosphates maybe more readily harvested than current rock phosphate sources, and regulatory hurdles to recycle them into poultry and livestock feeds may not be as difficult as many perceive. Projects are underway today because of the environmental potential, economic climate and mechanical capacity to harvest and recycle animal waste.

Introduction

Phosphorus (P) is an environmental issue in the Chesapeake Bay and beyond where soil levels in many fields are saturated beyond crop requirements. And while traditional fertilization of crops with livestock and poultry manure is good agronomic practice, only 5% of all U.S. cropland is fertilized with animal manures (USDA, 2009). This is largely because of the concentration of livestock and poultry outside the primary areas of crop production. This concentration of manure can pose environmental risks when stockpiled or applied in excessive amounts. The regional separation of manure and crop production raises the costs of using manure as a fertilizer since manure must be transported long distances to crops for application. Efforts to comply with environmental regulations on manure use and their associated costs are driving widespread interest in using manure as a feedstock for energy production. However, manure to energy projects are not currently in widespread use. According to the USDA, anaerobic digester systems including those being planned or under construction on U.S. dairies are associated with less than 3 percent of the cows and less than 1 percent of hogs. Combustion of manure is being applied at a single, centralized plant using litter from 6.6% of the U.S. turkey production while an idle California plant could utilize manure from about 3% of fed cattle.

The USDA report “Manure Use for Fertilizer and For Energy” considered these financial and societal impacts however; they did not consider recycling the minerals as feed supplements for poultry or livestock to replace dicalcium phosphate. This recycle option keeps transportation costs down by not exporting all the nutrients back to crop production regions of the country. Furthermore, it reduces the volume, weight and shipping costs of residual ash to approximately 10% of the original manure when export is warranted. Finally, feed phosphates are an expensive part of the diet and the report by Edwards, 2010 (Figure 1) documents the rising price trend and peak paid in 2009 ($/ ton). Lastly, this trend in feed phosphates is mirrored by the same trends in demand for rock phosphate and phosphoric acid (the precursor of feed and fertilizer phosphates) (Auman, 2010). Auman goes on to say that the quality of global phosphate reserves are declining, and therefore the cost of extraction and processing the ore is rising as the highest quality and most accessible reserves are depleted first.
Figure 1. 18% Phosphorous Cost/Ton

Recycling Results From the Past

Cattle Waste

Many studies with cattle and sheep have attempted to take advantage of the energy, protein and fiber in cattle waste by either ensiling with other forages or drying and feeding directly. Most of these studies indicate dry matter digestibility is low and they have limited protein and energy value (Lucas et al., 1975 and Harpster et al., 1978). Westing et al. (1985) fed cattle waste to steers (86.7% DM, 31% ash with 3.4, 2.0 and 1.1% Ca, K, Mg) to evaluate the mineral profile in tissues with special emphasis on As, Cu, Cd, Pb and Se. Results indicated the waste product did not increase tissue minerals to levels that would present a serious hazard to the health of humans consuming the tissues, nor were they especially useful as required macro-minerals because of their low concentrations.

Anaerobic digestion of livestock manure has been utilized to generate electricity, and other by-products with feeding value for both livestock and poultry. Universal Synergetics, Inc. (UNISYN) on the west coast received flushed manure from a 1200 cow dairy adjacent to their facility (McElvaney, 1990). The operation with a capacity of 185 wet tons per day would first mix then separate the grit, then the slurry would be pumped to one of ten 100,000 gal digester tanks. After 10 days digestion, the liquid was screened from the fiber and the bacterial solids harvested by centrifugation and drying using a spray dryer. Electricity produced from the biogas burn was used to power the facility and the carbon dioxide gas generated by the digester was used to grow Spirulina algae (a pigment, protein, and omega 3-fatty acid supplement).

Patterson and Loy (1992) reported on the feeding value of the UNISYN by-product for broiler chickens. Based on nutrient analysis it was estimated to contain 2199 kcal/kg metabolizable energy, 10.9% protein, 0.54% lysine, 0.32% methionine, 4.32% Ca, and 0.81% P. When supplemented to broiler chicks at both 1.5 and 3.0% of the diets in both a practical (corn/soy) and purified background to 3 wks of age, body weight was not statistically different between the control and anaerobic digester by-product
diets. However, body weight and F/G ratio were poorer in the purified background diets. Further studies with the UNISYN anaerobically digested dairy waste (ADDW) and the ash from this by-product (ADDWa) were conducted using broilers fed greater levels (3, 6, and 12%) to 21 days of age. This study indicated there was an upper limit for the ADDW with body weight and F/G negatively impacted by the 12% dietary level, whereas the highest body weight and F/G was realized for the 12% ADDWa ash by-product compared to the control and other treatment diets. Results from these trials indicated by-products such as those generated by anaerobic digestion of livestock waste can be incorporated back into the food chain, thereby reutilizing waste in a useful manner.

**Swine Waste**

In two balance studies Van Dyke et al. (1986) evaluated the energy and protein content of screened swine waste solids (SSWS) using gestating gilts. From Exp. 1 replacing 0, 25, 50 and 86% of the ME content of the basal diet the digestible energy of the diet decreased quadratically (85.2 to 58.3%) with increasing SSWS levels. The average DE and ME contents were calculated to be 1,998 and 1,854 kcal/kg DM respectively. In Exp. 2 where SSWS replaced 0, 25 or 50% of the CP in the basal diet, apparent digestibility of protein decreased linearly from 83.8 to 51.1% as the amount of SSWS in the diet increased. Results from these experiments indicated the SSWS can serve as a dietary energy source but has little value as a protein source for gestating gilts.

In another two trials, Cooke and Fontenot (1990) evaluated swine waste and broiler litter for their dietary minerals. With 15 wethers surgically equipped with duodenal and ileal cannulas the utilization of P, Ca, Mg, Cu, Fe and Cu was studied. Initially the animals were fed a low-P basal diet, and then allotted to ensiled diets (50% DM): the low-P basal, basal+swine waste, basal+broiler litter, basal+dical, and basal+soybean meal. Apparent P absorption was not different between wethers fed waste supplemented diets (37%) and those fed conventional diets (28%). Phosphorus absorption calculated by difference, tended to be higher for the waste supplements (59%) than the dical and soybean meal (37%). Less Ca was absorbed from the waste diets (0.62g/d) than from the conventional diets (1.28g/d), suggesting swine waste and broiler litter can be valuable sources of supplemental minerals including P and Mg.

Swine feces from finishing hogs both fresh and dried were evaluated as a nutrient supplement for gilts at 21.7 and 37.3% of the diet by Kornegay et al., (1977). Digestibility’s for dry matter, energy, crude protein and ash were 48.0, 46.7, 60.1 and 31.6% respectively. Amino acids digestibility ranged from 51.2 to 65.1% with the exception of ser, gly and cys which were even lower. The apparent absorption of most minerals was reduced as the level of dietary feces was increased, however, P was the only urinary component which increased significantly as the level of feces in the basal ration increased, indicating poor P retention.

**Poultry Waste**

Poultry waste has a long history of being fed to ruminants to take advantage of the non-protein nitrogen and other nutrients these animals and their bacteria can utilize. Broiler litter comprised of poultry excreta, bedding materials, wasted feed and feathers are generated in large quantities by growers in the broiler belt. Average litter nutrient composition includes 79% DM, 50% TDN, 23% CP, 24% CF and 2.9 and 1.6% Ca and P (Gadberry, 2006). The recommend use of litter for feeding was usually for beef brood cows or stocker cattle which were not destined for immediate slaughter. Litter was not recommended for fattening cattle, or dairy cows because of low energy and to ensure public safety from enteric pathogens and unapproved residues. According to McCaskey et al. (1990) 21 states had feed laws in 1989 that permitted the marketing of broiler litter as a feedstuff. In cattle diets broiler litter would require processing by either ensiling with other feed ingredients to encourage acid production, heat-treat with mechanical drying or pelleting, or the most economical was deep stacking that ensures heating to 130F
for a minimum of 5 days (Gadberry, 2006). Rations with from 50 to 80% broiler litter were very economical reducing feed costs nearly 50% compared with corn. However, today this practice has largely fallen in disfavor due to public perception and risks associated with pathogenic bacteria and residues (T. McCaskey, 2011, Auburn University, Auburn AL; D. Eversole, 2011, Virginia Tech., Blacksburg, VA; S. Gadberry, 2011, University of Arkansas, Fayetteville AR, personal communications).

In 1990, Muir et al. reported on broiler litter ash produced from burning pine shavings litter that had been used for one cycle of broilers. The furnace was a model E250 Wood Gun Furnace, Greencastle, PA located on the PSU University Park campus. In two experiments using 180 Peterson x Hubbard broiler chicks the biological value of P from the broiler litter ash was compared with dicalcium phosphate dibasic dehydrate as a standard. Three week body weight and tibia ash measures were used in non-linear bioassays to obtain the relative P biological value of the litter ash. The composition of the ash derived from the broiler litter included 15.45% Ca, 9.40% P and 7.12% K with a pH of 12.17. The P bioavailability of the ash determined from 3-wk body weight data was estimated at 79.1 and 82.9% of the standard in Exps. 1 and 2, respectively. Estimates based on tibia ash were 77.6% for Exp. 1 and 81.4% for Exp. 2. The authors indicated the experiments demonstrated that ash from the burning of broiler litter could be used as a dietary P ingredient for poultry.

Workers at Auburn University have shown experimentally that the mineral components in layer manure, when allowed to settle in lagoons, can be reclaimed as a dietary Ca/P source (30% Ca, 0.9% P) and re-fed to hens and broilers. Hen diets supplemented with 2.5%, 3.25% and 4.0% of the reclaimed minerals resulted in egg production, feed consumption and egg weight equal to hens fed traditional mineral sources (Rao et al., 1992). When fed to broilers in battery cages in a 2 x 4 factorial experiment comparing lagoon Ca (LG) with limestone (LS) Ca content, the diets were formulated to contain 0.6, 0.8, 1.0 and 1.2% Ca. At 3 and 6 weeks of age bone breaking strength, bone mineral content, bone density and body weight were measured to compare the relative Ca bioavailability of the two sources. Bioavailability of the LG at 3 weeks for the 0.6% Ca diet was between 82 and 97%, and at 0.8% between 95 and 100% depending on Ca parameter. When diets contained 1.0 and 1.2% Ca at either 3 or 6 weeks Ca bioavailability was 100% or more. The authors concluded that based on these and other experiments lagoon minerals can be recycled from commercial layer manure as dietary Ca and P sources for poultry (D.A. Roland, Sr., 2011, Auburn University, Auburn AL, personal communication).

**Recycling Results of Recent Efforts**

Recent efforts to utilize beef cattle manure as an energy source at an ethanol production facility indicated that 28 Mg of ash are generated for every 100 Mg of manure on a dry matter basis (Darapunein et al., 2009). When the ash was evaluated for soil characteristics, and plant growth it was determined that while some of the ash P is plant available, the P content was so low (0.99 to 2.17%) that extremely large amounts would have to be added to soil to supply meaningful amounts of P. These conclusions support the earlier findings of Mathers et al. (1972) who analyzed manure samples from 23 feedlots and determined the P concentration ranged from only 0.32 to 0.85%.

**FibroMinn**

Benson, Minnesota became home of the United States’ first commercial poultry litter fired electrical generation plant in October 2007. FibroMinn was pioneered and is operated by Fibrowatt LLC, with previous experience operating similar litter fired electrical plants in the UK (Fibrowatt, 2011). The 55 mega-watt power plant combuts more than 700,000 tons of litter and biomass annually with litter making up approximately 500,000 tons (G. Langmo, 2011, turkey producer, Litchfield MN, personal communication and Werblow, 2011). Fifty five mega watts is enough energy to provide approximately 40,000 homes with their electrical power needs. North American Fertilizer LLC processes and sells
110,000 tons of the ash fertilizer a year under the name, NAFmicro derived from the Fibrominn site (Morrison, 2011). The ash looks like fine, gray sand, but is screened, sprayed with water for better handling then stored in NAF warehouses. In addition to phosphorus and potassium the primary crop nutrients, it also contains other important nutrients including sulfur, zinc, copper, magnesium and boron. NAFmicro fertilizer is distributed by nine farm supply retailers in Minnesota, Iowa and South Dakota.

Auburn University

A series of experiments were conducted to evaluate the nutritional and economic value of poultry litter ash as a replacement for dicalcium phosphate in the diet of broiler chickens (Blake et al., 2006a). The project was initiated to evaluate the feasibility of an integrated ethanol, poultry production (IPEP) system in North Alabama that would use poultry litter as an alternative source of process energy for corn/ethanol production. The project was to explore an alternative use for broiler litter, generate dried distillers grains and solubles (DDGS) and a phosphate rich ash.

In Experiment 1, poultry litter ash was fed at graded levels (0, 25, 50, 75, and 100%) as a substitute for dicalcium phosphate on a weight:weight basis (Blake et al., 2006a). Broilers were fed the five experimental diets as starter (1-21d) and grower (22-41d) diets and housed in wire batteries with nine replicate pens fed each treatment and eight birds per pen. The ash contained 16.68% Ca, 10.08% P, 7.64% K and many trace minerals. While a growth was significantly reduced at the 100% substitution rate during the starting period, the effect disappeared by the conclusion of the study at 41 days. Results indicate that the complete substitution of dicalcium phosphate with poultry litter ash failed to compromise growth rate, feed consumption or feed conversion of broilers in battery cages. Although bone ash percentages varied among treatments there was no specific pattern that would indicate the integrity of the bones would be compromised by the addition of poultry litter ash to the diet.

Results from a similar Experiment 2, indicated pronounced differences in the dry matter digestibility of specific nutrients (Blake et al., 2006a). Digestibility of calcium and phosphorus increased significantly with greater levels of poultry litter ash. Such a relationship infers that the calcium and phosphorus component of the diet was more efficiently utilized as level of poultry litter ash increased. It is plausible that the calcium and phosphorus contained in the poultry litter ash may be more available to the bird compared to the dicalcium phosphate used in the control diet.

A third experiment was designed to evaluate the poultry litter ash under commercial conditions (Blake et al., 2006b). Broilers were fed graded levels of the poultry litter ash up to 100% replacement for dicalcium phosphate in the starter, grower and finisher diets fed to broilers in floor pens. All diets were formulated to meet the nutrient requirements of the broiler utilizing poultry litter ash as a replacement for dicalcium phosphate. Results indicate that there were no significant effects (P>.05) of poultry litter ash on body weight, gain or feed consumption when broilers were fed graded levels up to 100% replacement for dicalcium phosphate. However, body weights and body weight gains tended to trend downward (not significantly) for those birds that were fed the highest levels of poultry litter ash supplement in the starter and grower feeds. These differences diminished by day 41, indicating that compensatory growth may have been achieved to a slight degree. Processing performance of broilers at 41 days of age was also unaffected. Results indicate that the complete substitution of dicalcium phosphate with poultry litter ash failed to compromise growth and processing performance in market age broilers.

University of West Virginia

Researchers at the University of West Virginia are evaluating gasification of litter as a means for heating poultry houses (Shires et al., 2011). Feeding the resultant poultry litter ash (PLA) would recycle the nutrients and may represent a solution to manure application when nutrient management plans would
suggest otherwise. PLA may provide a cost effective essential nutrient for poultry by the partial or complete replacement of rock phosphate. The objectives of the study were to assess PLA as an alternative mineral source for broiler diets and to assess PLA’s effects on feed manufacture variables. The gasification byproduct contained 11.40% CP, 11.58% CF, 2.16% moisture and 74.63% ash. The ash contained 9.12% Ca, 5.52% P and 2.64% Na. Dietary treatments consisted of 7 different formulations with a positive control (PC) and negative control (NC) diet to create an adequate and deficient calcium and phosphorus baseline. Four diets were formulated with varying levels of phosphorus and calcium to create a standard curve. Diet 5 was formulated to be similar to the PC with PLA to be compared with the standard curve. Diet 6 was formulated as a commercial diet. Diet 7 was formulated as a commercial diet with PLA. In diets 5 and 7, PLA replaced rock phosphate additions. Diets 5, 6, and 7 were pelleted in random order each day, for 4 d to collect feed manufacture data.

Pellet mill relative electrical energy usage was similar between diets containing rock phosphate and diets with PLA, however mill rate (tonne/hr) was diminished (P=0.0319). In addition, PLA was shown to significantly improve pellet durability. The 7 diets were randomly assigned to 56 pens of 11 male and 11 female Cobb 500 broilers from 1 to 21 days to assess the efficacy of PLA via broiler performance. On day 21, 5 birds per pen were selected for tibia extraction to obtain bone mineralization data. Diets containing PLA (diets 5 and 7) demonstrated negative effects on feed intake, weight gain and feed conversion, and bone mineralization was reduced. These results indicate that PLA has available phosphorus and calcium potential, but arsenic content (99 ppm) was detrimental to bird performance.

Penn State University

Recent studies in Pennsylvania have looked into alternative energy sources for both contract grower propane and the electricity needs on hen complexes. At one field study site in central Pennsylvania with two 50 x 600ft houses (8250 bd/house) turkey litter (TL) ash (15.1 t) was generated during the 2010 brooding season from a 586kW (2 million BTU/hr) boiler made by Blue Flame Stoker, in Headingley, Manitoba (Patterson et al., 2010). Another experimental ash was generated from belted hen manure (HM) using a Coaltec Energy USA, Inc. gasification system. The ash products from these two study sites were evaluated as mineral supplements for growing commercial broilers (Burley et al., 2011). The TL and HM ash contained 11.6 and 3.8% P and 19.4 and 25.1% Ca, respectively. Three corn and soybean meal diets with 1% Celite (3100 kcal/kg, 22% CP) were formulated with 1% Ca and graded levels of available P (avP) (0.20, 0.25 and 0.35%). TL and HM ash diets were formulated to have 0.35 and 0.27% avP, respectively, with 1% Ca. In a 4 wk battery study, 280 Ross x Heritage males were fed these diets and provided water ad libitum, with 7 pens per diet and 8 chicks per pen. Weekly feed intake (FI), body weight (BW), and mobility was monitored. At 28 d, ileal digesta was collected from carcasses to determine P and Ca digestibility. Legs were removed for tibia bone ash determination.

Body weight was significantly reduced by the low P (0.20 and 0.25% avP), TL and HM ash diets compared with the control (0.35% avP) diet at wks 2 and 3. However, at the beginning, and end (wks 1 and 4) the TL ash diet did not differ significantly from the control. The control and TL ash diet weekly BW gain averaged 83, 178, 296 and 391 g/bird at wks 1, 2, 3, and 4, respectively, and FI averaged 104, 270, 464 and 646 g/bird in wks 1, 2, 3, and 4, respectively. Both gain and FI were reduced for the low P and HM ash diets. Bird mobility was excellent for the control and TL ash diets in wk 4 (100 and 98.2%, respectively), but was significantly reduced for the low P (0.20 and 0.25 avP) and HM ash diets (60.0, 76.0 and 60.7%, respectively). Overall, mortality was high for the low P (0.20 and 0.25 avP) and HM ash diets (85.7, 39.3, and 55.4%, respectively) and low for the control and TL ash diets (1.8 and 3.6%, respectively). Based on BW and FI, the P in HM ash was utilized at 83.7 and 68.1% efficiency, respectively, compared to monocalcium phosphate used in the 0.25% avP diet. For the TL ash, based on BW, gain, and FI, it was utilized at 88.3, 84.0, and 89.4% efficiency, respectively. Therefore despite the
lower P inclusion rate of the HM ash product (.27 vs. .35 avP), both HM and TL ash may have recycling potential as a dietary phosphate in commercial practice.

**Feed Authorization and Licensing with FDA and AAFCO**

Based on the data from these studies many recycled products from animal waste appear to have potential as feed ingredients. However, feed authorization and licensing is required with the Food and Drug Administration (FDA) and Association of American Feed Control Officials (AAFCO). Fortunately there already is an official feed definition section entitled 74. Recycled Animal Waste Products (Anonymous, 2010). They include:

74.1 Dried Poultry Waste,
74.2 Dried Poultry Waste –NPN Extracted,
74.3 Dried Poultry Litter,
74.4 Dried Ruminant Waste,
74.5 Dried Swine Waste,
74.6 Undried Processed Animal Waste Products, and
74.7 Processed Animal Waste Derivative.

Each definition is very specific and have clear indications of nutrient requirements e.g. 74.1 Dried Poultry Waste must be thermally dehydrated to a moisture content of not in excess of 15.0%, crude protein not less than 18.0%, and not more than 15.0% etc. etc. One definition that may fit the ashed poultry litter and manure products might be 74.7 Processed Animal Waste Derivative (Anonymous, 2010). This collective definition appears to be a catch all category with less well defined criteria. However, research results are still required to demonstrate efficacy and safety. This would be required for validating the Processed Animal Waste Derivative definition, or other routes of authorization including FDA’s Feed or Food Additive Petition (FDA, 2011). Section 571, Part 21 of the Code of Federal Regulations describes the kinds of data that must be submitted to FDA for a Feed Additive Petition. At a minimum each of the following subjects must be addressed: a. human food safety, b. target animal safety, c. environmental impact, d. utility, e. labeling, f. proposed regulation, g. assay methodology, and h. manufacturing process and controls.

Another option for approval that may represent a faster route to market is the GRAS exemption (Magnuson, 2011). Any substance added to an animal feed must be used in accordance with the FDA Feed Additive regulations unless, the substance is Generally Regarded as Safe (GRAS). Experts qualified by scientific training and experience must evaluate safety under conditions of its intended use. Animal feed GRAS must demonstrate utility as well as safety, while the former is not required by FDA for human foods. Finally the data the experts review must be in the public domain. Safety concerns on FDA’s list with ashed animal waste products include furans, dioxins, and heavy metals.

**Conclusions**

Energy production derived from animal or poultry waste combustion or anaerobic digestion is an incentive to consider in the overall management of this resource. Furthermore, the high density minerals concentrated by these processes have the potential to be valuable feed supplements. Critical nutrients would include appreciable phosphorus, calcium and trace minerals. Economically speaking, the most important mineral nutrient would be phosphorus based on current world supplies of quality, accessible rock phosphate. Because of the mechanized and efficient manure handling systems utilized in commercial poultry and animal production today, harvesting and extracting these nutrients is more plausible than ever. Despite regulatory hurdles to demonstrate utility and safety, recycled phosphates from animal waste may be in our future.
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NUTRIENT-DRIVEN APPROACHES TO OPTIMIZE INNATE IMMUNITY

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Summary

The innate immune system consists of constitutive cellular and molecular defenses responsible for managing non-self. The response of the innate immune system to changes in the amount or frequency of non-self has implications on how dietary nutrients are used for productivity. Given the diffuse nature of the immune system and inability to quantify nutrient requirements using traditional empirical approaches, nutritionists often attribute nutritional costs of immunity to increased maintenance costs. While this approach has helped to conceptually understand the impact of an immune response on nutrient utilization for growth, more clarity in this field will allow for further improvements in nutrient-driven approaches to influence immunity. By understanding altered nutrient metabolism during an immune response we can then begin to use hypothesis-driven approaches to understand how to better provide optimum nutrient profiles for desired immune responses and performance. This concept will be described using amino acids nutrition as a focal point in the proceedings, however the presentation will also address other nutrients as well.

Amino acid metabolism during an immune response

Microbial pathogens are typically used in experimental models used to examine altered nutrient metabolism during an immune response. Depending upon the species and route of infection (e.g. intramammary, sub-cutaneous, intravenous, intramuscular, oral gavage) this could include Escherichia coli, Salmonella, Campylobacter and Clostridium among others. Regardless of the route of infection, the innate immune system is present and provides the initial response to these pathogens. Depending upon the severity of microbial challenge, the innate immune system elicits a systemic response to help coordinate the immune response to the pathogen. The systemic response is manifest through behavioral changes in the animal in addition to decreased nutrient use for growth; however these changes are transient and may last hours to days. From an amino acid perspective, activation of an innate immune response results in skeletal muscle degradation and a negative nitrogen balance. Catabolism of skeletal muscle releases amino acids into the plasma and these substrates are available for use by tissues and cell types involved in host defense (Klasing et al., 1984). Consequently, activation of the innate immune system results in the repartitioning of amino acids from growth toward immunity (Humphrey et al., 2004). The shift in amino acid consumption from growth to immunity results in a shift in the amino acid composition of proteins being synthesized. These proteins differ in their amino acid composition and this change in amino acid profile may also be reflected in the diet. Reeds (Reeds et al., 1994) first hypothesized that the high rates of skeletal muscle catabolism during periods of infection are due to the high demand for specific amino acids whose proportions are particularly high in acute phase proteins. By comparing amino acid profiles of several acute phase proteins to skeletal muscle, aromatic amino acids appear to be in greatest demand due to their low proportion in skeletal muscle and high proportion in acute phase proteins (Reeds et al., 1994). Consequently, skeletal muscle is catabolized to supply amino acids for acute phase protein synthesis in an amount sufficient to supply aromatic amino acids, specifically phenylalanine, while the surplus levels of other amino acids liberated from skeletal muscle are excreted and contribute to the negative nitrogen balance of the animal (Reeds et al., 1994). Thus, catabolism of skeletal muscle is an important metabolic alteration during infection since this process provides amino acid substrate for the
liver to synthesize acute phase proteins. Rather than implementing nutritional support to prevent skeletal muscle degradation, efforts may be best served by providing the composition of amino acids that are ideal for the synthesis of protective factors, such as acute phase proteins. As an example, recent studies by Faure et al. (2007) have shown in rats that threonine utilization for synthesis of acute phase proteins and intestinal mucins is increased during infection.

**Amino acid needs for immunity**

Nutrition can regulate the type of immune response by a number of mechanisms (Klasing, 2007). Amino acids regulate immunity by serving as substrates for the development, maintenance and use of immune system. Consequently, the supply of amino acids at the appropriate amounts, times and ratios are important for immunity. The immune system consists of numerous tissue and cell types that are responsible for the production of a vast array of effector molecules involved in pathogen killing and in the regulation of the immune response (Table 1). The specific amino acid requirements for these cells, tissues, molecules and processes have been based upon empirical growth trials, yet it is not known if the amino acid requirements for growth are indeed optimum for immunity. Utilization of classical approaches for determining nutrient requirements may not be best suited for determination of amino acid needs for immunity. Based upon the diversity of immune cells, tissues, molecules and responses (Table 1), determining a dietary amino acid requirement that is optimum for all of these variables and their combinations is impractical since not all components of the immune system respond at all times in the same manner to all pathogens. Rather, more focused approaches that aim to determine optimum amino acid needs for specific cell types and specific responses to pathogens are needed.

**Table 1. Nutrient consuming components and processes of the immune system.**

<table>
<thead>
<tr>
<th>Lymphoid tissue</th>
<th>Leukocytes</th>
<th>Molecules</th>
<th>Processes</th>
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</thead>
<tbody>
<tr>
<td>Bursa</td>
<td>B cells: naïve, effector, memory</td>
<td>Mucus</td>
<td>Inflammation</td>
</tr>
<tr>
<td>Thymus</td>
<td>T cells: T helper (T&lt;sub&gt;H&lt;/sub&gt;), T cytotoxic (T&lt;sub&gt;C&lt;/sub&gt;), T&lt;sub&gt;H&lt;/sub&gt;/T&lt;sub&gt;C&lt;/sub&gt; naïve, T&lt;sub&gt;H&lt;/sub&gt;/T&lt;sub&gt;C&lt;/sub&gt; effector, T&lt;sub&gt;H&lt;/sub&gt;/T&lt;sub&gt;C&lt;/sub&gt; memory</td>
<td>Cytokines</td>
<td>Acute phase protein production</td>
</tr>
<tr>
<td>Lymph node</td>
<td>Natural killer cells</td>
<td>Antimicrobial peptides</td>
<td>Lymphocyte development</td>
</tr>
<tr>
<td>Spleen</td>
<td>Monocytes/ Macrophages</td>
<td>Antibody</td>
<td>Lymphocyte clonal proliferation</td>
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<tr>
<td>Mucosal associated lymphoid tissue</td>
<td>Dendritic cells</td>
<td>Reactive oxygen species</td>
<td>Synthesis of new leukocytes</td>
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<td>Hardarian gland</td>
<td>Neutrophils</td>
<td>Acute phase proteins</td>
<td>Phagocytosis</td>
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<tr>
<td>Blood</td>
<td>Eosinophils</td>
<td>Reactive nitrogen species</td>
<td>Chemotaxis</td>
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<td>Germinal Centers</td>
<td>Basophils</td>
<td>Antioxidants</td>
<td>Wound healing</td>
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<td></td>
<td>Thrombocytes</td>
<td>Complement</td>
<td>Mucosal barrier turnover</td>
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<td></td>
<td>M cells</td>
<td>Heat shock proteins</td>
<td>Allergy</td>
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<td>Autoimmunity</td>
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Supplying amino acids to the immune system: Push versus Pull

When considering diet modifications to help feed the immune system and optimize animal health, it is important to consider the concept of push versus pull in regards to amino acid partitioning to the immune system. A common approach aimed to increase activity of the immune system is to include more of a particular amino acid that is suspected to be in limited supply in the diet or during a particular physiological state. This “push” approach to feeding the immune system assumes that more is better and is fundamentally based upon the idea of tissue competition for amino acids, such as skeletal muscle versus immune tissue. The “push” approach to feeding the immune system assumes that providing more amino acids in the diet will increase their utilization by the immune system. However, simply increasing the supply of a particular amino acid does not necessarily directly translate to increased utilization. For example, increased dietary arginine levels will result in increased growth, yet this does not translate into increased growth of lymphoid tissue (Kidd et al., 2001). These events are also regulated by signaling systems that act independent of the amino acid supply, per se. Consequently, the “push” approach to feeding the immune system is based upon amino acid supply alone and does not consider the controls that couple amino acid supply with demand. In regards to feeding the immune system, it is important to understand how these cell types and tissues coordinate amino acid utilization.

The “pull” approach to feeding the immune system involves modulating the coordinated adaptations that direct nutrient partitioning toward immune function. These adaptations are complex and regulated by signals, often times cytokines, to help coordinate nutrient supply with nutrient demand across all tissues and within the animal (Humphrey et al., 2004). The coordinated adaptations of nutrient utilization throughout the body by signals from the immune system help to ensure that nutrient supply meets the nutritional demands associated with immune function.

Coordination of nutrient partitioning to the immune system is achieved in large part through the actions of cytokines. Leukocytes display many cytokine receptors, but other cell types have a much more limited expression pattern. This difference allows cytokines to act selectively upon cells of the immune system to increase their nutrient acquisition. Furthermore, the selective action of cytokines can affect nutrient acquisition by specific leukocyte populations. For example, activated T lymphocytes produce interleukin-2 (IL-2) that acts in an autocrine and paracrine manner to increase T lymphocyte proliferation. IL-2 also increases T lymphocyte glucose transporter-1 (GLUT-1) protein to provide energy for proliferation. During lymphocyte development, IL-3 also increases lymphocyte nutrient transporters for glucose, amino acids, lipids and metals (Edinger et al., 2002) and IL-7 maintains the metabolic activity of naïve T lymphocytes (Rathmell et al., 2001).

When formulating diets that are optimum for the immune system, it is important to consider the type of nutrients being offered to the immune system, i.e. supply, and to what specific aspect of the immune system that they are intended for, i.e. demand. Considering supply without demand can result in either no impact on immune function, or even decreased overall animal health, as evidenced by the severity of E. coli infection in iron supplemented newborn pigs (Kadis et al., 1984). Rather, nutritional approaches to enhance immune function should focus on supplying the nutrients at the appropriate times and in the appropriate amounts that complement the “pull” associated with increased nutrient partitioning for immune function.

References


DIETARY EGG ANTIBODY DIRECTED TO HOST GASTROINTESTINAL TARGETS TO OVERCOME INFLAMMATION-INDUCED LOSSES IN GROWTH AND FEED EFFICIENCY

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Summary

Animal scientists have clearly demonstrated that there are significant costs associated with the activation of the immune/inflammatory response. Preventing inflammatory damage associated with inflammation should be a standard management practice of animal producers. However, even with the best management program against inflammation (i.e., vaccination, sanitation, antimicrobial use, and biosecurity), the immune arsenal in modern animal facilities is unlikely to remain dormant, hence its destructive nature must be harnessed for the welfare of the animal. We have found that targeting host inflammatory products using egg antibody, particularly in the gastrointestinal tract of the animal is an effective way to generate novel products to both study inflammation and animal growth and feed efficiency, and to produce safe products for commercial animal agricultural uses.

Introduction

The movement of animals into confined hygienic animal facilities, where diseases can be prevented and controlled through the use of sanitation, bio-security, vaccination, and antimicrobials, has been a boon to modern animal agriculture. We now know that the marked improvements in animal performance using advanced disease control were primarily realized by minimizing the activation of the animal’s immune defenses (Cook, 1999). These immune defenses are a top priority to an animal, and animals will sacrifice growth and reproduction (by the diversion of nutrients) to defend against an invading pathogen or even prepare for the defense in the case of vaccination (Humphrey and Klasing, 2004). While we have effectively managed the microbial environment that animals encounter to achieve efficient animal performance and health, how has the immune system responded to these changes? Have we ignored a need to manage the animal’s defense machinery? One has to remember that the immune defenses of the animal have not evolved as fast as our modern disease management husbandry practices. Hence the animal still retains the necessary defense system to fight diseases that plagued the once wild flock and herds and in doing so, retains the ability to significantly affect animal performance. Is there a cost or unintended consequence associated with the “bench sitting” of the immune response in our bio-secure facilities? If recent studies of diseases in humans serve as an example, there is an apparent need for management of the immune and inflammatory response of our modernly managed animals.

Medical scientists have been observing an increased incidence of immune related disorders in human populations of developed countries. Immune dependent disorders of the gastrointestinal tract (irritable bowel syndrome), airway (asthma and allergies), skin (eczema) and other autoimmune diseases are rare in developing countries, but seem to increase as countries become more urbanized and industrialized. Surprisingly these diseases emerge as the prevalence of infections decline in the population. While improved nutrition is associated with improved affluence, diet per se is not the cause of these “disorders of civilization” (Gwee, 2005). Scientists have begun suspecting that improved disease
control through vaccination and the use of improved sanitation has reduced environmental immune stimulation to a point that the human’s immune defense system, instead of becoming dormant, is becoming increasingly activated, dysfunctional or imbalanced. The theory behind the emergence of immune related disorders in industrialized societies was first published by Strachan in 1989 and now is known as the “hygiene hypothesis” (Carpenter, 1999; Guarner, 2006; Liu, 2006; Schaub, 2006; Renz, 2006). Since Strachan’s first observation on a relationship between hygiene and airway disease, many studies have been published showing that as the microbial hygiene of the human environment becomes more “sanitized,” the incidence and severity of immune-induced diseases increased. For example, children raised on farms in industrialized countries have less asthma than those not exposed to a farm like microbial environment (Riedler et al., 2001). Some have hypothesized that the genes that are involved in causing human disorders are the same genes that are beneficial to the fighting of microorganisms. The hygiene hypothesis continues to receive broad support among the scientific community even though the underlying mechanism by which exposure to microorganisms reduces inflammatory disease is not well understood. The concepts behind the hygiene hypothesis have not been a subject of discussion or directed research within the animal science community. Could improved hygiene in modern agricultural facilities be a limiting factor in animal growth and development? Are there needs for immune/inflammatory regulation in our modern day animal facilities? Evidence suggested that managing the inflammatory processes of the modern-day animal can result in a significant improvement in performance.

Proposals for the treatment of human immune related disorder involve exposure to mild pathogenic agents (Elston, 2006 discusses the use of Trichuris suis in the treatment of Crohn’s disease). Such probiotic therapy or a return to poorer sanitation and elimination of disease prevention management strategies would be counter-productive to our continued progress in improving animal performance standards. If animal performance is being limited by inappropriately directed inflammation, then the decrease in animal performance caused by such an imbalanced immune response must be addressed using new strategies.

All animal producers are well aware of performance losses associated with the activation of immune/inflammatory responses (Cook, 2010). Loss of productivity is not only observed in overt diseases, but in simple procedures of immune activation, such as vaccination (Cook, 1999). Active immune processes that have no clear use in the prevention or defense against an infectious disease agent will result in a loss of feed efficiency and animal growth. Strategies to overcome lost animal performance during unwanted inflammatory responses should offer new ways to manage the animal’s response to modern husbandry techniques.

Estimating the cost of unwarranted immune activation

Table one provides an estimate of the total decrease in performance of broilers and swine due to immune activation. The transition from a sterile environment to one where commensal organisms begin to colonize the host is well established to have considerable negative effects on animal performance. One could argue that these costs are unavoidable, however, can these cost be minimized? Sanitation has been described as a means of improving the health of flocks and herds by minimizing infectious disease. Also important is the role in sanitation at improving animal performance even in the absence of disease. As reviewed by Cook (2010), there are clear examples where the level of sanitation offered during the growing of swine and poultry had considerable effects on the efficiency of production. Performance gains realized when producers switched from multi-age growing facilities to all-in all-out management practices illustrates the importance of minimizing immune activation as a means of improving animal growth. Vaccination, as a potent immune activator, also suppresses growth and feed efficiency. While vaccination is essential insurance against costly infectious disease outbreaks, apart from the cost of the vaccine and its delivery few consider the cost of vaccination on overall performance of the growing animal. A study was conducted where young ducklings were housed in a “sanitized” research facility where exposure to
disease was limited. Ducks were vaccinated twice with a killed bacterin the first 3 weeks of life and final market carcass weight was assessed. Market weight and breast meat percent yield was reduced 13 and 9%, respectively (Table 2). Chamberlee et al., (1992) also reported significant losses in growth rate, feed efficiency and increased mortality in broiler vaccinated compared to unvaccinated counterparts, when infectious disease was absent.

Table 1. Conservative estimated losses in growth and feed efficiency as a result of immune activation.

<table>
<thead>
<tr>
<th>Immune Activator</th>
<th>change in feed efficiency</th>
<th>corn loss (bus)</th>
<th>$ US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth¹</td>
<td>+12%</td>
<td>203 m</td>
<td>609m</td>
</tr>
<tr>
<td>Sanitation²</td>
<td>+10%</td>
<td>169 m</td>
<td>507m</td>
</tr>
<tr>
<td>Vaccination³</td>
<td>+7%</td>
<td>118 m</td>
<td>354m</td>
</tr>
<tr>
<td>Total</td>
<td>+29%</td>
<td>490</td>
<td>1,470m</td>
</tr>
</tbody>
</table>

Drew et al., 2003; Muramatsu et al., 1988; Loynachan et al., 2005; Roura et al., 1992, Renaudeau, 2009; Chamberlee et al., 1992, M.E. Cook unpublished.

Table 2. Effects of vaccinating ducklings with a killed *Riemerella anatipestifer* bacterin twice during the first two week of life on final carcass weight and breast meat yield.

Ducklings were housed in an isolation facility to prevent exposure to infectious disease. Half the ducks were injected with the killed bacterin subcutaneously at day 11 and 20 and the other half received no vaccination. Ducks were processed at approximately 2.5 Kg for determination of final carcass weight and deboned breast meat yield. (Data collected courtesy of Maple Leaf Farms, WI)

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Carcass/neck (Kg)</th>
<th>diff (%)</th>
<th>Breast meat (Kg)</th>
<th>diff (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>1.99</td>
<td>.255</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.80</td>
<td>9</td>
<td>.221</td>
<td>14</td>
</tr>
</tbody>
</table>

Key players involved in suppressed growth and feed efficiency

Before attempting to find a solution to overcome the growth suppressive action of the immune/inflammatory response, one must determine what the key processes responsible for reduced growth are. Figure 1 divides contributors into 4 categories: The immune stimulant (e.g. bacteria and vaccination), the immune system, the mechanism for redistributing or removing nutrients from muscle, and over reactive inflammatory processes that exceed the need for adequate defense. Improved animal growth that has been observed in animals housed in germ free environments as compared to conventional environments provides ample evidence that the presence of microbes slows animal growth and feed efficiency. Regulation of the microflora of the animal has been the principal method that animal producers have improved animal growth. The best example of improved growth of animals by controlling the microflora has involved the use of broad-spectrum antibiotics. Recent attempts at finding alternatives for antibiotic use have focused on the regulation of gut microbes through the use of feed additives, such as probiotics. Probiotics are mainly used to influence the microbial flora away from pathogenic bacteria to
more innocuous bacteria. Probiotics have also been shown to increase growth rates and feed efficiencies of chickens in sanitary housing (Jin et al., 1998). Regulation of the microbial species that activate the immune response will continue to be an important strategy in improving animal growth; however, as has been shown with the monoaasociation of germ free animals with “healthy” commensal organism such as Lactobacillus, even healthy bacteria can depress growth (Loynachan et al., 2005). Strategies that target a specific species/stain of microorganism as a potent immune activator (e.g., an infectious pathogen) may be useful when the agent is present, but is unlikely to have broad appeal as a growth promoter since pathogens in modern agricultural facilities are rare and well controlled.

Figure 1. Elements that play a role in the regulation of animal growth and development.

Environmental immune stimulants are initiators of the immune/inflammatory response. The immune system is a host defense mechanism that is essential in the prevention of the progression of an infection. The mechanisms for the movement of nutrient reserves found in the host are elements that induce skeletal muscle degradation during the immune response. The over reactive inflammatory elements are feed-forward products of the immune system that can ultimately bring harm to the host. IC- immune cells, LB lactobacillus.

Suppressing the immune response will improve animal growth and feed efficiency. Studies have shown that when animals are genetically selected for reduced immunological function, they grow at greater rates than those selected for increased immune function (Seigel et al., 1982). Cook (2004) discusses the apparent imbalance between growth and immunity. However, the immune capacity of an animal, even in rigorously controlled environments such as found in modern animal agriculture systems is still needed to assure defense against potential pathogens, and to assure that adaptive immune defense is prepared through the use of vaccination. Immune suppression for the purpose of stimulating animal growth and feed efficiency would not appear to be a sustainable approach.

A third possible mechanism to prevent immune-induced regulation of body weight gain and feed efficiency is to minimize the withdrawal of stored nutrients, particularly skeletal muscle during an immune/inflammatory response. This approach should be considered useful if one considers that the immune system draws more nutrients from tissues than is actually needed for generating an effective defense against a pathogen. Humphrey et al. (2004) showed the cost of lipopolysaccaride (LPS) challenged chicks on acute phase proteins, leucopoiesis, immunoglobulin (Ig) synthesis and its effects on body weight gain. LPS challenged chicks doubled the amount of protein production, a slight increase in Ig production, 700 time increase in acute phase protein production, and this caused a decrease in body weight of 15%. To accomplish this goal, a systemic approach is needed to prevent wasting responses to cytokines such as TNFα and IL-1β. Cook (1999) discussed the use of derivatives of linoleic acid, known as conjugated linoleic acid (CLA) as protectants against immune-induced muscle wasting. Reduced endotoxin-induced wasting was repeatedly demonstrated (Cook et al., 1993; Miller et al., 1994). Currently CLA has had only limited entry into the marketplace at this time. Other approaches to reduce
muscle wasting during immune challenge, particularly in farmed animals, have not been developed sufficiently and may eventually become a strategy for improving animal growth.

The fourth possible means of preventing immune-induced regulation of growth and feed efficiency is to regulate immune/inflammatory responses that actually may be harmful to the overall health of the animal. As suggested in the introduction, the inflammatory response can clearly be over reactive and cause damage to the host. Classic diseases of over-reactivity include autoimmune disorders, and responses to pathogens that lead to organ failure, such as systemic inflammatory response syndrome in the septic animal. Like any physiological function, the immune and inflammatory response needs to have feedback control systems that bring the response back to a homeostatic state. For reasons fully not understood, there are situations where there is apparent failure in the down-regulation of immune responses. Unchecked immunity can have devastating effects on host health. In the case of the farm animal, where diseases are generally controlled through sanitation, vaccination, the occasional use of antibiotics, or the frank elimination of diseased animals, regulation of an overactive immune/inflammatory response may be very productive, since the immune system is likely not responding to a true threat to animal health, but a perceived threat. In the following paragraphs, we will explore the regulation of elements of the immune response that are not critical to immune defense, but may represent an unwarranted response. Ultimately, for any strategy to be useful in the regulation of an over reactive response, it must have no adverse effects on disease resistance and adaptive immunity. We believe that by regulation of inflammatory reactions that can damage the host, disease resistance and adaptive immunity can actually be improved since, through this control, host mechanisms to down-regulate the immune response will not be activated.

Targeting the over reactive immune/inflammatory response

Control of over-reactive inflammatory processes is not new in the medical care of animals and humans. Examples of anti-inflammatory agents include both the steroidal (i.e., glucocorticoids) and the nonsteroidal cyclooxygenase inhibitors (i.e., aspirin and ibuprofen). Studies have shown that the use of both types of anti-inflammatory stimulate growth in pigs (Gaines et al., 2002; Xu et al., 1990). The problem with using systemic inhibitors of inflammation is that they regulate physiological processes that are essential for other important function and result in side effects, and their use in food animal could be viewed as problematic due to residues. Cook (2010) outlined the ideal nature of an anti-inflammatory method to improve growth: will not leave residues, will not effect systemic physiological processes essential to normal health and defense, and the anti-inflammatory agent would improve animal growth even in the absence of overt disease. To develop such products, a focus on processes that reside outside of systemic circulation would be critical. A site of considerable inflammatory activity, even in the healthy animal would involve the gastrointestinal tract (GIT) of the animal. The growth depression due to the transition from a germ free to conventional state does not specifically involve the invasion of pathogens into systemic circulation, hence there may be capacity to target growth suppressing effects in the GIT.

There is increasing evidence that the host’s inflammatory process extends into the lumen of the GIT. A number of inflammatory related mediators, including cytokines, enzymes, and gut peptides are released to luminal secretions. In addition, the host has surface receptors in cells of the mucosa that monitor and respond to luminal environmental stimuli. Splichal et al., (2007) showed that during exposure to a relatively low virulent strain of Salmonella enterica serovar Typhimurium, secretion of IL-18 into the lumen of the pig was observed. Luminal cytokine secretion appeared even in the absence detectable levels of circulating IL-18. During a systemic inflammatory response, the host releases to the lumen of the GIT secretory phospholipase A2 (sPLA2) (Zayat et al., 2008). More importantly, luminaly released sPLA2 appeared to have an adverse effect on animal health and may actually serve as a feed-forward loop for driving an inflammatory process (Rozenfield et al., 2001; Zayat et al., 2008). Daun and McCarthy (1993) showed that systemic administration of IL-1 (an inflammatory cytokine) induces the
release of cholecystokinin (CCK), which in turn reduced GIT motility (e.g., gut emptying). Gut peptides, such as CCK are released into the lumen of the GIT (Furuse, 1999) and the biological relevance of this release is not clearly defined. The presence of inflammatory mediators in the lumen of the GIT suggest that, if they play a role in promulgating the inflammatory process and ultimately suppress animal growth and feed efficiency, these products could be accessed without applying systemic regulators of inflammation. One other class of targets on the apical side of cells that interface with the GIT lumen is receptors. Toll like receptors are well known to be found on epithelial cells. Toll-like receptors (TLRs) are found to be expressed on epithelial cells in the intestinal lumen. TLRs are known to be important molecules for sensing bacteria. TLR2 has been found to sense gram-positive bacteria and is known to be expressed in low levels in non-inflamed intestines, however it is upregulated in inflamed intestines. TLR4 has been found to sense gram-negative bacteria, while minimally expressed in non-inflamed intestines, and is found to be upregulated in inflamed intestines (Cario, 2010). These TLRs sample luminal microbial antigens. Since the Toll receptors are critical initiators of secondary signaling processes during inflammatory events, these receptors may also represent potential targets to regulate inflammatory processes in the GIT that may suppress growth and feed efficiency.

Figure 2 represents a model for the targeting of over reactive and unwanted inflammatory events that may serve as an unnecessary response to the animal’s environment. The ideal agent that would target the mediators of the inflammatory process would need to inactivate the mediator in the lumen of the GIT. After neutralizing the inflammatory mediator, the targeting agent would be either excreted with undigested matter or would be destroyed by digestive enzymes. Ideally, the platform for making inhibitors of inflammatory molecules would be malleable, such that a single platform could be used to manufacture many different inhibitors. Designing specific pharmaceuticals that inhibit a specific activity and results in no residues is an incomprehensible undertaking. Fortunately, nature gave us the perfect platform, the antibody.

**Figure 2. Inflammatory response in the intestinal lumen.**

During an inflammatory stimulus in the lumen of the gastrointestinal tract, the host detects the environmental threat through receptors and releases host inflammatory mediators into the lumen of the GIT. Some of these inflammatory products may have direct effects on microbial species, but others may induce feed forward signals that enhance GIT inflammation. If the GIT inflammation is not regionally contained, the inflammatory process could become systemic resulting in systemic release of catabolic cytokines into circulation. IC-immune cells, C-CCK, P-sPLA2, B-Bacteria.

**Antibody platform and its use in improving animal growth and feed efficiency**

Antibodies are malleable tools, simple to design to neutralize targets, and have recently gained wide use as pharmaceuticals. Humanized antibodies are currently in broad use for the treatment of inflammatory diseases such as Crohn’s and rheumatoid arthritis (Zidi et al., 2010; Bickston et al., 2010). The use of antibodies as oral supplements have not been recognized in human medicine, however, there
have been a number of uses of antibodies in agriculture (Cook and Trott, 2010). A limitation to using antibody to treat agricultural animals is the cost of producing antibody. Cook and Trott (2010) recently reviewed the potential of using egg antibody as a source of cost effective antibodies for animal agriculture. Most importantly, a single laying hen can produce gram quantities of antibody specific for essentially any target of interest. Antibodies retain binding or neutralizing activity in the GIT, and can be used effectively in the diet to target a broad range of inflammatory products produced by the host. Antibodies are not absorbed by the host, hence there are no residual activities found in the eatable portion of the farm animal. Lastly, the egg laying industry is currently structured in a manner that allows for the mass production of antibodies at a relative low cost. Hence, oral egg antibodies were selected as an ideal method to develop and study new methods to minimize the effects of inflammatory products released into the lumen of the GIT.

Using hypothesis driven experiments, based on peer-reviewed publications, we sought to make antibody to some key targets involved in inflammatory responses. In some cases the literature was not clear on the presence of these targets in the lumen of the GIT. Products selected were gut peptides, cytokines, pathogen pattern recognition receptors and inflammatory enzymes. In Cook (2004), we reported on the usefulness of two gut peptide antibodies that improved broiler growth, antibody to CCK and Neuropeptide Y (NPY). In a study involving anti-CCK antibody, feed efficiency was improved approximately 7% when healthy broilers were fed egg yolk powder containing the antibody compared to control egg yolk powder. Marked improvements in both feed efficiency and weight gain were also observed in broiler fed antibody to NPY, however, antibodies to other peptides (ie. anti-bombesin and anti-motilin) did not improve broiler performance. Several surprises were observed when feeding antibody to CCK. First, the tyrosine on CCK-8 had to be in its natural sulfated form during vaccination in order to generate a useful antibody with regards to feed efficiency. Also, the animal’s performance was very sensitive to dose of the antibody. When the egg powder containing the antibody was fed at a high dose, improved feed efficiency was lost. Beginning in the late 1990s we found that antibody to sPLA2 was also an effective growth stimulant, and the results of this antibody were consistent (also see Cook, 2004, 2010). In the synthesis of this antibody, whole enzyme preparations were made for vaccine production. The antibody was found to have substantially better effects when used in species other than poultry (see below for further advances). Antibodies to toll-like receptor-4 were also found to have promising but variable effects on broiler growth and feed efficiency, but to date these antibodies have not received as much research as some of the others. Antibodies to dozens of other antigens have also been made and tested in broiler chicks, and the ones listed above are the few that seem to have positive effects on animal growth and feed efficiency.

Future developments

Advancing the use of host targeted egg antibodies for growth promotion has some hurdles. First, egg antibodies have limited stability when subjected to feed heat processing. Cold pelleting, often associated with the manufacture of swine starter diets, appears to not adversely affect antibody activity. However, in animal feed manufacture that has extended steam preconditioning or involves the use of extrusion technology, antibody egg powder added to the diet often loses most of its bioactivity and has to be added post-pellet formation. We have found some methods for improving the heat stability of egg antibody, however, new advances in this area, particularly where components of the egg itself can be used as a stabilizing ingredient would greatly improve the usefulness of egg antibody in feeds processed using high temperatures.

In studies aimed at determining the quantity of antigen specific antibody in each egg, it has been found that as little as 10% of the 150mg of IgY found in each egg is specific for the target selected (Cook and Trott, 2010). Eggs are expensive for use in animal feeds. Since eggs are the carrier for the antibody, the price of the product to achieve a level of benefit remains high. Attempts have been made to find new
approaches to increase the level of specific egg antibody in each egg. These approaches have examined different strains of laying hens at different periods of the productive cycle (Trott et al., 2008a) and the use of different adjuvants in the vaccine used to stimulate egg antibody production (Trott et al., 2008). Methods that can increase the level of egg antibody at least two-fold would result in a decreased use level by 50%. Additional discoveries in improving the amount of antigen-specific antibody in the egg would have commercial benefit for the producer and end user and would likely expand the use of products containing egg antibody.

A third area that we have been recently exploring involves improving the specificity of the antibody to avian targets. When entire proteins are injected, the laying hen will not make antibody to self epitopes. In order to make antibody that has great effectiveness in the chicken, we have to design the vaccine in a manner such that the antibody made will react more effectively with the chicken target. The current approach involves the identification of a peptide sequence in the protein of interest. The peptide is made, and a vaccine is engineered such that when injected into the hen, the antibody produced will react with the chicken target (Figure 3).

**Figure 3. Peptide selection for antibody production.**

Using current protein databases containing amino acid sequence information, regions of a protein that is linked to the potential bioactive portion of the molecule are selected. Peptides are synthesized, attached to a carrier and injected in the hens. Effectiveness of the antibodies generated is evaluated using either cell culture models of chick bioassays. This technique “tricks” the hen into making antibody to regions of the protein that would not be normally produced using a whole protein.

**Additional reading:**


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FEEDING MANAGEMENT TO DECREASE METHANE EMISSIONS FROM RUMINANTS

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Summary

Ruminant livestock contribute significantly to methane emissions to the atmosphere. Whereas, methane is 20 to 50 times stronger per mole as a greenhouse gas than carbon dioxide, methane emissions are a significant environmental concern. Enteric methane emissions are largely a function of organic matter fermentability in the rumen and to a lesser extent in the large intestine. However, several factors have been observed to decrease methane emissions per unit energy fermentation in the rumen. Firstly, higher energy diets lead to decreased methane emissions per unit energy digested in the rumen. Secondly, addition of fats to the diet appears to inhibit protozoa and methanogens to decrease methane emissions. Thirdly, use of ionophores to inhibit gram-positive acetic acid-producing bacteria shifts fermentation toward propionic acid and decreases methane production. Some have proposed that the addition of tannins or saponins may further decrease methane emissions. The use of vaccines or probiotics has also been proposed to decrease methane emissions. Many feed additives or ingredients only temporarily decrease methane emissions but animals adapt and methane emissions return close to original amounts. We propose a model to explain and predict methane emissions based on stoichiometry, kinetics, and thermodynamics. This model will improve understanding of the ways in which various dietary manipulations affect emissions. It highlights the possibility for additional feed additives that could decrease methane emissions.
BIOLOGY AND TROUBLESHOOTING OF LOW MILK FAT ON DAIRY FARMS

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Summary

The evolution of milk pricing systems in the US has intensified interest in the factors that affect milk fat content and yield on commercial dairy farms. Troubleshooting milk fat issues on dairy farms remains one of the more challenging tasks within overall nutritional management of dairy cows. Research evidence largely discounts commonly held theories of milk fat depression (related to insufficient substrate supply or insulin as a regulator of substrate availability for milk fat synthesis). A substantial body of research conducted during the past few years has resulted in the development of the biohydrogenation theory of milk fat depression, which suggests that specific intermediates of fatty acid metabolism in the rumen (e.g., trans-10, cis-12 conjugated linoleic acid and related intermediates) are potent inhibitors of milk fat synthesis. Small quantities of these fatty acid intermediates flowing from the rumen to the lower tract are sufficient to induce substantial decreases in milk fat content and yield. Available data support that this provides a unifying concept for diet-induced milk fat depression that in turn can be used to focus troubleshooting efforts on commercial dairy farms. Specific characteristics of ration formulation (e.g., fatty acid profile, carbohydrate profile, use of ionophores) likely interact with factors related to producing an altered ruminal environment (e.g., rumen pH, rate of passage, aspects of feeding management, overcrowding of facilities) to result in economically significant decreases in milk fat content and yield. Ongoing university- and industry-based research efforts should improve specific guidelines for preventing or troubleshooting challenges with milk fat on commercial dairy farms.

Introduction

Although the topic of milk fat depression (MFD) is not a new topic in the dairy industry, both industry and research interest in MFD in North America has intensified dramatically during recent years. Most pricing systems in the U.S. are now based on yields of milk components, with greater value placed upon yields of milk fat and milk protein. Recent data summaries indicate that approximately 38% of herds shipping milk into the Mideast Federal Order 33 (primarily Indiana, Michigan, Ohio, and Pennsylvania) experienced a short-term (one- to three-month period in any year) decrease (more than 1 SD decrease below the mean; milk fat test < 3.46%) in milk fat test (Bailey et al., 2005).

During the past decade, substantial evolution has occurred in our understanding of the causes of MFD. As will be discussed below, we do not fully understand all of the ruminal conditions that can result in predisposition for MFD; however, this new understanding of the mechanisms for MFD has facilitated our ability to troubleshoot milk fat problems on commercial dairy farms. These mechanisms (and the reasons why previously held theories for MFD likely are not applicable) will be reviewed briefly in this paper; however, the reader is referred to other recent reviews that describe these in more detail (Bauman and Griinari, 2001, 2003; Perfield and Bauman, 2005; Bauman and Lock, 2006). The subsequent emphasis of this paper will be to discuss dietary and management factors that affect the predisposition of cows and herds to MFD. Our understanding of many of these remains conceptual; we expect that after
research conducted during the next few years is summarized our recommendations will be more quantitative.

Theories of milk fat depression

In general, theories of the cause of MFD can be divided into two broad categories – those suggesting that substrate supply for milk fat synthesis is limiting in situations in which MFD occurs and those suggesting that MFD is caused by direct inhibition of milk fat synthesis in the mammary gland (Bauman and Griinari, 2001). Theories relating to substrate limitation of milk fat synthesis that have been discussed include acetate insufficiency, beta-hydroxybutyrate (BHBA) insufficiency, and the glucogenic-insulin theory of MFD. Approximately 50% of milk fatty acids are synthesized de novo from acetate and BHBA (formed from butyrate produced in the rumen) in the mammary gland -- these typically are the short- and medium-chain fatty acids, and approximately half of the 16-carbon fatty acids (Bauman and Griinari, 2001). Although the substrate supply theories are attractive (e.g., acetate and butyrate are required for fatty acid synthesis by the mammary gland; therefore a deficiency of ruminal production of these VFA in scenarios of low ruminal fiber digestion must cause MFD) and are still referred to in the dairy industry, it is unlikely that they explain MFD. This argument is summarized in Table 1. Concurrent with the substantial decrease in milk fat yield when a high-grain, low forage diet was fed was a substantial decrease in the molar percentage of acetate and a small decrease in the molar percentage of butyrate in ruminal fluid. The molar percentage of propionate was increased sharply, resulting in a dramatic decrease in the acetate to propionate ratio in ruminal fluid. These types of approaches continue to be used commonly by researchers and others to imply that a change in molar percentage of a VFA in ruminal fluid must reflect a change in production rate. However, if we refer to data for ruminal production (measured using isotopic approaches) of VFA from cows fed these two types of diets at the bottom of Table 1, it is evident that the changes in molar proportions of VFA in the top part of the table were driven only by substantially increased production of propionate and that production rates of acetate and butyrate were not affected by diet.

Table 1. Acetate and butyrate shortage theories and milk fat depression

<table>
<thead>
<tr>
<th>Item</th>
<th>Normal diet</th>
<th>High grain, low forage diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield</td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td>Milk fat, g/d</td>
<td>683</td>
<td>363</td>
</tr>
<tr>
<td>Ruminal VFA, molar percentage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>67</td>
<td>46</td>
</tr>
<tr>
<td>Propionate</td>
<td>21</td>
<td>46</td>
</tr>
<tr>
<td>Butyrate</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Acetate/propionate</td>
<td>3.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Ruminal VFA production, moles/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>29.4</td>
<td>28.1</td>
</tr>
<tr>
<td>Propionate</td>
<td>13.3</td>
<td>31.0</td>
</tr>
<tr>
<td>Whole-body entry of butyrate (moles/d)</td>
<td>7.0</td>
<td>9.1</td>
</tr>
</tbody>
</table>

1 Data compiled from Davis et al. (1967); Bauman et al. (1971); Palmquist et al. (1969). Adapted from Bauman and Griinari, 2001.

The increased molar percentage and production rate of propionate when a high grain, low forage diet was fed that is depicted in Table 1 led some to consider the glucogenic-insulin theory of MFD. This theory suggests that large amounts of propionate produced in the rumen results in production of large amounts of glucose by the liver and subsequently increased circulating insulin concentrations. The mammary gland is considered to be somewhat insensitive to insulin compared with tissues such as
adipose tissue and muscle; therefore it was proposed, milk fat synthesis decreases due to a “competition” among tissues for substrates for milk fat synthesis with diets that increase circulating insulin causing a preferential channeling of substrates to non-mammary. However, cows subjected to a long-term hyperinsulinemic-euglycemic clamp (experimental technique in which the effect of insulin can be determined without the confounding effects of hypoglycemia) did not decrease milk fat synthesis compared to control cows (McGuire et al., 1995); in fact, insulin or glucose infusion results in very different profiles of milk fatty acids compared to diet-induced MFD (Bauman and Griinari, 2001), suggesting that this mechanism does not explain diet-induced MFD.

The second category of theories for the cause of MFD relates to the production of specific fatty acids in the rumen in situations of diet-induced MFD that directly inhibit milk fat synthesis in the mammary gland. Davis and Brown (1970) observed that MFD commonly was associated with increased concentrations of trans-fatty acids in milk fat. Trans-fatty acids are produced in the rumen as intermediates of the biohydrogenation of linoleic and linolenic acids to stearic acid (Figure 1). Linoleic and linolenic acids represent a large percentage of the fatty acids found in most forages and other plant-based feedstuffs fed to dairy cattle (cereal grains, oilseeds, etc.). Biohydrogenation of these fatty acids in the rumen by rumen bacteria is extensive, and most of the linoleic and linolenic acid consumed by cows is biohydrogenated fully to stearic acid before leaving the rumen (Lock et al., 2005); however, advances in analytical techniques during the past few years have led to the determination that varying quantities of a large number of trans-C18:1 monoenes and conjugated linoleic acids pass from the rumen to the lower tract for absorption (Bauman and Lock, 2006; Table 2). These findings, coupled with the finding that only certain trans-fatty acids and isomers of conjugated linoleic acid are associated with MFD led Bauman and Griinari (2001) to evolve the “trans-theory” of MFD into the “biohydrogenation theory”, through which they hypothesized that “under certain dietary conditions the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates which are potent inhibitors of milk fat synthesis.” These fatty acid intermediates are absorbed and directly inhibit expression of key genes in the mammary gland related to milk fatty acid synthesis and secretion (Piperova et al., 2000; Harvatine and Bauman, 2006).

**Figure 1.** Generalized scheme of ruminal biohydrogenation of linoleic and linolenic acids to stearic acid. Adapted from Harfoot and Hazlewood, 1997.
Figure 2. Generalized scheme of ruminal biohydrogenation of linoleic acid under normal conditions and during diet-induced milk fat depression (dotted line). Adapted from Griinari and Bauman (1999).

Research conducted before the development of the biohydrogenation theory of MFD and that conducted during the past few years since it was advanced suggest that this theory represents a unifying theory for diet-induced MFD. The most well-studied “altered pathway” of ruminal biohydrogenation of linoleic acid is depicted in Figure 2, in which under situations of altered ruminal fermentation (commonly low ruminal pH) linoleic acid (C18:2) is first isomerized to trans-10, cis-12 conjugated linoleic acid (CLA) and then reduced to trans-10 C18:1 before being further reduced to stearic acid (C18:0). Using pure isomers of CLA infused into the abomasum, Baumgard et al. (2000) determined that trans-10, cis-12 CLA was a potent inhibitor of milk fat synthesis. In contrast, infusion of cis-9, trans-11 CLA (the CLA isomer produced through normal ruminal biohydrogenation; Figure 1) into the abomasum did not affect milk fat synthesis. In subsequent experiments, it was found that the response of milk fat to infusion of trans-10, cis-12 CLA was dose-dependent (de Veth et al., 2004). Passage to the intestine of as little as 1.5 to 2 grams per day of this fatty acid isomer would be sufficient to reduce milk fat synthesis by 10 to 15%, which is within the magnitude of MFD that has economic consequences for dairy producers in North America.

Although the potent effects of trans-10, cis-12 CLA on milk fat synthesis are the most well-characterized, it is likely that other related fatty acid isomers have effects on milk fat synthesis. As mentioned above, advances in analytical techniques have enabled the characterization of a large number of isomers of trans-C18:1 and CLA (Table 2); the specific biological functions (if any) of many of these isomers remain uncharacterized. Recently, Perfield et al. (2005) showed that trans-9, cis-11 CLA caused a reduction in milk fat synthesis and another report indicated that the cis-10, trans-12 CLA also reduced milk fat synthesis in lactating dairy cows (Sæbo et al., 2005). Nevertheless, results from a large field study conducted by our research group and collaborators (Nydam et al., 2009) suggests that commercial dairy farms with low milk fat percentage have increased concentrations of the fatty acids in milk that would represent situations of altered ruminal biohydrogenation.
Table 2. Range of positional and geometric isomers of trans 18:1 and conjugated linoleic acids (CLA) and their ruminal outflow (g/d) in lactating dairy cows\(^1\)

<table>
<thead>
<tr>
<th>Trans-C18:1</th>
<th>Ruminal Outflow</th>
<th>Conjugated Linoleic Acids</th>
<th>Ruminal Outflow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isomer</td>
<td>Min</td>
<td>Max</td>
<td>Isomer</td>
</tr>
<tr>
<td>Trans-4</td>
<td>0.4</td>
<td>2.0</td>
<td>trans-7, cis-9</td>
</tr>
<tr>
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<td>3.4</td>
<td>trans-7, trans-9</td>
</tr>
<tr>
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<td>16.2</td>
<td>trans-8, cis-10</td>
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<tr>
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<td>1.4</td>
<td>13.1</td>
<td>trans-8, trans-10</td>
</tr>
<tr>
<td>Trans-10</td>
<td>1.5</td>
<td>114.0</td>
<td>cis-9, trans-11</td>
</tr>
<tr>
<td>Trans-11</td>
<td>17.0</td>
<td>148.0</td>
<td>trans-9, trans-11</td>
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<tr>
<td>Trans-12</td>
<td>1.9</td>
<td>20.8</td>
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<tr>
<td>Trans-13 + 14</td>
<td>4.2</td>
<td>60.3</td>
<td>trans-10, trans-12</td>
</tr>
<tr>
<td>Trans-15</td>
<td>2.0</td>
<td>29.0</td>
<td>cis-10, trans-12</td>
</tr>
<tr>
<td>Trans-16</td>
<td>2.3</td>
<td>18.2</td>
<td>cis-11, trans-13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>trans-11, cis-13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>trans-11, trans-13</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>cis-12, trans-14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>trans-12, trans-14</td>
</tr>
</tbody>
</table>

\(^1\) Adapted from Bauman and Lock, 2006. Data were derived from five studies where samples were collected from either the omasum or duodenum of lactating dairy cows (Piperova et al., 2002; Shingfield et al., 2003; Qiu et al., 2004; Loor et al., 2004; 2005).

Application of the biohydrogenation theory to troubleshooting diet-induced MFD on commercial dairy farms

The biohydrogenation theory provides an attractive unifying conceptual framework for troubleshooting milk fat issues on commercial dairy farms for several reasons. First, changes in milk fatty acid profile caused by infusion of trans-10, cis-12 CLA into the abomasum are consistent with common dietary situations (high grain, low forage; high unsaturated oil intake) that cause MFD and are unlike the changes in milk fatty acid profile caused by glucose or insulin infusion as described above. Second, this theory helps reconcile problems with low milk fat percentage absent overt signs of ruminal acidosis (e.g., by conventional evaluation on a dairy farm, rumen health seems excellent yet the herd has low milk fat test). Third, it enables us to conceptualize the potential roles of known modifiers of the ruminal environment (e.g., monensin or other compounds that may modify ruminal fermentation) in interacting with other factors of the ruminal environment to result in MFD in some cases. Finally, field experience troubleshooting milk fat issues on dairy farms suggests that MFD occurs as a result of several concurrent diet or management factors rather than as a result of a single factor, and our understanding of the biohydrogenation theory offers many opportunities for interactions of diet and management components to result in MFD.

We can divide the factors that can contribute to MFD into four general categories: 1) Factors that affect substrate supply and availability; 2) Factors that result in an altered ruminal environment; 3) Factors that influence biohydrogenation rate; and 4) Factors that influence rate of passage.

1) Factors that affect substrate supply and availability:

Given that the fatty acid isomers that cause MFD are intermediates in the pathways of ruminal biohydrogenation, it is logical that the amount of initial substrate (linoleic acid and perhaps linolenic acid) may be related to the amount of the key fatty acid intermediates that are produced and hence are subject
to passage from the rumen. As indicated above, linoleic acid (C18:2) is the predominant long-chain fatty acid in corn and corn byproducts. Estimates of C18:2 intake using CPM-Dairy in herds in the Northeastern US in which corn silage comprises the majority of the forage base in the ration and oilseeds are essentially the sole source of added dietary fat can approach or exceed 400 to 500 g/d. Furthermore, ready availability of corn byproducts (e.g., distillers grains) at low-cost in the feed industry can result in substantial inclusion of these byproducts in “least-cost” rations. Commonly, book values are used for the fat content of distillers grains in ration formulation; however, interaction with a number of feed industry professionals suggests that the fat content of distillers grains can vary widely. Based upon general survey of the literature, an effect of grain processing (in addition to potentially impacting the ruminal environment) may be to increase fatty acid availability in the rumen in some situations. Although our recent work (Nydam et al., 2009) suggests that there is no threshold level for C18:2 that substantially affects risk for low milk fat, we have had success in fixing low milk fat by decreasing C18:2 intake in a number of situations.

2) Factors that result in an altered ruminal environment:

These factors adhere most closely to those traditionally considered when troubleshooting MFD on dairy farms, although it is likely that some factors not commonly considered also may interact with diet formulation to produce an altered ruminal environment leading to the production of trans-10, cis-12 CLA or related biohydrogenation intermediates. One major factor that leads to flux of fatty acids through alternate pathways of ruminal biohydrogenation is low ruminal pH. Ruminal pH is thought to represent the balance between acid production from ruminal fermentation of carbohydrates, buffer production from salivary and dietary sources, and the rate of removal of fermentation acids from the rumen by absorption or passage (Allen, 1997). Dynamic interactions of these factors can result in marked changes in ruminal pH through any 24-h period. These factors have been well-reviewed (Shaver, 2005) and include dietary carbohydrate profile and rates of degradation of these carbohydrate fractions as affected by source, processing, and moisture; physically effective NDF (peNDF) supply as affected by source and particle size; and production of salivary buffers as a function of peNDF supply and source. Shaver (1995) also illustrated that the amount of chewing (and salivary buffer produced) per unit of rumen-fermentable organic matter (RFOM) consumed decreases as RFOM increases. This has implications for the risk of higher producing cows (who also have higher DMI) to have lower ruminal pH or different dynamics of ruminal pH during any 24-h period. In addition to factors associated with diet formulation, practicing nutritionists are well-aware of the on-farm factors related to feeding management (DM changes, variation in mixing and measurement of ingredient quantities) that affect the translation of the ration on paper to the ration in the feed bunk. Finally, other aspects related to management or the environment (feeding frequency, stocking density, heat stress, among others) can have marked effects on meal patterns of dairy cows and hence the dynamics of ruminal pH in any 24-h period (reviewed by Shaver, 2005; Von Keyserlingk and DeVries, 2005). Despite our general understanding of these factors, the degree and duration of low ruminal pH required to cause sufficient flux of linoleic acid through alternative pathways of ruminal biohydrogenation is not known.

Although the implications of low ruminal pH for production of the MFD-causing intermediates have been well-considered, it is not known which other factors can also cause changes in the rumen bacteria population resulting in an increased flow of fatty acids through alternate pathways of ruminal biohydrogenation. There is strong anecdotal evidence from our experience to suggest that factors such as ensiled feeds with abnormal fermentation profiles (particularly high acetic acid corn silages) or feeds with high yeast and mold counts or the presence of mycotoxins also may result in changes in biohydrogenation and cause MFD; however, these factors remain unstudied in a controlled manner.
3) Factors that influence biohydrogenation rate:

It is also logical that factors that affect the rate of biohydrogenation of fatty acids in the rumen may change the likelihood that intermediates responsible for MFD will pass from the rumen to the lower tract where they can be absorbed and directly inhibit milk fat synthesis in the mammary gland (Harvatine and Allen, 2006). In particular, the final step of conversion of C18:1 to C18:0 in ruminal biohydrogenation appears to be rate-limiting and slowing of this step likely increases passage of altered fatty acid intermediates to the intestine that in turn can decrease milk fat.

Diet components appear to have an effect on this rate-limiting step. For example, fish oil affects those rumen bacteria catalyzing the terminal step in biohydrogenation and as a result the rumen outflow of trans fatty acids increases (Bauman and Griinari, 2003). In vitro studies with mixed cultures of rumen bacteria have established that docosahexaenoic acid, one of the long chain omega-3 fatty acids in fish oil, is are responsible for this effect (AbuGhazaleh and Jenkins, 2004). In addition, evidence from other work in addition to our recent work (Nydam et al., 2009) suggests that excessive intake of C18:1 likely contributes to low milk fat in some situations, and we have had success in some herds decreasing C18:1 intake to less than 150 grams/day. Feeds that tend to be higher in C18:1 include many animal/vegetable oil fat blends that can be fed along with some commercial fat sources.

4) Factors that influence rate of passage:

A fourth area that influences the likelihood that biohydrogenation intermediates responsible for MFD may pass from the rumen to the lower tract is rate of passage. This has been less well-characterized than the other factors, but the possibility is logical. As described above, cows consuming greater amounts of RFOM have less chewing activity and buffer production per unit of RFOM than cows consuming smaller amounts of RFOM. Cows that are consuming larger amounts of RFOM are those cows with higher DMI, hence those cows also will have greater rates of passage from the rumen. This simply means that those cows (or herds) with higher DMI likely will be more at risk for MFD, and thus the margin of error is smaller in those herds.

Summary

Low milk fat percentage and yield is an important economic issue to dairy farms in most parts of the world. Research conducted during the past decade has markedly heightened our understanding of the etiology of milk fat depression, and this understanding can be translated into conceptual approaches for troubleshooting milk fat issues on commercial dairy farms. Ongoing university- and industry-based research will further enhance our ability to diagnose the causes of milk fat issues on individual farms and to provide detailed guidelines for preventing or troubleshooting milk fat problems on dairy farms.

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Davis, C.L. 1967. Acetate production in the rumen of cows fed either control or low fiber, high grain diets. J. Dairy Sci. 50:1621-1625.


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USING CORN COPRODUCTS FROM ETHANOL PRODUCTION-OPPORTUNITIES AND CHALLENGES IN FEEDING

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Summary

The ethanol industry in the United States continues to mature with the predominant grain in use being corn grain. Nearly 200 plants are producing fuel ethanol with its feed coproduct, distillers dried grains with soluble (DDGS) continuing to grow in use. For every gallon of ethanol produced about 6.5 pounds of distillers dried grains are also created. Approximately 34 million tons of distillers grains were produced in 2010, second only to soybean meal at 38 million tons in terms of total tons of feed ingredient produced. Nearly 9 million tons of these DDGS were exported from the US last calendar year. The feed value of these distillers grains continue to be evaluated with upper limits in many rations being tested. As nutritionists and feeders adjust rations to take advantage of nutrient value and cost savings, more animals are adapting to rations with this feed ingredient. Process differences as well as additional coproduct separation continue to change feeding values of specific DDGS. Even though the industry is beginning to mature, developmental changes to the DDGS are likely to continue as manufacturers continue to optimize returns from existing plants. Fractionation prior to fermentation and deoiling after fermentation are the most common changes seen today. These will affect the feeding value of the DDGS used and also which species are able to capitalize on the altered nutritional properties. Higher protein and lower fat content are the most frequently seen changes in nutrient composition. Variations in fiber content, particle size as well as color are also encountered. Feeding value of distillers grains will continue to be modified as manufacturers tweak processes and accommodate changes requested by customers. Optimization of these DDGS by various species will require diligence by nutritionists and formulators to gain full value from their use. The outlook is for more variation in DDGS and products from fuel ethanol production as we move forward. This will offer more opportunities for utilization in a greater variety of rations.
ROLE OF RUMEN-PROTECTED NUTRIENTS IN DIETS THAT ARE HIGH IN ETHANOL BYPRODUCTS

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Summary

As protein prices continue to increase and environmental regulations dictate that nutritionists reduce the protein level of their rations, it is becoming more important that alternative ingredients are utilized in dairy rations. Distillers grains and rumen-protected amino acids are both viable options that allow nutritionists to reduce ration costs and lower dietary protein levels while maintaining or improving the nutrient content of rations. Feeding DDGS at significant levels in rations has traditionally been avoided because of the perceived lower protein quality that they contribute to the ration relative to other high protein feedstuffs such as soybean and canola meal. One of the major nutritional problems that results when DDGS replace greater amounts of soybean or canola meal in rations is the reduction in MP-Lys which potentially can cause a limitation in this AA. However, with the recent development of several RP-Lys sources in addition to the RP-Met sources which have been available for many years, dairy nutritionists now have the ability to include higher levels of DDGS in rations without having a significant negative impact on the MP-Lys and MP-Met content of their rations. Utilizing DDGS can also allow nutritionists greater ration balancing flexibility in terms of being able to select a wider variety of protein sources which will allow them to reduce overall ration protein levels while maintaining or improving the nutritional quality of the ration. This will not only result in a lower level of nitrogen excretion into the environment but it can also help nutritionists reduce ration costs.

Introduction

Protein continues to be a relatively high input cost in dairy rations. In addition nitrogen excretion into the environment is an area of increasing concern and regulation by state and federal agencies. Managing both the cost of protein in the diet and nitrogen excretion into the environment is dependent on maximizing the efficiency with which protein is used by the cow. Recent growth of the ethanol production industry has resulted in an ever-increasing amount of distillers grains (DDGS) available as a feedstuff for lactating dairy cows. Nutritionally, DDGS present both an opportunity and a challenge for the dairy industry as they tend to be a relatively inexpensive protein and energy source but can also present several nutritional challenges as well. However, with the availability of several rumen-protected lysine (RP-Lys) and methionine (RP-Met) products in the marketplace, nutritionists can overcome many of the amino acid challenges that feeding higher amounts of DDGS create and reduce the level of overall protein in their rations which will reduce the amount of nitrogen excretion into the environment.
Background

Cows actually do not have a requirement for protein per se but rather require amino acids (AA). Proteins are made up of long chains of amino acids and can be classified as either essential (EAA), which cannot be synthesized by animal tissues or cannot be synthesized in sufficient amounts to meet requirements (arginine and histidine), or non-essential, which can be synthesized by the cow (NRC, 2001). Essential amino acids must be provided in the diet in the form of rumen undegradable protein (RUP) or produced by rumen bacteria during microbial protein synthesis in the rumen. Lysine (Lys) is one of the ten EAA and its predominant biological function is almost exclusively for synthesis of protein (milk protein, growth, pregnancy, maintenance) and non-essential amino acids (Lapierre, 2009). Methionine (Met) is also one of the ten EAA and plays a myriad of biological roles in the body that include: the initiator AA for protein synthesis in all eukaryotic organisms, methyl donation (via S-adenosyl-methionine, SAM), source of sulfur, biotin synthesis, lipoic acid synthesis, intermediate in the biosynthesis of cysteine, carnitine, taurine, lecithin, and phosphatidylcholine and other phospholipids.

Efficient utilization of dietary protein depends on the ability to formulate diets that deliver the optimal amount of metabolizable AA (AA actually absorbed from the intestine) in the right proportions to meet the needs of the cow. Once a single EAA becomes limiting the other absorbed AA cannot be utilized to produce proteins and protein efficiency begins to decline. Lysine and Met have often been shown to be the most-frequently limiting AA in MP in lactating dairy cows (NRC, 2001). Of particular interest to dairy nutritionists, is the impact of these two AA for milk protein production (Schwab et al., 1976; Polan et al., 1991; Armentano et al., 1997). It should not be surprising that Lys and Met are generally considered to be limiting in most U.S. dairy cattle rations as most commonly used feedstuffs are low in Lys and Met content relative to the Lys and Met content of milk protein, bacterial protein, and lean tissue (NRC, 2001; Table 1).
Table 1. A comparison of the essential amino acid composition of body lean tissue, milk, and ruminal bacteria with that of some common feeds.\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Arg</th>
<th>His</th>
<th>Ile</th>
<th>Leu</th>
<th>Lys</th>
<th>Met</th>
<th>Phe</th>
<th>Thr</th>
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<th>Val</th>
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<td>3.7</td>
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</tbody>
</table>

\(^1\) Amino acid values for lean tissue, milk, and ruminal bacteria are from O’Connor et al. (1993) and amino acid values for feeds are from NRC (2001).

Increasing metabolizable Lys (MP-Lys) and Met (MP-Met) levels in deficient diets can occur through increasing microbial protein production or by feeding high quality feeds containing relatively high amounts of Lys (blood meal, fish meal, heat-processed soybeans and soybean meals) and Met (fish meal, corn gluten meal, DDGS, brewers grains, canola meal). A significant potential challenge with meeting the requirement for Lys is the variability in level of intestinal digestibility of Lys. Lysine in the presence of heat, moisture and reducing sugars can undergo Maillard reactions resulting in indigestible end products. This will reduce the bioavailability of the Lys and this process can occur even under controlled conditions designed to increase protein bypass as well as randomly, such as in overheated silages.

As DDGS production continues to increase in the United States (Table 2), the availability and relatively low cost of DDGS present a significant opportunity for dairy nutritionists to use greater amounts of them in their rations to meet the nutritional requirements of dairy cows. Kononoff et al.
(2007) recently evaluated both the rumen undegradable protein (RUP) values and the intestinal digestibility of this protein. Using 16 h in situ incubations, these researchers found that the RUP portion of DDGS averaged 43.0% of CP, which was actually higher than soybean meal (28.4% of CP). As would be expected, the RUP of DDGS was not as high as non-enzymatically browned SBM (75.7% of CP). Interestingly, the RUP portion of DDGS had an intestinal digestibility of 86.2% of CP, which although this value was slightly lower than soybean meal (98%) and non-enzymatically browned SBM (96%), this digestibility is still relatively high and demonstrates that distillers grains can contribute a quality source of digestible AA to the ration.

Table 2. Annual production of distillers grains in the United States from 2005 to 2011.

<table>
<thead>
<tr>
<th>Year</th>
<th>Million (short) tons (DM basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>11</td>
</tr>
<tr>
<td>2006</td>
<td>14</td>
</tr>
<tr>
<td>2007</td>
<td>16</td>
</tr>
<tr>
<td>2008</td>
<td>26</td>
</tr>
<tr>
<td>2009</td>
<td>32</td>
</tr>
<tr>
<td>2010</td>
<td>39</td>
</tr>
<tr>
<td>2011</td>
<td>40</td>
</tr>
</tbody>
</table>

Figure adapted from Kononoff (2010).

With the advent of modeling programs that more accurately predict the delivery of metabolizable protein (MP) and AA to the cow there has been significant interest in formulating specifically for AA. In addition, sources of concentrated rumen protected Lys and Met now provide nutritionists with tools to more accurately formulate diets for AA by providing the specific AA without feeding excessive levels of MP to achieve those levels. This has the benefits of increasing the efficiency of protein utilization while increasing milk and milk component production. This will lead to increased profitability for dairies and reduced excretion of nitrogen into the environment helping producers meet more stringent environmental restrictions in the future.
Balancing Rations Using Distillers Grains and Rumen-Protected Lysine and Methionine

Feeding ethanol containing byproducts such as DDGS has traditionally been viewed by many nutritionists as a lower-cost, lower nutrient quality method of balancing rations. However, this stigma has been changed in recent years as several RP-Lys products have been introduced into the dairy nutrition marketplace. Now, nutritionists have the ability to substitute DDGS for higher Lys containing feedstuffs such as soybeans and not sacrifice the AA quality of the ration or needing to feed higher levels of dietary crude protein to achieve the same level of MP-Lys in the ration. Coupled with RP-Met products that have been available in the marketplace, utilizing DDGS can not only be a cost-effective ingredient choice, but can actually improve the nutrient quality and density of rations. There are several points that should be discussed when balancing rations for AA that will help nutritionists be more informed when balancing rations for AA, particularly using DDGS.

The question is often asked whether or not it is more important to focus on the amount of Lys and Met being supplied to the cow or the ratio in which they are supplied. The simple answer is that both are important. Ultimately, cows require amounts of nutrients, not percentages and ratios; however, the ratio is important for ensuring that the animal is utilizing the AA as efficiently as possible. As demonstrated by Schwab et al. (2004), maximizing the amount of Lys and Met in the ration while maintaining an approximate 3:1 ratio of Lys to Met will provide the cow with the maximum availability of digestible AA to help meet her productive requirements.

When balancing diets for Lys and Met, it is analogous to taking three legs to make a solid stool. In this case the efficient use of protein assuming Lys and Met are first limiting requires; the optimal ratio Lys to Met, the optimal levels of Lys and Met expressed as a percentage of MP and the optimal amounts in grams to meet the requirement of the cow. Most nutritionists recognize the need to provide Lys and Met in the proper ratio although there is some debate as to what the “correct” ratio should be. A realistic target is between 2.9:1 and 3.0:1. This alone is not enough as you could have three molecules of Lys and one of Met and thus the correct ratio but the cow would not be able to produce a hundred pounds of milk with it. The second leg of the stool is Lys and Met expressed as a percentage of MP. This is particularly important in terms of achieving efficiency. If Lys and Met percentages are too high (not generally a problem) these expensive nutrients are wasted since other limiting AA will limit performance. If the percentages are too low then all of the other AA are utilized inefficiently and protein efficiency suffers.

Whitehouse et al. (2009) used three different commonly used programs for balancing amino acids to estimate the requirement values of Lys and Met as percentages of MP to maximize milk protein content and milk protein yield and subsequently, determined the optimal ratios of Lys and Met generated from these results. Schwab and Foster (2009) utilized the data from Whitehouse et al. (2009) and included \( r^2 \)-values which are presented in Table 3. There are several interesting points to take away from this table. First, while the recommended optimal Lys to Met ratios have been adjusted downward when maximizing both milk protein content and yield, the ratios are still relatively close to the 3:1 ratio that have been previously recommended. Additionally, these recommendations are still relatively new and have not been universally tested in the field to determine whether it is economically worthwhile adjusting rations to achieve the new ratios. Second, economics will ultimately dictate the maximum amount of grams of metabolizable Lys and Met that will be fed to maximize milk yield and milk protein yield. However, if nutritionists do choose to adjust their rations to reflect the new recommended Lys to Met ratios, they should achieve these ratios not by reducing the amount of metabolizable Lys in their rations but rather by increasing the current level of metabolizable Met in their rations to obtain the new recommended ratio of...
Lys to Met. As was previously stated, cows ultimately require amounts of nutrients, not percentages and it has been the experience of the authors that most commercial dairy rations currently are not meeting the grams of metabolizable Lys and Met required to maximize milk yield and milk protein synthesis as a result of economics. Hence, reducing the grams of metabolizable Lys to meet a lower ratio of Lys to Met rather than increasing the level of metabolizable Met to meet this ratio may result in lost milk yield and milk protein yield.

Table 3. Breakpoint estimates for required concentrations of Lys and Met in MP for maximal content and yield of milk protein for the NRC, CPM, and AMTS models.¹

<table>
<thead>
<tr>
<th>Item</th>
<th>NRC Model</th>
<th>CPM Model</th>
<th>AMTS Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optimal Lys</td>
<td>Optimal Met</td>
<td>Lys r²</td>
</tr>
<tr>
<td>Content of milk protein</td>
<td>6.80</td>
<td>2.29</td>
<td>.82</td>
</tr>
<tr>
<td>Yield of milk protein</td>
<td>7.10</td>
<td>2.52</td>
<td>.65</td>
</tr>
</tbody>
</table>

1 Adapted from Schwab and Foster (2009) and Whitehouse et al. (2009).

The third leg of the stool is the total grams of metabolizable AA fed. Cows have a requirement for grams not percentages. As production increases the cow needs more grams to meet her increased production. Consequently placing any artificial cap on the number of grams required can result in reduced performance and efficiency of protein use. It is important for nutritionists to remember that although the Lys to Met ratio is important, it is only one leg of the AA balancing stool and that it is also important to maximize the total grams of Lys and Met in an economically feasible manner to truly maximize milk yield and milk component production. In many areas of the U.S. where the value of milk fat and particularly, milk protein makes up a large portion of dairy producer’s milk checks, striving to maximizing the grams of metabolizable Lys and Met, maximizing the concentrations of Lys and Met in MP, and maintaining a ratio of Lys to Met of approximately 3:1 will result in a positive return on investment and maximize profitability.

Feeding Distillers Grains
Feeding of DDGS to dairy cattle is not a new concept and has been extensively studied for more than one-hundred years (Kononoff, 2010). Distillers grains continue to be readily available and a relatively inexpensive source of protein and energy and as a result, they have become a commonplace ingredient in many dairy cattle rations.

The most widespread and predominant source of protein in dairy cattle rations in the United States has traditionally been soybeans, particularly, soybean meal. However, soybeans have dramatically increased in cost over the past few years and have become an expensive source of protein which has helped to necessitate the incorporation of alternative protein sources such as DDGS into dairy rations. The nutritional AA challenge this creates is that DDGS contain a lower level of Lys relative to soybeans, although it is important to note that their Met content is slightly higher (Table 1). As a result, as greater amounts of DDGS replace the protein portion previously filled by soybeans, diets that were once more limiting in Met than Lys, may now actually become more limiting in Lys. Table 4 depicts a very simplistic ration example demonstrating the impact of replacing 1-lb DM of 48% soybean meal with 1-lb DM of DDGS. As can be observed in this scenario, MP-Lys decreased by 4.6 g/d or about 3% of the total MP-Lys when DDGS replaced only 1-lb DM of 48% soybean meal. Several points need to be made in this scenario: 1) In this example, the ration contained 0.5 lb blood meal and as a result, lowering soybean meal still resulted in a total MP-Lys that was in excess relative to MP-Met; therefore, no supplemental Lys needed to be added in the ration, 2) Diet costs were decreased by $0.10/head/day and the ratio of Lys to Met actually improved which demonstrates that DDGS can be used as both a nutritional and cost effective feed ingredient without negatively impacting the quality of the ration, 3) This simplistic example of a real-world ration demonstrates the key point that when DDGS are used to replace a portion of the soybean meal in a ration, MP-Met will generally be unaffected; however, MP-Lys may be decreased significantly and in many cases, supplemental MP-Lys from an RP-Lys source may be needed to be used to offset the loss of MP-Lys in the ration, 4) Total ration protein was decreased by 0.5% which equates to 0.04 lb less nitrogen being fed per cow per day or 4.35 lb less nitrogen being fed and excreted per 100 cows every day.

Table 4. Effect of replacing 1-lb DM of 48% soybean meal with 1-lb DM of distillers grains on metabolizable amino acid supply in a typical lactating dairy ration\textsuperscript{1,2,3}.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Before Ration</th>
<th>After Ration</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, lbs</td>
<td>51.0</td>
<td>51.0</td>
</tr>
<tr>
<td>CP, %</td>
<td>17.2</td>
<td>16.7</td>
</tr>
<tr>
<td>MP, g/d</td>
<td>2560</td>
<td>2526</td>
</tr>
<tr>
<td>ME, Mcal/d</td>
<td>66.3</td>
<td>66.1</td>
</tr>
<tr>
<td>MP balance, g/d</td>
<td>75.3</td>
<td>34.3</td>
</tr>
<tr>
<td>ME balance, g/d</td>
<td>6.8</td>
<td>6.7</td>
</tr>
<tr>
<td>Lys, g/d (% of MP)</td>
<td>176.8 (6.90)</td>
<td>172.2 (6.82)</td>
</tr>
<tr>
<td>Met, g/d (% of MP)</td>
<td>56.6 (2.21)</td>
<td>56.4 (2.23)</td>
</tr>
<tr>
<td>Lys/Met ratio</td>
<td>3.12:1</td>
<td>3.06:1</td>
</tr>
</tbody>
</table>

\textsuperscript{1} CPM Dairy v3.0.10 predictions.  
\textsuperscript{2} Before ration contained (% of DM): Corn silage (30.4%), MMG haylage (31.8%), grass hay (1.5%), corn meal (17.7%), protein/mineral/vitamin mix (9.3%), 48% soybean meal (9.3%).  
\textsuperscript{3} After ration contained (% of DM): Corn silage (30.4%), MMG haylage (31.8%), grass hay (1.5%), corn meal (17.7%), protein/mineral/vitamin mix (9.3%), 48% soybean meal (7.3%), and ethanol dried distillers grains (2.0%).
Conclusions

Feeding DDGS at significant levels in rations has traditionally been avoided because of the perceived lower protein quality that they contribute to the ration relative to other high protein feedstuffs such as soybean and canola meal. One of the major nutritional problems that results when DDGS replace greater amounts of soybean or canola meal in rations is the reduction in MP-Lys which potentially can cause a limitation in this AA. However, with the recent development of several RP-Lys sources in addition to the RP-Met sources which have been available for many years, dairy nutritionists now have the ability to include higher levels of DDGS in rations without having a significant negative impact on the MP-Lys and MP-Met content of their rations. Utilizing DDGS can also allow nutritionists greater ration balancing flexibility in terms of being able to select a wider variety of protein sources which will allow them to reduce overall ration protein levels while maintaining or improving the nutritional quality of the ration. This will not only result in a lower level of nitrogen excretion into the environment but it can also help nutritionists reduce ration costs.

References


Whitehouse, N., C. Schwab, D. Luchini, T. Tylutki, and B. Sloan. 2009. Comparison of optimal lysine and methionine concentrations in metabolizable protein estimated by the NRC (2001), CPM-Dairy (v.3.0.10) and AMTS-Cattle (v.2.1.1) models. J. Dairy Sci. 92 (Suppl. 1):103. (Abstr.)
FEED EFFICIENCIES IN GROWING DAIRY HEIFERS

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Summary

There are limited published data available for feed efficiency (FE) calculations of growing dairy heifers. The 2001 Dairy NRC publication dry matter intake (DMI) prediction equation illustrates considerable variation in observed vs. predicted DMI, and this variation increased with increasing DMI. Growth of large breed (Holstein) dairy heifers is impacted by primarily dietary protein and energy levels, and should normally be within 0.8 to 0.9 kg daily. But genetics can affect DMI as influenced by mature body weight of heifers. Feed efficiency does not take into account height of heifers which has a distinctly different pattern of increase being greater within the first 6 months of age, and then decreasing curvilinearly as opposed to the more linear increase in daily body weight gain post-weaning. Limit feeding of dairy heifers impacts FE as limit fed diets typically have lower forage concentrations which results in less rumen/gut fill and more true body weight increase. Published data indicate that limit feeding lower forage diets results in less DMI/gain vs. conventional heifer diets. But these data are limited by most often being calculated for the entire trial of 4 up to 35 weeks, rather than at more frequent intervals. In calves fed diets ranging from 4 to 60% hay, FE were confounded in that these calves at 83 days of age had increasing gut fill with increasing proportion of dietary hay concentration. Similarly, calves fed a range of dietary crude protein and ADF concentrations had better FE with increasing crude protein up to 15% but poorer FE with increasing ADF up to 25% at 20 weeks of age. In a commercial dairy, implementation of limit feeding increased the DMI/gain ratio in heifers with increasing body weight. But limit feeding was later discontinued because feed economics had changed, and there were diet formulation and behavior concerns. Data from a large commercial calf and heifer ranch illustrated greatest conversion of nutrients in dry matter intake to body weight prior to weaning. Feed efficiency then became poorer with increasing age because of greater maintenance requirements with heavier body weights. Because of so many variables affecting FE, it is difficult to compare FE across studies or across operations. Thus, it is better to compare FE within a study or within an operation and on similar diets. Ultimately, economic FE is the most critical as long as the desired growth is achieved without problems.

Introduction

Over the last 40 years, heifer-growing programs have gone through various phases. At one time, heifers were the forgotten animal on a dairy, often banished to the proverbial “back 40” acres. They had access to only poor pasture or poor quality forages. Then in the 1970s and 1980s, corn silage became popular for feeding to dairy cows, and to heifers. Too often corn silage was available free choice to heifers, which resulted in their fattening even if protein supplementation was adequate and grass or other hay was also available. In a classic study, Jahn and Chandler (1976) showed that with high fiber/low
quality forage, there was a practical limit to how much protein could compensate to try to attain average daily gain (ADG) objectives. Stobo et al. (1966) found that with younger calves, increasing forage level had a double negative effect in that ADG was decreased as forage level increased from 4 to 61% while gut fill also increased distorting true body growth. As increasing emphasis was placed on growing and harvesting higher quality forages for dairy cows, these forages were often fed to heifers too. At the same time, increasing emphasis was placed on higher genetic merit for cows. Consequently, intake of heifers increased with genetic merit too. And with higher quality forage, heifers could now get fat when fed virtually forage only diets. Some producers then began to seek and use some poorer quality roughage to reduce dietary energy concentration and to thereby limit energy intake when diets were fed free choice to heifers. That led to others seeking another approach to feeding heifers—limiting their intake. There are limited data available in the literature and from the field on feed efficiencies (FE) in growing heifers. This paper will explore the background and data available to better characterize FE in growing heifers. Feed efficiencies (FE) in this paper will be expressed as (dry matter intake) DMI/ADG.

**Dry Matter Intake and Protein/Energy Relationship**

Phenotypic variables in raising heifers are many as Hoffman (2004) listed 17 affecting performance other than nutrition. Nutrition itself is comprised of many variables, among which the largest is DMI. Dry matter intake prediction for heifers has been difficult. In the 1989 Nutrient Requirements of Dairy Cattle (NRC), the prediction was based mainly on what energy requirements were estimated to be, and then back calculating what the DMI would need to be to meet those requirements. This resulted in a similar curve to the 2001 NRC DMI prediction except when heifers exceeded 450 kg of body weight (BW). That segment then deviated upwards from the 2001 NRC DMI projection because the 1989 DMI estimation was based on the assumption that lower quality/energy forages would be used for these larger heifers, which would then require this amount of DMI to meet these heifers’ energy needs. In fact, lower quality forage reduces DMI because of lower rate and extent of digestibility, which would also increase gut fill. In the 2001 NRC, actual dairy heifer DMI (Figure 1) were gathered (mainly from trials done over a 20 year period at the then Purina Mills Dairy Research Center) and plotted versus DMI prediction using the 1996 Beef NRC equation for beef heifers. The observed DMI (data points) were initially lower than predicted DMI (line), but then shifted higher and above the line when DMI was ≥ 5 kg/d. This inflection corresponds to heifers over 6 months (mo) of age. Greater DMI for dairy heifers versus beef heifers above this point may simply reflect the greater size (especially height) of dairy heifers along with their genetic predisposition. Another key point is that variation of actual DMI progressively increased as heifers ate more. In fact, DMI variation in heifers is likely greater than in lactating cows. This may be due to greater genetic diversity in heifers, but is more likely due to greater differences in the nature of feeding programs for heifers along with their environmentally different situations.

If it is assumed that ADG was 0.8 kg (not likely a safe assumption) across the range of DMI in Figure 1, increased DMI/ADG would result with likely more variation as DMI increased. But with the range in DMI, and with likely variation in ADG commensurate with increased DMI (age and body weight), DMI/gain could overlap considerably at a given age, body weight (BW), and DMI.
Dry matter intake of heifers progressively increases as heifers grow larger, but their DMI as a percentage of BW progressively decreases from a high of about 3% around weaning to a low of about 1.8% near calving. Along with this change in relative DMI, there is also a suggested change in the ratio of dietary protein to energy in g of crude protein/Mcal of metabolizable energy/kg of DM (VandeHaar, 1998). For the first 6 mo of age, this ratio is recommended to be 66, declines to 63 for mo 8 to 12, declines further to 60 for mo 12 to 16, bottoms out at 56 for mo 14 to 23, and then increases to 60 for mo 23 to calving at 24 mo of age.

Growth Related Factors

How should growth targets be established? While Mature Body Weights (MBW) have been proposed to establish target weights for growing heifers as a percentage of their MBW (BANM, Hoffman 2007, Van Amburgh and Meyer 2005) this presumes that MBWs are known and that genetic variability is low. In many large commercial herds, genetic history is obscure because in start-up or expansion phases of such herds, bred heifers were purchased with unknown genetic history and from a variety of geographic locations. An example of within breed variation was studied (Hansen et al., 1999) due to divergent genetic selection for large versus small body size of Holstein cows from 1966 to 1994. Coefficient of variation (CV) % ranged from 0.6 to 1.2% for BW and 0.16 to 0.36% for WH (wither height) for first parity (n = 217) to third parity (n = 51) animals. These results contrast with a 5-year (yr) Holstein herd database of mixed genetic lines (Kertz et al., 1997) with a CV of 8 to 9% for BW and 2.3 to 3.1% for WH for first parity (n = 281) to 113 for third or fourth parity (n = 61) animals.

There are several significant limitations of the MBW approach. First is the need for a simple method (Kertz, 2007) to gather BW and WH measurements of either mature cows or by their 2nd lactation to use as a lactation factor to compute MBW. Another very practical problem is grouping and movement of heifers. Bach et al. (2006) evaluated the impact of delaying heifers (n = 2,817 Holsteins) from moving from one group to the next when they had not reached targeted BW for that group. This was done at a large calf/heifer ranch in Spain (disclosure, I have consulted for this operation) which raises for 140 other
dairies. Hence there is a lot of potential genetic variation. Animals that were delayed, and then regrouped with a new set of younger incoming heifers, were weighed every 15 days (d) until they reached the target BW and then were moved up one group with their initial acquaintances. This resulted in improved daily gain of the older heifers. The overall increased daily gain observed for the first 18 d of regrouping disappeared 45 d later, as the ADG of delayed animals was then similar to their non-delayed cohorts. Whether this was due to social order, environmental differences, or differences in BW or age is not known.

Lastly, there is a need to grow heifers without fattening. Some key biological principles apply. First, calves are born with low body fat levels, as low as ~4%. Conversely, their body water level is the highest it will likely ever be for there is an inverse relationship between body fat and body water percentages (Reid et al., 1955). When animals grow and deposit fat early in life, this is primarily by hyperplasia—an increase in the number of adipocytes or fat cells. Later increases in body fat are primarily by an increase in the size of those fat cells—hypertrophy. The more fat cells present at this later stage, the greater the propensity to fatten. That is why there is much concern in the human population with avoiding fatter infants and toddlers. There are too many situations with dairy heifers where they are grown too fast leading to fattening.

What is too fast? An ADG of 0.68 to 0.77 kg during the first 2 mo of a calf’s life results in a doubling of BW by 2 mo of age. An ADG of .82 to .91 kg during the subsequent 22 mo results in a BW of 636 kg at 22-24 mo of age. This is a reasonable pattern of growth for heifers to first calve at 24 mo of age. What about ADG of 1.14 to 1.36 kg? Based on the maximum rate of protein deposition being ~1 kg per day (M. Meyer, Ph.D. thesis, 2007 Cornell University), growth rates greater than that would be fattening. The other element of growth that is often overlooked and seldom measured is height. Total WH increase from birth to first calving is ~61 cm. One half of that occurs during the first 6 mo of a calf’s life at the rate of 5 cm/mo. Another 25% of that occurs during 7 to 12 mo of life, and then only 25% occurs during the last 12 mo before calving (Kertz et al., 1998). (To convert hip height to wither height simply add 0.8 cm to the latter.) Since this height increase is under biological control at given age periods, there is no compensatory gain for height as can occur for BW. Feed efficiency does not account for height increase.

An Israeli study (Murkusfeld and Ezra, 1993) concluded that “the relative importance of height as a predictor of future milk yield is underestimated and should be stressed more in replacement heifers.” This does not mean that more emphasis should be placed on genetic selection for height, but rather that more attention should be placed on raising heifers so that they reach their height genetic potential in order that they achieve body mass consistent with reaching their genetic potential for milk yield.

Limit-Feeding Strategies and Results

Limit feeding has been shown to be useful to control growth in other livestock animals such as beef cows (Loerch, 1996), feedlot steers (Loerch, 1990), ewes (Susin et al., 1995), and beef heifers (Wertz et al., 2001). Potential benefits are to reduce feed costs, reduce nutrient excretion, and reduce feedstuffs needed. These studies may also provide some insights into factors impacting FE.

Lammers et al. (1999) used a 2 x 2 factorial arrangement with or without an estradiol implant treatment at growth rates of 0.7 kg/d or 1.0 kg/d. All heifers received the same diet, but DMI was adjusted weekly to achieve the target rate of gain. No negative effects were noted in first lactation performance, but there were differences in growth parameters due to rate of ADG (Table 1). Use of the implant lowered (P<0.06) DMI and decreased (P<0.10) FE. (Often in literature, FE data are limited by not calculating it over several time intervals within the overall trial period. That single FE would cover studies as short as 4 wk but up to 35 wk long.) In this study, only one FE was calculated, and that
covered the entire 20-wk period. Since dietary protein concentration was increased for heifers limit fed to equalize protein amount consumed by accelerated fed heifers, lower (P<0.05) ADG, WH, heart girth, and body condition score (BCS) were due to less energy consumption as a consequence of lower DMI (P<0.05). Heifers may have grown the same on both treatments if energy intake had been equalized.

Table 1. Limit feeding effects on growth and performance of Holstein heifers from 19 to 39 wk of age (from Lammers et al., 1999).

<table>
<thead>
<tr>
<th></th>
<th>Limit fed</th>
<th>Accelerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average daily gain (ADG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial age, d</td>
<td>132</td>
<td>134</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>130.6</td>
<td>129.2</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>.71a</td>
<td>1.01b</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>3.22a</td>
<td>4.36b</td>
</tr>
<tr>
<td>DMI/ADG</td>
<td>4.57a</td>
<td>4.39b</td>
</tr>
<tr>
<td>Wither height increase, cm/4wk</td>
<td>2.80a</td>
<td>3.17b</td>
</tr>
<tr>
<td>Heart girth increase, cm/4wk</td>
<td>5.18a</td>
<td>6.57b</td>
</tr>
<tr>
<td>Body condition score (BCS), initial</td>
<td>2.25</td>
<td>2.24</td>
</tr>
<tr>
<td>BCS, change</td>
<td>.49a</td>
<td>.73b</td>
</tr>
</tbody>
</table>

a,b P < 0.05.

Zanton and Heinrichs (2007) fed Holstein heifers for 245 d either high-forage (75% equally from corn and grass silage—HF) or low forage (25% equally from corn and grass silage—LF) total mixed rations (TMR) to achieve the same ADG beginning at 125 kg of BW and 117 d of age. Each treatment was in a separate lot bedded with sand so bedding consumption and lack of bunk access were not issues. Growth parameters were not different except for greater (P<0.05) paunch girth with heifers fed the LF TMR. Average daily gain did not differ by design, and were 0.828 for HF and 0.827 for LF. But DMI did differ (P<0.001) at 5.96-HF and 5.32 LF with associated different FE (P<0.003) of 7.04 and 6.4. In a later study, Zanton and Heinrichs (2008) quantified manure excretion using Holstein heifers at 340 kg of BW and 14.5 mo of age. They were fed a high forage diet (grass silage, grass hay, and corn silage) at 1.25, 1.50, 1.75, and 2.0 % of BW. Dry matter intake increased in proportion to feed offered until reaching a plateau at 1.92% of BW. But experimental periods were only 35 d, so meaningful FE could not be calculated. Manure excretion increased at a rate of 2.5 times DMI, and this excretion was entirely due to greater wet feces excretion as amount of urine excretion did not change. Overall nutrient efficiency decreased with lower DMI down to 1.5% of BW, with all levels of feed offered above this level resulting in similar efficiencies.

Effects of low forage (33% corn silage—LCS) and high forage (77% corn silage—HCS) TMR on rumen parameters were evaluated (Moody et al., 2007) in 298 kg Holstein heifers; and in a second trial, the effects on nutrient utilization and fecal excretion were evaluated in Holstein heifers at 6 mo (172 kg of BW) and 12 mo (337 kg of BW). Experimental periods were only 21 d. Mass of rumen contents was lower (P<0.05) for heifers fed LCS. Major differences were in less (P<0.01) wet and dry feces produced by heifers fed HCS for both age groups. Moody et al. (2007) found that heifers fed high or low forage diets, and restricted-fed, had wet vs. dry rumen contents of 18% and 16%, respectively. While not directly compared, this would indicate that heifers fed restricted amounts of low forage diets could have the same BW gains as heifers fed non-restricted higher forage diets; but, because of less rumen fill, would have truer BW gain. Zanton and Heinrichs (2007) found that heifers fed a high forage diet had 11.5% more wet rumen contents than heifers fed a low forage diet.

Hoffman et al. (2007) explored a multitude of elements in one trial with pregnant Holstein heifers beginning at 464 kg of BW and 17.5 mo of age. There were 9 pens containing 6 heifers per pen, with 3 replicate pens randomly assigned to one of 3 TMR. Control groups were fed a diet that contained 11.3%
CP and 2.46 Mcal ME/kg of DM in ad libitum amounts (100%) based on NRC (2001) recommendations for a 450-kg heifer. The 90% and 80% dietary treatments contained 12.7 and 14.2% CP and 2.55 and 2.69 Mcal of ME, respectively. These two diets were formulated to provide similar daily intakes of CP, energy, vitamins, and minerals as compared with feeding the control diet. No differences in the size or body condition scores of the heifers were observed after a 111-d feeding period (Table 4), but there was a 30% linear improvement (P<0.01) in FE as heifers reduced intake from 100 to 80%. Fecal DM excretion was also reduced linearly (P<0.10) with heifers fed the 80% diet producing 40% less total DM excretion compared to heifers fed the 100% diet.

Table 2. Growth, feed efficiency (FE), excretion, and subsequent first lactation milk production for heifers fed diets at 100, 90, or 80% of ad libitum intake during the prepartum period (Hoffman et al., 2007).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Feeding level, % of ad libitum amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>470</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>0.75</td>
</tr>
<tr>
<td>Initial hip height, cm</td>
<td>138</td>
</tr>
<tr>
<td>hip height, cm/111 d</td>
<td>4.6</td>
</tr>
<tr>
<td>Initial heart girth, cm</td>
<td>186</td>
</tr>
<tr>
<td>heart girth, cm/111 d</td>
<td>16</td>
</tr>
<tr>
<td>FE, kg of DMI/kg of BW gain&lt;</td>
<td>12.8</td>
</tr>
<tr>
<td>Excretion</td>
<td></td>
</tr>
<tr>
<td>DM, kg/d&lt;</td>
<td>3.5</td>
</tr>
<tr>
<td>N, g/d&lt;</td>
<td>140</td>
</tr>
<tr>
<td>P, g/d&lt;</td>
<td>27</td>
</tr>
</tbody>
</table>

*a* Linear effect (P < 0.01).

Calculated FE from Literature and Field Data

Stobo et al. (1966) weaned calves at 5 wk of age after hay and water were offered ad libitum beginning at 3 wk of age. A concentrate mixture was provided for up to 83 d to maximum intakes of 0.45, 0.91, 1.36, 1.81 or 2.27 kg/d in treatments 1-5 respectively. Calves were then sacrificed for gut content and rumen papillae measurements. As hay level in diets increased, DMI decreased, ADG decreased, but FE resulted in a more inconsistent effect (Table 3). This is most likely related to true BW increase being confounded with increasing gut fill as hay level in diets increased. Additionally, rumen papillae length was maximized at the lowest dietary hay level, consistent with the negative impact on rumen functional development with forage in diets fed to young calves (Warner, 1991).
Table 3. Intake, gain and gut measurements for calves at 83 d of age (Stobo et al., 1966).

<table>
<thead>
<tr>
<th></th>
<th>1.15</th>
<th>1.32</th>
<th>1.77</th>
<th>2.15</th>
<th>2.36</th>
</tr>
</thead>
<tbody>
<tr>
<td>% hay in DMI</td>
<td>61</td>
<td>31</td>
<td>25</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Daily gain, kg</td>
<td>0.32</td>
<td>0.42</td>
<td>0.47</td>
<td>0.60</td>
<td>0.59</td>
</tr>
<tr>
<td>DMI/gain(FE)</td>
<td>3.59</td>
<td>3.14</td>
<td>3.77</td>
<td>3.58</td>
<td>4.00</td>
</tr>
<tr>
<td>Live weight, kg</td>
<td>59</td>
<td>63</td>
<td>69</td>
<td>78</td>
<td>77</td>
</tr>
<tr>
<td>Rumen papillae, mm</td>
<td>4.2</td>
<td>5.2</td>
<td>5.5</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td>% of live weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reticulo-rumen</td>
<td>18</td>
<td>15</td>
<td>14</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Alimentary tract</td>
<td>23</td>
<td>20</td>
<td>19</td>
<td>15</td>
<td>14</td>
</tr>
</tbody>
</table>

Jahn and Chandler (1976) ad libitum fed Holstein calves diets varying in CP (8.0, 11.5, 15.0, and 18.5%) and in acid detergent fiber—ADF(11, 18, and 25%) for 2 consecutive 6-wk periods beginning after 8 wk of age. There were 2 males and 3 females assigned per treatment in a randomized complete block design. Initial starting BW averaged 64 kg. Roughage source used to vary ADF levels consisted of a blend of 75% corn cobs and 25% orchard grass hay. From regression equations created by data analysis, DMI and ADG were calculated for each level of ADF and CP. Then FE were calculated from those DMI and ADG. While there were only 5 calves per treatment group, the distinct pattern, except for 8% CP/11% ADF, was as dietary CP increased DMI/gain decreased (Figure 3). Within % CP treatments, increasing ADF % increased DMI/ gain.

Figure 3. Feed/gain for various dietary crude protein (CP) and acid detergent fiber (ADF) levels (Jahn and Chandler 1976).

Mason Dixon Farms in Gettysburg, PA began utilizing limit feeding of high energy diets for their heifers in July 2006 under the management of Alan Waybright, initial recommendations by A. J. Heinrichs of Pennsylvania State University, and on-going diet formulation by nutritionist Robert Fry of Atlantic Dairy Management Services. By October 2006, all heifers were on this program, and as of January 2009, about 2000 had their first calf. At the time of this analysis (January 2009), most Holsteins were being bred to Normande Reds. About 250 of those 2,000 heifers were cross-bred, and only 30 cross-bred cows were in lactation. First-calf heifer age averaged 23 mo with Holstein pre-calving BW ranging
from 556 to 636 kg, and cross-bred BW ranging from 477 to 511 kg. Heifers started the limit feeding program at ~ 4 mo of age and weighing ~125 to 136 kg BW. Primary benefits realized at that time were lower feed costs and less manure. The 5 groupings of heifers, each with their own ration are noted in Table 4. These diets were developed by trial and error at Mason Dixon Farms by the nutritionist and the calf/heifer manager. Limit feeding was discontinued in January 2010 due to cost head/d then being less with traditional rations, nutritionist not being comfortable balancing rations to meet heifer needs--mostly CP, and having more bad behavior such as sucking, etc.

Table 4. Mason Dixon Farms typical limit-fed heifer rations in January 2009, with this author’s assumed daily gain of 0.725 kg (A. Waybright and R. Fry, personal correspondence).

<table>
<thead>
<tr>
<th>Heifer Group</th>
<th>DM fed kg</th>
<th>DMI % BW</th>
<th>Estimated DMI/gain</th>
<th>% Forage</th>
<th>Forage sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>159 kg</td>
<td>3.63</td>
<td>2.28</td>
<td>5.01</td>
<td>58</td>
<td>Corn/barley silages, haylage</td>
</tr>
<tr>
<td>223 kg</td>
<td>4.63</td>
<td>2.08</td>
<td>6.39</td>
<td>50</td>
<td>Barley silage, corn silage</td>
</tr>
<tr>
<td>272 kg</td>
<td>5.72</td>
<td>2.10</td>
<td>7.89</td>
<td>50</td>
<td>Barley silage</td>
</tr>
<tr>
<td>386 kg</td>
<td>6.68</td>
<td>1.73</td>
<td>9.21</td>
<td>54</td>
<td>Barley silage</td>
</tr>
<tr>
<td>477 kg</td>
<td>7.37</td>
<td>1.55</td>
<td>10.17</td>
<td>62</td>
<td>Barley silage</td>
</tr>
</tbody>
</table>

Another source of field data is from the Rancho Las Nieves in Spain (Table 5). About 6,000 calves/heifers annually are raised for around 140 dairies (Bach et al., 2006). Group 1 represents calves up to weaning, and reflects their being the most efficient in converting nutrients to growth. With increasing age and BW, along with introduction of forage and fed at higher proportions, FE numbers progressively increases. This reflects the increasing maintenance needs with more BW, and increasingly less efficient conversion of nutrients to ADG.

Table 5. Dry matter intake (DMI), average daily gain (ADG), and feed efficiency (FE) as DMI/ADG from Rancho Las Nieves in Spain (A. Bach, personal communication).

<table>
<thead>
<tr>
<th>Group</th>
<th>Age at end, d</th>
<th>DMI, kg/d</th>
<th>ADG, kg/d</th>
<th>FE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>1.32</td>
<td>0.757</td>
<td>1.74</td>
</tr>
<tr>
<td>2</td>
<td>111</td>
<td>2.56</td>
<td>1.001</td>
<td>2.56</td>
</tr>
<tr>
<td>3</td>
<td>162</td>
<td>5.16</td>
<td>1.030</td>
<td>5.01</td>
</tr>
<tr>
<td>4</td>
<td>226</td>
<td>6.63</td>
<td>0.986</td>
<td>6.72</td>
</tr>
<tr>
<td>5</td>
<td>295</td>
<td>7.43</td>
<td>0.961</td>
<td>7.73</td>
</tr>
<tr>
<td>6</td>
<td>406</td>
<td>8.91</td>
<td>0.847</td>
<td>10.52</td>
</tr>
<tr>
<td>7</td>
<td>650</td>
<td>10.56</td>
<td>0.840</td>
<td>12.57</td>
</tr>
</tbody>
</table>

Economics

The last aspect to be briefly addressed is economics. In terms of FE, this could vary considerably given differences in feedstuffs costs, and in a multitude of other factors. A field data study was initially done (Hoard’s Dairyman, 2000) with 62 herds in WI. This study was essentially repeated in 2007 using 49 dairy operations, with four being custom calf grower operations (Zwald et al., 2007). In both data sets, once calves were beyond the weaning phase, daily feed cost per heifer increased. Not because the feed sources progressively became more expensive per kg, but because maintenance costs progressively increase with more BW resulting in less nutrients being available for growth until maintenance needs were met first. This principle is too often not recognized.
Conclusions

- Feed efficiency (FE) is subject to many variables in growing dairy heifers.
- FE does not take into account height increase which is especially critical in dairy heifers less than 6 mo old. Height increase is curvilinear as opposed to BW increase which is primarily linear.
- Typically, younger heifers with less BW are the most efficient in converting nutrients to BW. Consequently, they typically have lower DMI/gain FE.
- Higher forage rations typically result in higher DMI/gain FE.
- Because of so many variables affecting FE, it is difficult to compare FE across studies or across operations. Thus, it is better to compare FE within a study or within an operation.
- Ultimately, economic FE is most critical as long as desired growth is achieved without problems.

References


Hoard’s Dairymen. 2000. Real herds, real heifers…Here’s the low down on daily growing costs. Pages 302-303. April 25,


GENETIC SELECTION IN DAIRY COWS USING RESIDUAL FEED INTAKE AND OTHER FEED EFFICIENCY MEASURES

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Summary

There is renewed interest in selecting for cows with enhanced feed efficiency due to a recent spike in feed costs. While this interest has focused discussion on feed efficiency, a historical perspective suggests that past genetic selection practices have done much to improve feed utilization in the dairy industry by encouraging higher yield and a subsequent reduction in the size of the national dairy cow population. Selecting for moderation in cow body size is a more recent development that is intended, in part, to limit feed intake. While simultaneous selection for higher yield and moderation in cow body size are expected to improve feed efficiency, direct selection may help improve feed utilization traits more rapidly. The concept of feed efficiency is straightforward on its surface, but there are multiple ways to define feed efficiency. Dry matter efficiency and other measures of gross feed efficiency (generally defined as yield / intake) are easy to calculate and recognized by those outside of dairy cattle genetic research. Such measures are generally correlated with higher yield and smaller body size, which makes comparisons for efficiency among cows at different yield and body weight problematic to some researchers. Gross efficiency is also inflated by early lactation body tissue mobilization. Residual feed intake is independent of yield, body weight, and (sometimes) body condition. Gains in parameters such as digestive efficiency, or lower maintenance costs at equivalent body weight are the theoretical goals of selecting for residual feed intake, but have not been demonstrated in practice. It is also possible to have an improvement in residual feed intake, but an actual increase in the amount of feed required to produce a unit of milk. Heritability estimates are generally higher for dry matter efficiency than for residual feed intake and both measures of efficiency can be unfavorable correlated with reproductive performance and other economically important traits if not formulated properly. Recently, measures of residual yield have been considered. This is similar to the concept residual feed intake, except that yield is regressed in intake, body weight, and body condition levels. Regardless of the definition we prefer for feed efficiency, underfeeding of cows with high genetic merit for yield limits feed efficiency. This is particularly true for large cows and those that naturally carry higher levels of body condition. Direct selection for feed utilization is now closer to becoming feasible due to the advent of genomic selection techniques that will allow selection for novel traits that we cannot measure on a whole-population basis. This should be viewed as a refinement of past efforts to improve feed efficiency and not a new direction of selection in our dairy cattle populations.

Current and historical selection for feed efficiency

Rising feed costs have focused efforts on developing selection opportunities to improve feed efficiency. However, it is important to recognize that selection for feed efficiency in the dairy industry is well established and has been extremely successful. Most improvement in feed efficiency has come through selection for higher yield, with more recent effort to limit excessive feed intake. It was recently estimated that the current dairy production requires 23% of the feedstuffs to produce an equivalent amount of milk when compared to the 1944 dairy industry (Capper et al., 2009). Nearly all of this gain in
feed efficiency is due to higher output per cow, which has led to dramatic reductions in the number of dairy cows and replacements.

Efforts to curb further increases in cow intake rely on indirect selection against body weight. The Lifetime Net Merit index currently places 6% of its emphasis on limiting increase in body size and has placed negative weight on body size since 2000 (Cole et al., 2010). The index places weight on milk, fat, and protein yield in proportion to their projected economic value minus associated changes in feed intake. Simultaneous selection for higher yield and reduced feed expenses is effectively selection for a higher income over feed cost. While this type of indirect selection has proven extremely successful, it is generally believed that direct selection for feed utilization traits would allow faster genetic improvement in efficiency.

Efforts to select directly for feed efficiency are limited by the lack of feed intake measures on individual cows. Genetic improvement for traits such as yield has been facilitated by the collection of phenotypic records on a large proportion of the dairy cow population. Such an effort would be economically and practically infeasible for feed intake. However, recent advances in genomic selection offer hope for an alternative approach where feed intake is measured on a smaller number of individuals in order to develop genomic selection tools.

Definitions of feed efficiency

Defining feed efficiency is not a trivial issue. Gross feed efficiency, commonly referred to as dry matter efficiency (DME) in the US, is generally expressed as a ratio of energy corrected yield to dry matter intake. DME is the most widely recognized measure of feed efficiency and is used by nutritionists and other consultants in the dairy industry. It is not, however, a clear indicator of digestive efficiency or the efficiency of energy use for basic metabolic function because it is highly correlated with yield and body size. This complicates the comparison of cows at different yield and body weights. A more practical problem when evaluating DME is that early lactation yield is supported by body tissue mobilization. A cow with high DME may not be efficient, but simply mobilizing a relatively large proportion of body condition. Such a cow may actually be very inefficient because more energy is required to deposit body tissue than is available to support subsequent yield. In one recent study, cows were evaluated for DME before and after adjustment for BCS and changes in BCS (Vallimont et al., in press). Failure to account for body condition biased the evaluation of efficiency toward those cows that peaked higher and earlier.

Residual feed intake (RFI) is most commonly derived by regressing feed intake on milk, fat and protein yield in addition to functions of body weight and body weight change. Thus, it is independent of yield and body weight. RFI is the most widely considered measure of feed efficiency in beef cattle production and has been increasingly considered by dairy cattle researchers. Whether RFI reflects true differences in digestive or metabolic efficiency has not been conclusively demonstrated. In a study of Angus cattle (Lines et al., 2009) selected for high RFI (low efficiency) or low RFI (high efficiency) for four generations, the high efficiency line grew at the same rate and consumed less feed that the low efficiency line as predicted. However, there were important differences in the body composition among the lines. The low efficiency animals had more back fat than the high efficiency line, and the researchers concluded that there were no differences between the lines in basal metabolic rate which was the trait researchers had hoped to improve. Body composition has been considered when deriving RFI in some more recent dairy cattle studies (Coleman et al., 2010, Vallimont et al., in press).

Coleman et al. (2010) recently pointed out an additional limitation of RFI in regards to dairy cattle selection schemes. Cows at the same level of production may have equivalent RFI despite large differences in feed intake because body weight is used in the calculation of RFI. Some improvement in metabolic efficiencies may occur, but the amount of feed actually required to produce a unit of milk could
actually increase. This would defeat the purpose of selecting for efficiency. Moreover, a very small and low yielding animal can have a favorable RFI, but would not be an economically efficient cow. The authors derived a measure of residual solids production by regression of milk solids yield on feed intake, body weight, changes in body weight, and body condition. This measure was correlated favorably with yield, gross efficiency and RFI, but needs further development.

**Heritability and relationships with other traits**

Due to the practical limitation of obtaining large amounts of feed intake data on individual cows, estimates of heritability for various measures of feed intake are relatively imprecise. Nevertheless, gross efficiency was generally more heritable in studies that have considered both gross feed efficiency and RFI. Vallimont et al. (*in press*) obtained heritability estimates for gross efficiency that ranged from 0.14 (fat corrected milk yield / dry matter intake) to 0.21 (protein yield / crude protein intake), but only 0.01 for a measure of RFI that accounted for differences in BCS. Van Arendonk (1993) reported heritability estimates of 0.37 for gross efficiency versus 0.19 for a measure of RFI that did not consider BCS. Other heritability estimates for RFI range are as high as 0.69 and generally consider body weight differences but not BCS (Veerkamp, 1998).

A primary difference among various measures of feed efficiency is the manner in which they relate to other traits. RFI is defined to be independent of other measureable traits, though success in achieving that goal can vary. The genetic correlation estimate between DME and yield was recently estimated to be 0.87, whereas DME was negatively correlated with body weight (-0.66) and BCS (-0.70) (Vallimont *et al.*, *in press*). The genetic correlation estimate between RFI and gross efficiency has been estimated to be -0.73 in two studies (Vallimont *et al.*, *in press*; Van Arendonk *et al.*, 1993).

The correlation estimates of DME with yield and body weight suggest that efforts to increase yield while limiting body size will succeed in improving feed efficiency. However, relationships of feed efficiency with other traits of economic importance are critical in developing selection goals that will improve total economic efficiency. We have recently estimated the genetic correlation estimate between DME and days open (0.58), and between RFI and days open (-0.85) to be unfavorable. Such correlations highlight the need for multiple trait selection goals that focus on improving all aspects of economic efficiency and not solely feed efficiency.

**Interactions of feeding management and genetic response**

A factor rarely considered in discussion of feed efficiency is the interaction of feeding management with cow genotype. In a recent evaluation of feed intake data in 11 Pennsylvania tie-stall herds, Dekleva (2010) contrasted selection response in herds with high and low levels of feed refusals. Herds were split into the six herds that had the highest rate of feed refusal and five herds with lowest rate of feed refusal. Milk, fat and protein yield was regressed on sire predicted transmitting abilities for yield. The expectation is a 1 kg increase in yield for every 1 kg increase in PTA. The regression coefficients in the herds with high refusals ranged from 0.87 (fat yield) to 1.23 (protein yield), which indicated feeding practices were allowing cows in those herds to fully express their genetic potential. However, herds feeding to a clean bunk or with low levels of refusals had a significantly lower response with regression coefficients ranging from 0.34 (fat and protein yield) to 0.44 (milk yield).

Dekleva (2010) also evaluated the relationships among body weight, BCS and yield in the same two groups of herds. The genetic correlation estimate between yield and body weight was near 0 in the herds feeding to a high rate of refusals, whereas the genetic correlation was strong and negative (-0.80) in the herds that fed to a low rate of refusals. The ability of a large cow to fully express her genetic potential for yield was more severely impacted by limiting feed availability than for a small cow. Likewise, the
relationship between yield and body condition score was more unfavorable in the low feed refusal herds (-0.21) than in the high feed refusal herds (-0.63).

**The unknowns**

Selection for feed efficiency is more complex when one considers the potential to feed diets that vary in nutrient density and costs at different points during lactation. If body condition can be deposited at low cost near the end of lactation, the tendency to mobilize larger amounts of body tissue in early lactation switches from a feed efficiency liability to a potential economic gain if adverse effects on cow health are not encountered. Many breeders have also suggested that large cows can produce large quantities of milk while eating more low quality, high roughage diets when compared to smaller cows. While this has not been demonstrated and appears unlikely except at extremes milk yield levels, the relationship of body size and economic efficiency could vary depending on diet.

**Conclusion**

Dairy cattle breeders have made tremendous gains in feed efficiency through indirect selection for higher yield and, to a lesser extent, moderation of body size. However, advances in genomic selection may allow for more direct and rapid improvements in feed efficiency. The ideal manner of defining feed efficiency remains elusive at this point, but measures as simple as dry matter intake would enhance the accuracy of current selection for income over feed cost. Rising feed costs encourage efforts to minimize feed wastage and reduce feed refusals. This may reduce response to genetic selection for yield, particularly for larger cows, and result in lower realized feed efficiency. Future genetic selection programs will allow more precise predictions of feed utilization, but should be viewed as a refinement of current genetic selection practices and not a new direction in dairy cattle selection.

**References**


MONITORING FEED EFFICIENCY IN DAIRY COWS USING FAT-CORRECTED MILK PER UNIT DRY MATTER INTAKE

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Introduction

Anyone associated with the dairy industry is painfully aware of the rapid increases in feed costs that have occurred over the last three years. While the milk prices have gradually recovered from the crash of 2009, profitability has not returned as increased feed prices have consumed most of the increased producer income. There are many reasons for the increase in feed prices. Most certainly, the diversion of traditional livestock feeds such as corn into ethanol production along with increased demand for food following the global recession of 2009 are at the top of everyone’s list. While feed costs have traditionally accounted for 50% of the cost of milk production, more recently that number has increased. Do you remember when you could feed the average lactating cow for $3-4? That is certainly is not the case anymore and likely will not be the case unless there is a major change in the demand for feed grains.

In spite of the impact of feed prices on dairy profitability, the dairy industry is the only livestock industry in the U.S. that does not have a standard index of feed utilization efficiency. The beef feedlot, growing and finishing swine, and poultry broiler industries all use feed per gain as their standard index of feeding efficiency. Each industry has common benchmarks for feed efficiency that are used to evaluate farm or feed yard performance. Increased animal performance due to improved genetics, nutrition and management in these industries has resulted in improved feed efficiency over time. This improvement is largely a function of dilution of the growing animal’s maintenance requirements in respect to their total feed requirements. A higher proportion of feed is used for growth and a lower proportion for maintenance.

While feed per gain can be used in growing dairy heifers, it is not appropriate for lactating cows. Recently, there has been interest in the use of fat-corrected milk (FCM) per unit feed dry matter intake (DMI) as an index of feed efficiency in dairy cows. I will refer to as FCM efficiency (FE) from here on. The use of FCM as the numerator in calculating FE makes sense in that it standardizes milk yield on an energy yield basis. It is easily calculated from the milk yield and fat content of a herd or a group within a herd. The most commonly suggested FE formula uses 3.5% FCM as the standard measure of milk production (the numerator) and DMI as the denominator where:

\[
\text{FCM Efficiency} = \frac{\text{FCM}}{\text{DMI}} 
\]

Where: \( \text{FCM} = 3.5\% \text{ fat corrected milk, kg/d (See Equation 5)} \)
\( \text{DMI} = \text{Dry matter intake, kg/d} \)

Most confinement dairies routinely monitor DMI, milk yield and milk composition. Therefore calculation of 3.5% FCM and FE is relatively straightforward. The real questions become: 1) What are the factors that affect FCM efficiency? and 2) Are there benchmarks that dairy producers and nutritionists can use as standards to compare FE in individual herds?

The Feed Efficiency Numerator: Fat-Corrected Milk

Heats of combustion of milk where used by Gaines and Davidson to develop the standard formula for 4% fat-corrected milk (FCM) in 1923. The FCM formula was developed as means to standardize milk
production records on an energy equivalent basis for use in genetic analysis. Milk fat normally accounts for 50% or more of the total energy content in milk and milk fat is by far the most variable of the major milk components both within and across breeds and within a cow’s lactation (Sutton, 1989).

Usually referred to as the Gaines formula, FCM was derived from the caloric values of 4.09 and 9.28 kcal/g respectively for solids-non-fat and milk fat that had previously been determined by Stocking and Brew (1920). Gaines and Davidson (1923) found that milk fat could be used as a predictor of the heat of combustion [H] of milk. Remarkably, the caloric value they used for milk fat is nearly identical to the value (9.29 kcal/g) used by the NRC (2001). The use of a constant caloric value (4.09 kcal/g) for nonfat milk solids assumed that there was a constant ratio among individual nonfat solids components (protein, lactose, and ash). Ash has caloric value of 0, while lactose and crude protein have values of 3.95 and 5.45 kcal/kg, respectively (NRC, 2001). Based on these [H], a milk with an ash, lactose, and crude protein content of 0.70, 4.85, and 3.20%, respectively would have a [H] of 4.18 kcal/g. This value is slightly greater than the value of 4.09 kcal/g used in deriving the Gaines formula. In fact after deriving the FCM formula Gaines and Davidson (1923) found that the actual energy concentration predicted from their FCM equation under predicted milk energy by 3%.

While final derivation of the Gaines formula used milk energy values expressed as kcal/quart, I have converted their equations to kcal/kg to illustrate how the equation predicts the energy concentration in milk (M):

\[ E \ (\text{Mcal/kg}) = 109.21M \ (2.66 + f) \]  
\[ \text{Where:} \quad E = \text{heat of combustion of milk} \]  
\[ M = \text{amount of milk produced} \]  
\[ f = \text{milk fat\%} \]

For milk containing 4% fat:

\[ E \ (\text{Mcal/kg}) = 109.21M(2.66 + 4) \]  
\[ = 109.21M \times 6.66 \]  
\[ = 727 \text{ kcal/kg} \]  
\[ = 0.727 \text{ Mcal/kg} \]

To standardize milk production to a constant energy value of milk with 4% fat:

\[ E' = \frac{\text{total energy value of the entire quantity of milk}}{\text{energy value of 1 kg of 4 percent milk}} \]  
\[ = \frac{109.21M \ (2.66 + f)}{109.21M \ (2.66 + 4)} \]  
\[ = \frac{2.66M + Mf}{6.66} \]  
\[ = \frac{2.66M + 100F}{6.66} \]  
\[ = 0.3994M + 15.15F \]

Where:  
\[ M = \text{kg milk} \]  
\[ F = \text{kg milk fat} \]

After Gaines and Davidson (1923) did same exercise using milk energy equations developed by others and finding similar relationships, they decided round their coefficients to make life simpler such that the final equation for 4% FCM became:

\[ 4\% \text{ FCM} = 0.4M + 15F \]
The formula stuck! Since the coefficients were so easy to remember “FCM equals 0.4 times pounds of milk plus 15 times pounds of fat”, the 4% FCM equation quickly became ubiquitous in the dairy science community and has been used for the last 90 years.

One item that I mentioned earlier was that Gaines and Davidson found that the predicted energy value of one unit of 4% milk predicted from Equation 4 was low. Realizing that their value was about 3% low compared with others (0.727 vs. 0.749 Mcal/kg), they assumed their number was wrong. So they increased (fudged) their number to conform with other results. Thus the [H] or net energy NE₁ for one kilogram of 4% FCM became 0.749 (0.75) Mcal.

Later on as our dairy cows improved and milk production increased and correspondingly, average milk fat percent declined, someone (We do not know who?) decided that it would be more appropriate standardize records to 3.5% FCM. Derivation of the 3.5% FCM formula uses the same process:

\[
E' = \frac{\text{total energy value of the entire quantity of milk}}{\text{energy value of 1 kg of 3.5 percent milk}} = \frac{109.21M (2.66 + f)}{109.21M(2.66 + 3.5)} = \frac{2.66M + Mf}{6.16} = \frac{2.66M + 100F}{6.16} = 0.4318M + 16.23F
\]

In contrast to the 4% FCM formula the 3.5% FCM coefficients are not so easy to remember. To keep it straight, I have them posted on a tack board in front of my desk when I need them. Using Gaines and Davidson’s (1923) 3% fudge factor, the net energy of one kilogram of 3.5% FCM would be 0.692 Mcal. For the most part the Gaines formula is adequate for predicting milk energy. However, one must remember that it does not account for changes in the proportions of milk lactose, protein, and ash as the energy contribution of those constituents are indirectly predicted from milk fat content. This approach is probably appropriate for breed differences in nonfat milk solids but not among cows within breed or for the changes in milk protein and fat that occur during a lactation. Tyrrell and Reid found that the Gaines formula under predicted milk energy when milk fat was low (< 3%) and then developed a new solids corrected milk (SCM) equation with the same energy concentration (0.75 Mcal/kg) as the Gaines formula to overcome this problem. Similarly, DHIA uses energy corrected milk (ECM) derived from regression equations developed by Tyrrell and Reid (1965) to standardize lactation records. While somewhat better than the Gaines formula, these equations also use some of the same assumptions which create small but inherent errors. In the end, these formulas have not gained the same usage that the 3.5% FCM formula has for calculating dairy feed efficiency.

The Feed Efficiency Denominator: Dry Matter Intake Effects

Cows that eat more will give more milk and as intake and milk production increases FE increases. The reason for the improved FE is that a larger and larger portion of the cow’s feed intake is being used for productive purposes and a smaller proportion for maintenance. The maintenance dilution effect is illustrated in Figure 1-A where the intake effects on diet digestibility has not been adjusted for feed intake. Each increment of feed above maintenance results in an equal increment in 3.5% FCM and FE increases from 1.23 to 1.96 as intake increases from 1X to 5X maintenance.

Unfortunately increasing feed intake also affects diet digestibility. In this case about the expected decline (NRC, 2001) is about 3 digestibility units or 0.03 Mcal/lb net energy for lactation (NEₐ) for each multiple of maintenance. This is illustrated both numerically and graphically in Figure 1-B. At 1X maintenance the diet has a NEₐ of 0.77 Mcal/lb while at 5X maintenance, the diet has an NEₐ of 0.64Mcal/lb. Each increment of feed above maintenance results in a smaller increase in 3.5% FCM. Here, FE increases from 1.12 to only 1.54 as intake increases from 1X to 5X maintenance. Note that there is
little improvement in FE at greater than 4X maintenance feeding. This response illustrates why high milk production does not necessarily lead to huge increases in FE.

Figure 1. Unadjusted and adjusted 3.5% FCM production and 3.5% FCM/DMI in response to increasing feed intake from 1 to 5X multiples of maintenance.

<table>
<thead>
<tr>
<th>DMI, lb/d (X Maint)</th>
<th>NE\textsubscript{l} Mecal/Mcal/lb</th>
<th>3.5% FCM, lb/d</th>
<th>FCM/DMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 (1)</td>
<td>10 (.77)</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>26 (2)</td>
<td>20 (.77)</td>
<td>32</td>
<td>1.23</td>
</tr>
<tr>
<td>39 (3)</td>
<td>30 (.77)</td>
<td>64</td>
<td>1.64</td>
</tr>
<tr>
<td>52 (4)</td>
<td>40 (.77)</td>
<td>96</td>
<td>1.84</td>
</tr>
<tr>
<td>65 (5)</td>
<td>50 (.77)</td>
<td>127</td>
<td>1.96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DMI, lb/d (X Maint)</th>
<th>NE\textsubscript{l} Mecal/Mcal/lb</th>
<th>3.5% FCM, lb/d</th>
<th>FCM/DMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 (1)</td>
<td>10 (.77)</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>26 (2)</td>
<td>19.1 (.74)</td>
<td>29</td>
<td>1.12</td>
</tr>
<tr>
<td>39 (3)</td>
<td>27.4 (.70)</td>
<td>56</td>
<td>1.43</td>
</tr>
<tr>
<td>52 (4)</td>
<td>34.8 (.67)</td>
<td>79</td>
<td>1.53</td>
</tr>
<tr>
<td>65 (5)</td>
<td>41.3 (.64)</td>
<td>100</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Assumes a 1375 lb consuming a diet with and 1X multiple of maintenance NE\textsubscript{l} value of 0.77 Mcal/l feed DM (NRC, 2001) where diet digestibility declines 3 percentage units per multiple of maintenance feeding.

What are the Benchmarks for FE?

In order to establish benchmark FCM feed efficiencies, the published 50\textsuperscript{th} percentile milk production data from 2009 DHIA summary for the Holstein and Jersey breeds (AIPL, 2010) were used. Lactation curves for milk, milk fat percent, and 3.5% FCM were generated for both breeds using Wood’s (1969) formula with adjusted coefficients as reported by Dunlap et al. (2000) for Holsteins. These coefficients were also used for the Jersey breed since more recent coefficients specific to Jerseys are not available. Dry matter intake was predicted using the NRC (2001) intake prediction formula and along the lactation curve based on the predicted milk production and milk fat percent. The proportion first, second, and third or greater parity cows were assumed to be 34, 27, and 39%, with bodyweights (BW) of 536, 582, and 626 kg for Holsteins, respectively (Dunlap et al., 2000). The respective BW used for Jerseys were 395, 427, and 454 kg. Feed efficiency was then calculated as in Equation 1.

Figure 2 shows parity and days-in-milk (DIM) effects on FE in a 2009 50\textsuperscript{th} percentile Holstein herd with a 305 day lactation average of 9820 kg (21,629 lb). By far that largest factor affecting FE is stage of lactation where FE declines from approximately 2.25 at the beginning of lactation to about 1.30 at the end of lactation. The rapid decline in FE with DIM is due to two factors: 1) Cows are in a negative energy balance at the beginning of lactation such that a portion of the milk produced is due to energy from tissue mobilization and not from feed; and 2) feed intake lags behind the lactation curve and peaks later on making the denominator (DMI) component of the FE equation larger and FE smaller. Once peak DMI is achieved, FE declines linearly at about 100 DIM. While there is a parity effect where mature cows have higher FE than first lactation cows, this is largely due to differences in milk production and to a lesser extent, the fact that first parity cows have “flatter” lactation curves.

Figure 3 shows parity and days-in-milk (DIM) effects on FE in a 2009 50\textsuperscript{th} percentile Jersey herd with a 305 day lactation average of 6774 kg (14,920 lb). Similar to Holsteins, there is a rapid decline with DIM. Here the advantage of 3\textsuperscript{rd} lactation vs. 2\textsuperscript{nd} lactation is reversed. This may be more due to the effect of BW on predicted feed intake in the NRC 2001 equation rather than a real advantage with 2\textsuperscript{nd} parity cows. Remember that the NRC (2001) was primarily based on Holstein cows. Just as with Holsteins, DIM is the most important factor affecting FE. In comparing the two breeds, average FE across a lactation for a 50\textsuperscript{th} percentile Jersey herd was 1.45 as compared with 1.49 for the 50\textsuperscript{th} percentile Holstein herd. Since the
reduced Jersey effect is primarily due to disparity in the 3rd lactation, I doubt that there is a real breed effect as the values during the 1st and 2nd parities were similar.

Figure 2. Parity and days-in-milk effects on 3.5% FCM per dry matter intake in 2009 50th percentile Holstein herds with DHIA 305 day lactation averages of 8980 kg (21,769 lb) milk.

Figure 3. Parity and days-in-milk effects on 3.5% FCM per dry matter intake in 2009 50th percentile Jersey herds with DHIA 305 day lactation averages of 6774 kg (14,902 lb) milk.
**Production Level Effects on FE**

Figure 4 shows the effect of production level on FE using the 30th through 90th percentile Holstein herds from the AIPL (2009) summary. These herds had a range in 305 day lactation yields from 19,717 to 26,190 lb milk. The corresponding average daily 3.5% milk production in those herds would be approximately 66, 72.5, 78.6, and 87.5 lb per cow per day. Expected average feed intakes were respectively 45.1, 47.1, 49.1, and 51.9 lb. I would caution that the feed intake estimates may be low, especially at the higher end of production, since we assumed similar BW for all herds. Generally, herds with higher production also have larger cows which would increase feed consumption.

The FE at 150 DIM increased from 1.42 to 1.62 with increasing herd production. The curves showing the change in FE with DIM were nearly parallel suggesting a linear increase with increasing lactation yield. This suggests a standardized FE should be adjusted for level of milk production. In this example the change in FE was roughly a 0.01 FE units per lb 3.5% FCM meaning that comparisons of FE data from year-to-year should include an adjustment for milk production.

**Figure 4. Milk production effects on 3.5% FCM per dry matter intake in 2009 30th, 50th, 70th, and 90th percentile Holstein herds with DHIA 305 day lactation averages of 8952 9820, 10672, and 11890 kg (19,717, 21,629, 23,506, and 26,190 lb).**

**150 Day Feed Efficiency**

Because of the rapid decline in FE with increasing DIM, evaluating overall FE in cows in very early lactation (<60 DIM) probably has little meaning. So much of the high FE in early lactation is due to use of body tissue energy in support of milk production that FE at that stage of lactation may be a meaningless statistic. Also you would not necessarily want to have groups of early lactation cows that have an extremely high FE. That might reflect a greater loss of body condition resulting in poor feed intake and possibly subclinical or clinical ketosis.
As shown in Figures 2-4, once milk production and more importantly DMI peaks by 100 DIM, FE declines linearly for the remainder of lactation. This makes it possible to determine a DIM adjusted FE for a herd or group. DHIA calculates 150 day milk for comparing herd production on a month-to-month basis where daily milk production from individual cows cow is adjusted to a constant 150 DIM. This statistic is very useful for evaluating feeding and management changes in herd as it adjusts for the known effects of stage of lactation on milk production. Similarly a 3.5% FCM efficiency adjusted to a constant DIM would also be valuable in evaluating feeding changes that affect FE.

Using curve pealing techniques, it was found that the effect of DIM could be modeled well by an equation consisting of both linear and exponential decay components (model not shown). More importantly, it was also found that once the linear portion of the decline in FE occurred at 100 DIM, the percent decline was similar, about 0.1% per day. Conversion of actual FE to 150d FE is done by subtracting 150 from the actual DIM from 150 and correcting FE up or down by 0.1% per day.

Table 1 illustrates the correction to 150d FE for herds or groups of cows with varying DIM. Cows in Herd 1 averaging 125 DIM had the greatest measured FE (1.47) while those in Herd 4 at 200 DIM the lowest measured FE (1.43). When adjusted to a constant 150 DIM (150d FE) feed efficiency was actually greatest in Herd 4 at 200 DIM (1.502). In addition to the DIM adjustment, the expected 150d FE within a herd should also be adjusted for milk production. Herds with greater production should be expected to have a greater average FE. The suggested adjustment taken from above would be .01 FE units per lb 3.5% FCM production. Similar to the use of 150 day milk, 150 day FE could be used to evaluate feeding and management changes that affect feeding efficiency.

Table 1. Adjustment of measured FE (3.5% FCM/DMI) to a 150 day FE.

<table>
<thead>
<tr>
<th>Herd</th>
<th>Days in Milk</th>
<th>Measured FE (3.5% FCM/DMI)</th>
<th>DIM minus 150</th>
<th>% Change (Difference *0.1)</th>
<th>Adjustment Factor</th>
<th>150d Adjusted FE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125</td>
<td>1.47</td>
<td>-25</td>
<td>-2.50</td>
<td>0.975</td>
<td>1.433</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>1.44</td>
<td>0</td>
<td>0.00</td>
<td>1.000</td>
<td>1.440</td>
</tr>
<tr>
<td>3</td>
<td>175</td>
<td>1.46</td>
<td>25</td>
<td>2.50</td>
<td>1.025</td>
<td>1.497</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>1.43</td>
<td>50</td>
<td>5.00</td>
<td>1.050</td>
<td>1.502</td>
</tr>
</tbody>
</table>

Feed Effects on FCM Efficiency

There are numerous examples in the literature of feeding factors that can influence FE. On the dairy farm, effective management of feed resources may be one of the most important tools to improve feed utilization. Avoiding excessive handling of feeds and ensuring proper storage to reduce shrink losses improves farm FE. Proper silo management pays huge rewards in terms of silage storage losses. Proper feed bunk design and feeding management can reduce feed losses at the feeding row. Close attention to feed refusals such that they do not exceed 2-3% of the total amount fed reduces the loss of valuable dairy cow TMR. Even though cow feed refusals are typically is fed to pregnant heifers so they are not a total loss, the cost of the dairy cow ration is considerably greater than that for the heifer ration. That said, I will focus on specific feeds and feed additives that can impact FE.

Monensin Effects

Monensin has been used in the beef industry as a feed efficiency enhancer for 40 years. It was approved for use in lactating dairy cows in December 2005. In a meta-analysis of studies with lactating dairy cows Duffield et al. (2008) reported weighted mean monensin responses of -0.3 and +0.7kg/d for DMI and milk production, respectively. Unfortunately, FE was not reported nor was there sufficient information to calculate FE from that report. Perhaps the largest coordinated monensin experiment with
lactating dairy cows involved 9 universities with 858 cows that was first reported by Symanoski et al. (1999). Feed efficiency as measured by 3.5% FCM/DMI increased from 1.50 to 1.56 at the highest level of monensin feeding. These responses would have been even greater had milk fat test been maintained with monensin feeding. Fat test has and continues to be a concern among dairy producers with monensin use. Anticipated FE responses would be about .06 units when feeding 300 mg/d monensin to dairy cows.

Table 2. Feed intake, milk production, and 3.5% FCM feed efficiency responses to monensin.

<table>
<thead>
<tr>
<th>Monensin, g/ton:</th>
<th>0</th>
<th>11</th>
<th>15</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter intake, lb/d</td>
<td>43.9</td>
<td>43.4</td>
<td>42.8</td>
<td>42.3</td>
</tr>
<tr>
<td>Milk yield, lb/d</td>
<td>65.0</td>
<td>66.7</td>
<td>66.8</td>
<td>67.5</td>
</tr>
<tr>
<td>Milk fat, %</td>
<td>3.65</td>
<td>3.53</td>
<td>3.49</td>
<td>3.38</td>
</tr>
<tr>
<td>3.5% FCM milk (lb)</td>
<td>66.1</td>
<td>66.8</td>
<td>66.7</td>
<td>66.0</td>
</tr>
<tr>
<td>3.5% FCM/DMI</td>
<td>1.50</td>
<td>1.54</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>Energy efficiency (%)</td>
<td>--</td>
<td>+2.0</td>
<td>+2.5</td>
<td>+4.0</td>
</tr>
</tbody>
</table>

1 Grams per ton of total mixed ration dry matter. Corresponding amounts of monensin using treatment means are: 0, 238, 321, and 465 mg per cow per day.

**Dietary Protein Effects**

In the 1970’s and 1980’s there were a series of energy metabolism experiments conducted at both USDA-Beltsville and the University of New Hampshire on the effects of dietary crude protein (CP) on digestibility and milk production. In those studies increasing CP increased digestibility and milk production, especially in diets containing less than 15% crude protein. These results were most likely due to deficiencies in rumen degradable protein required to optimize rumen microbial growth and rumen fermentation. More recently, Kalscheur et al. (2006) measured the production responses in cows fed diets ranging from 12.3 to 17.1% CP where rumen undegradable protein was held nearly constant (5.5 to 5.9%) and rumen degradable protein ranged from 6.8 to 11.2%. The results from that experiment and those of Holter et al. (1982) are shown in Table 1.

Table 3. Summary of diet crude protein effects on 3.5% FCM feed efficiency

<table>
<thead>
<tr>
<th>Study</th>
<th>Diet crude protein, %</th>
<th>Dry matter intake, kg/d</th>
<th>3.5% FCM, kg/d</th>
<th>FCM/DMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalscheur et al., (2006)</td>
<td>12.3</td>
<td>13.9</td>
<td>15.5</td>
<td>17.1</td>
</tr>
<tr>
<td></td>
<td>20.5</td>
<td>21</td>
<td>21.2</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>32.9</td>
<td>33.4</td>
<td>34.9</td>
<td>35.9</td>
</tr>
<tr>
<td></td>
<td>1.604</td>
<td>1.592</td>
<td>1.649</td>
<td>1.679</td>
</tr>
<tr>
<td>Holter et al. (1982)</td>
<td>11.0</td>
<td>13.7</td>
<td>15.7</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>17.5</td>
<td>18.3</td>
<td>18.7</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>22.8</td>
<td>28.2</td>
<td>31.2</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>1.309</td>
<td>1.543</td>
<td>1.670</td>
<td>1.601</td>
</tr>
</tbody>
</table>

In both experiments, diets containing less that 15.5% CP had reduced FE. There was no consistent response in FE to feeding more that 15.5% CP. In the study by Holter et al. (1982) diet DM digestibility increased linearly from 57.2 to 68.2% with increasing CP. Holter et al. (1982) also reported the results of a 2nd experiment where dietary CP ranged from 13.8 to 20.9% of diet DM. Dry matter digestibility increased from 68.6 to 76.3% but there were no FE responses. I would conclude that protein
effects on FE are apparent when diet RDP is less than required, particularly when less than 9% of diet DM. Expected FE responses would be on the order of 0.015 to 0.03 units per percentage unit CP up to 15.5% diet CP or 9% RDP in the ration DM.

**Dietary Fat Effects**

Because of its increased energy density, dietary fat should improve FE in proportion to the difference between fat source NE\textsubscript{f} concentration and the NE\textsubscript{f} concentration the current diet being fed. Assuming that fat does not change the energy partition between tissue and milk production, the improvement in FE can occur in one of two scenarios: 1) Added fat maintains NE\textsubscript{f} intake and 3.5% FCM output while reducing DMI; or 2) Added fat maintains DMI while increasing NE\textsubscript{f} which is used to increase 3.5% FCM. Alternatively, the response could be a combination of Scenarios 1 and 2. Table 1 shows theoretical outcome of Scenarios 1 and 2 using the substitution of 2% additional dietary fat in a diet fed to mature Holstein cows producing 35 kg/d FCM, eating 23.6 kg/d DMI of a diet containing 1.55 Mcal NE\textsubscript{f} /kg DM and a baseline FE of 1.48. The published NE\textsubscript{f} values of various fat sources from the NRC (2001) were used in the calculations.

If fat addition causes feed intake to decrease and milk production is constant (Scenario 1), FE increases by 0.06 to 0.07 units depending on the fat source and on a percentage basis, by 3.8 to 5.0 percentage units. However, if DMI is increased causing energy intake and milk production to increase (Scenario 2), FE is increased by 0.16 to 0.20 units and on a percentage basis, by 12 to 13 percentage units. The reality probably lies somewhere in the middle between no change in feed intake and no change in milk production. However, the addition of dietary fat theoretically could increase FE quite dramatically depending on its effect on DMI and milk production. The expected response to dietary fat would be on the order .03 to .10 FE units per percentage unit fat addition.

**Table 4. Theoretical changes in 3.5% FCM feed efficiency with substitution of 2% fat in the diet.**

<table>
<thead>
<tr>
<th>Diet/Fat Source:</th>
<th>Basal Diet</th>
<th>Ca Soaps</th>
<th>Hydrolyzed Tallow</th>
<th>Tallow</th>
</tr>
</thead>
<tbody>
<tr>
<td>3X NE\textsubscript{f}, Mcal/kg</td>
<td>1.55</td>
<td>5.02</td>
<td>5.41</td>
<td>4.53</td>
</tr>
<tr>
<td>DMI ↓, NE\textsubscript{f} Intake ↔, 3.5% FCM ↔</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCM, kg/d</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>23.6</td>
<td>22.6</td>
<td>22.5</td>
<td>22.7</td>
</tr>
<tr>
<td>3.5% FCM/kg DMI</td>
<td>1.48</td>
<td>1.55</td>
<td>1.56</td>
<td>1.54</td>
</tr>
<tr>
<td>Change in 3.5% FCM/kg DMI</td>
<td>0.07</td>
<td>0.07</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>% Change in 3.5% FCM/kg DMI</td>
<td>4.5</td>
<td>5.0</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>DMI ↔, NE\textsubscript{f} Intake ↑, 3.5% FCM ↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCM, kg/d</td>
<td>35</td>
<td>39.3</td>
<td>39.7</td>
<td>38.7</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>23.6</td>
<td>23.6</td>
<td>23.6</td>
<td>23.6</td>
</tr>
<tr>
<td>3.5% FCM/kg DMI</td>
<td>1.48</td>
<td>1.66</td>
<td>1.68</td>
<td>1.64</td>
</tr>
<tr>
<td>Change in 3.5% FCM/kg DMI</td>
<td>0.18</td>
<td>0.20</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>% Change in 3.5% FCM/kg DMI</td>
<td>12.2</td>
<td>13.4</td>
<td>10.7</td>
<td></td>
</tr>
</tbody>
</table>

**Dietary Cation Anion Difference (DCAD) Effects**

Ruminants typically are fed diets with dietary cation anion differences (DCAD) ranging from 250 to 450 meq/kg DM. Most of the active cation is potassium as forages are a rich source of this element. Added sodium typically comes from sodium bicarbonate (Salt, NaCl is cation anion neutral). Hu and Murphy (2004) summarized the literature responses to increasing DCAD in dairy cows and found
maximal DMI and 4% FCM at 400 and 490 meq/kg diet DM, respectively. We reported responses to added potassium (Erdman et al., 2008) in an experiment with 45 Holstein cows during the first 20 weeks postpartum. This experiment was designed to examine the effects of mineral supplementation in corn silage based diets as compared to 50:50 alfalfa hay-corn silage diets. In that study, potassium carbonate and calcium carbonate were added to a corn silage (corn silage + DCAD) diet to match the potassium and calcium concentrations in the alfalfa hay-corn silage diets. We found a 0.14 unit increase in FE with potassium addition that raised the dietary DCAD in the corn silage based diet from 251 to 336 meq/kg DM (Table 4). White et al. (2008) reported similar results in a study which raised the DCAD from 250 to 420 meq/kg using potassium carbonate. Using the equations for DMI and FCM responses to dietary DCAD from the meta-analysis of Hu and Murphy (2004), the calculated FE by increasing DCAD from 250 to 400 meq/kg DM was increased from 1.31 to 1.36.

Using principal components analysis on the dataset of Hu and Murphy (2004), we (Erdman unpublished, 2010) found that FCM increases in response to DCAD using either sodium (bicarbonate) or potassium (carbonate or bicarbonate). With sodium the increase in FCM was associated with an increase in feed intake. However, with potassium, the increase in FCM was not associated with increased feed intake. This suggests that potassium is potentially stimulating a change in digestibility which may correspond to the marked improvement in FE with potassium supplementation (Erdman et al., 2008; White et. al., 2008). The expected response to dietary potassium would be on the order of 0.07 to 0.15 FE units depending on the basal DCAD concentration.

Table 4. DCAD Effects on FE in corn silage based diets (Adapted from Erdman et al., 2008)

<table>
<thead>
<tr>
<th>Item</th>
<th>Alfalfa Hay-Corn Silage</th>
<th>Corn Silage + DCAD</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCAD, meq/kg DM</td>
<td>281</td>
<td>251</td>
<td>336</td>
<td>---</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>22.0</td>
<td>22.7</td>
<td>20.7</td>
<td>0.52</td>
</tr>
<tr>
<td>Milk kg/d</td>
<td>35.6</td>
<td>35.5</td>
<td>37.8</td>
<td>1.25</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.91</td>
<td>4.32</td>
<td>4.08</td>
<td>0.113</td>
</tr>
<tr>
<td>3.5% FCM, kg/d</td>
<td>37.9</td>
<td>40.4</td>
<td>41.4</td>
<td>1.45</td>
</tr>
<tr>
<td>FCM / DMI</td>
<td>1.76</td>
<td>1.80</td>
<td>1.94</td>
<td>0.050</td>
</tr>
</tbody>
</table>

References


UNRAVELING THE ROLE OF INFLAMMATION IN EQUINE LAMINITIS:
WHAT WE’VE LEARNED FROM A TOXIC DISEASE MODEL

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Summary

Equine laminitis is a devastating disease of the foot of horses and ponies, affecting animals from all ages, breeds, sexes, and disciplines. Laminitis causes unacceptably high morbidity and mortality in equine populations, with horses and ponies often euthanized on humane grounds due to the intractably painful and chronic nature of the disease. A strong and concerted research effort has been undertaken within the equine research community within the past two decades in an attempt to discover the key pathophysiologic mechanisms underlying laminitis; while previous reports had characterized laminitic lesions as minimally inflammatory (Hunt, 1991), more recent work with several experimental models has revealed the presence and importance of inflammation in equine laminitis. Further investigation, including work to clarify the role of inflammation in laminitis related to endocrine disease, is required to enable veterinarians to make appropriate therapeutic decisions when presented with these difficult cases.

Introduction

Laminitis is a devastating disease of the equine foot that is characterized by failure of the integrity of the structures supporting the distal phalanx (coffin bone) within the hoof capsule (Eades, 2010). While certain breeds have been reported to be predisposed to laminitis (Arabians, Morgans, and ponies, for example; Frank, 2006), the condition can potentially affect all ages, breeds, sexes, and disciplines of horse and pony. The pain and disability suffered by affected horses and the economic losses incurred by the equine industry due to this disease have made laminitis research a stated priority for investigators performing equine veterinary research (American Association of Equine Practitioners, 2009). The condition is a common sequel to a diverse group of primary disease states in adult horses, including alimentary carbohydrate overload, enterocolitis, surgical gastrointestinal disease, septic pleuropneumonia, retained placenta and metritis, insulin resistance, and mechanical overload (Parsons, 2007; Peloso, 1996; Frank, 2010).

Such a diverse range of causes might also suggest a diversity of structural lesions within the hoof capsule of laminitic horses; however, the histologic appearance of the laminae of acutely laminitic horses is remarkably consistent, particularly from horses with laminitis secondary to sepsis. Initially (within 6-12 hours of experimental induction), vascular changes, edema, and evidence of sluggish blood flow through laminar capillaries are noted (Hood, 1993). During this same time point, white blood cells can be observed in the perivascular areas of the laminae as they extravasate into the tissue (Black, 2006). Changes in the histologic appearance of the laminae themselves are also evident by this time frame, with lengthening and flattening of the laminae, deformation of the epithelial cells, and, ultimately, separation of the basal epidermal cells from the laminar basement membrane. This separation is a structural change that precedes detachment of the distal phalanx from its support structures, allowing displacement of the bone within the hoof capsule (rotation and/or sinking; Pollitt, 1996). Through the intensive study of experimentally-induced laminitis, information regarding the roles of vascular dysfunction, extracellular
matrix remodeling/destruction, and inflammation has become available. Recent research efforts have placed emphasis on elucidating the role of the inflammatory response in the hoof in the pathophysiology of experimentally-induced laminitis; the information gleaned from these studies has clarified the importance of inflammation and validated roles of anti-inflammatory medications in the treatment of laminitis, a disease for which very few effective prophylactic or therapeutic treatments exist.

While the precipitating cause(s) of laminitis can vary widely, the clinical signs observed in the evaluation of an affected individual are very characteristic. Affected horses display variable degrees of the following: increased temperature of the hoof capsules, increased amplitude of the peripheral arterial pulse in the palmar/plantar digital arteries, incessant shifting of weight between limbs, responsiveness to hoof testers applied over the toe and periphery of the sole, swelling and/or troughing of the coronary bands, redistribution of weight bearing to the hind limbs, and overt lameness (Stokes, 2010). A scale has been developed to describe the severity of clinical lameness associated with laminitis, which is in common use in both clinical and research settings (Obel, 1948; Table 1). While one or more limbs can be affected more severely than the others, and the front feet often display more severe structural changes than the hind feet (Hood, 1999), laminitis occurring secondary to a systemic insult is commonly held to affect all four feet to some degree (B.S. Leise, unpublished data). This is likely not true for support limb laminitis, which occurs in a single foot associated with excessive weight bearing on a limb.

Table 1. A scale developed to describe the severity of clinical lameness associated with laminitis, which is in common use in both clinical and research settings (Obel, 1948).

<table>
<thead>
<tr>
<th>Obel Grade</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Shifting weight; lameness noted at trot</td>
</tr>
<tr>
<td>II</td>
<td>Lameness noted at walk; forelimb can be easily elevated</td>
</tr>
<tr>
<td>III</td>
<td>Lameness noted at walk; forelimb elevated with difficulty</td>
</tr>
<tr>
<td>IV</td>
<td>Refusal to move, often recumbent</td>
</tr>
</tbody>
</table>

The black walnut extract model

One of two common experimental models used to study equine laminitis, the black walnut extract model was developed following the observation that horses bedded on shavings containing black walnut heartwood often spontaneously developed laminitis (Ralston, 1983). Since the initial observations, the model has been thoroughly characterized and used to delineate the role of inflammation in experimental equine laminitis. To induce laminitis, 2 g/kg of black walnut heartwood extract from a tree harvested in the fall (northern hemisphere) are soaked in 8 liters of water for 12 hours. The shavings are filtered out, and the resultant extract solution is administered to horses via nasogastric tube (Minnick, 1987). Approximately 80% of horses given this material show a response and develop laminitis. The initial clinical evidence of this response includes a drop in white blood cell count in peripheral blood and fever present within 4 hours of administration; responding animals typically become laminitic (Obel grade I) at ∼12 hours, but displacement of the distal phalanx is uncommon with this model (Belknap, 2010).

Hallmarks of inflammation, including migration of white blood cells into tissue from the peripheral blood and elaboration of pro-inflammatory cytokines and chemokines, have been documented in laminar tissue obtained from horses with laminitis experimentally induced with black walnut extract. Gene expression of interleukin-1β (IL-1β), IL-6, and IL-8 in laminar tissue has been shown to be increased at 1.5 hours, 3 hours, and 12 hours (onset of lameness) after administration of black walnut extract (Belknap, 2007; Figure 1).
Given the rapidity with which inflammatory changes are documented in the digital laminae (prior to any signs of lameness), it has been suggested that a pathogen-associated molecular pattern (PAMP)-like molecule(s) contained within the black walnut heartwood is absorbed in the small intestine and delivered hematogenously to the foot. Here, this substance(s) may stimulate innate immune responses through binding pattern-recognition receptors (PRRs), such as Toll-like receptors, as well as participate in activation of peripheral white blood cells (Belknap, 2010). Endothelial activation within the laminar vasculature, with increased expression of chemoattractant chemokines (such as CXCL8 and CXCL1) and adhesion molecules (such as selectins and ICAM-1), leads to increased leukocyte adherence and emigration into inflamed tissue. Dramatically increased laminar gene expression of ICAM-1 and E-selectin have been reported at the 1.5 hour time point in the black walnut model of laminitis (Loftus, 2007); increased laminar expression of CXCL8 and CXCL1 have been reported at this time as well (Loftus, 2007; Faleiros, 2009).

Leukocyte infiltration of tissue is a hallmark of inflammation, and products of activated leukocytes (primarily neutrophils and macrophages) contribute to the inflammatory response. Activated leukocytes elaborate pro-inflammatory cytokines and chemokines, reactive oxygen and nitrogen species, and a diverse array of proteases; all of these can contribute to the tissue damage that occurs during a robust inflammatory response and likely play an important (but as yet incompletely characterized) role in the development of laminitis. Mononuclear cells are present in the laminae of normal horses, and the numbers of CD163 (+) macrophages in the laminae have been shown to increase transiently with the induction of carbohydrate-induced laminitis (Faleiros, 2011b; Figure 2 displays three images of CD163(+) leukocytes within the laminar epidermis [a,c] and laminar vasculature [b] of horses with BWE-induced laminitis).
The carbohydrate overload models

While the black walnut extract model of laminitis has proven very reliable and useful for exploring the molecular events occurring during the development of laminitis, clinical features of horses with BWE-induced laminitis differ somewhat from those that are observed in horses with naturally occurring, sepsis-associated laminitis. For example, horses with BWE-induced laminitis do not typically progress to laminar structural failure, with displacement of the distal phalanx (Belknap 2010); this is common (and devastating) in clinically-affected horses. Further, horses appear to be able to potentially completely recover from BWE-induced laminitis following a single bolus administration; clinically affected horses often progress to the chronic stage, again, often accompanied by structural changes within the hoof capsule. For these reasons, an experimental model that more closely replicated clinical disease was needed; therefore, the models of carbohydrate-overload laminitis were developed. Using either starch (17.6 g/kg of a mixture of 85% corn starch/15% wood flour; Leise 2011) or oligofructose (10 g/kg; Van Eps, 2006) administered as a bolus via nasogastric tube, laminitis can reliably be induced in ~80% of horses. The onset of signs of fever (~8-12 hours) and Obel grade I laminitis (~24 hours) are delayed somewhat compared to the black walnut model. Laminar expression of pro-inflammatory cytokine and chemokine genes (including IL-1β, IL-6, IL-12p35, COX-2, E-selectin, ICAM-1, and IL-8; Figure 3) has recently been shown to be upregulated at the Obel grade I time point in the carbohydrate-overload model; however, in contrast to findings in BWE-induced laminitis, laminar inflammatory mediator production does not appear to be prominent at the developmental stages (i.e., at the onset of fever) in the carbohydrate model (Leise, 2011).

Figure 3. Laminar expression of pro-inflammatory cytokine and chemokine genes (including IL-1β, IL-6, IL-12p35, COX-2, E-selectin, ICAM-1, and IL-8).
Leukocyte infiltration into the laminae of horses with carbohydrate-induced laminitis has recently been investigated; while leukocytes (primarily neutrophils and macrophages) have been shown to emigrate into laminar tissue prior to the onset of epithelial stress and basement membrane degradation in the BWE model (Faleiros, 2009a), this information was not available for carbohydrate-induced disease until very recently. A recent investigation found calprotectin (+) leukocytes emigrating into the laminae at the developmental (onset of fever) time point in the carbohydrate model; the calprotectin (+) laminar leukocyte content was substantially increased at the onset of lameness, as was the content of CD163(+) macrophages (Faleiros, 2011a). Again, animals with carbohydrate-induced laminitis often progress to laminar failure, and since leukocytes infiltrate the laminae prior to this point, it is possible that they (along with the inflammation that frequently accompanies their presence) play a critical role in laminar failure. Inflammation is clearly present and may be pathophysiologically critical in the model of laminitis that most closely resembles clinical disease, creating the opportunity for therapeutic intervention with anti-inflammatory therapy. Further investigation, including clinical trials, is required.

Endocrinopathic laminitis

Laminitis that occurs secondary to endocrine disease in horses (such as that accompanying obesity and insulin resistance or pituitary pars intermedia dysfunction [PPID]) has been reported to be the most common form of the disease in the United States, with 46% of reported cases associated with pasture exposure (USDA-NAHMS, 2000). However, the role of inflammation in the pathophysiology of endocrinopathic laminitis has been poorly characterized to date. With the recent experimental induction of laminitis through infusion of exogenous insulin in normal ponies (Asplin, 2007) and Standardbred horses (De Laat, 2010) and further characterization of experimental models of pasture-associated laminitis, the ability to study the laminar events that occur in endocrinopathic laminitis should advance rapidly. Preliminary data obtained in the author’s laboratory suggest that the laminar lesion of ponies affected with pasture-associated laminitis is minimally inflammatory, with little elevation in the expression of pro-inflammatory cytokine genes apparent in the laminae of ponies administered a dietary carbohydrate challenge for one week (Burns, T.A. and J. K. Belknap, unpublished observations). Furthermore, conditions associated with increased glucocorticoid tone, such as PPID or exogenous corticosteroid administration, have been linked to laminitis; since corticosteroids have potent anti-inflammatory properties when administered at pharmacologic doses, this suggests a less prominent role for inflammation in laminitis occurring secondary to steroid use. Further investigation is necessary to more fully characterize the pathophysiology of endocrinopathic laminitis and what role, if any, anti-inflammatory medications may play in modulation of the disease in this setting.

References


DEATH IN THE FEED ROOM - IONOPHORE TOXICITY IN THE HORSE

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Summary

Ionophore toxicity is a very real and constant threat to the horse population. Errors in feed production and the accidental exposure of horses to contaminated feed or feed intended for other species are the prime methods of poisoning. There are no treatments for this type of poisoning and no ante-mortem diagnostics available. Ingestion is usually fatal, often without clinical signs. Stomach content of suspicious feed should be tested for a definitive diagnosis and chronic Ionophore poisoning should be investigated in cases of poor performance and unthriftiness if other more common causes have been ruled out. In the case of Ionophores, it is up to the quality control of the companies that make our horse feeds and the watchfulness of the owners and barn managers who have their hands on the feed scoop to insure that what we give our horses will not hurt them.

Introduction

There is an old farmer’s saying that “Where there is livestock- there is dead stock”, and it is commonly understood that no matter how rigidly one controls pasture, fencing, feed, water and the hundred other management issues related to owning and caring for animals, some losses will occur. It is fitting that at this conference focusing on “nutritional biosecurity”- essentially the safety of all feeds, hays and products feed to horses, that some attention be paid to a relatively uncommon but very real threat. Ionophore toxicity is a serious problem in the horse and only quality control by feed producers and extreme watchfulness by owners keeps it from becoming more commonly seen. Still cases of Ionophore poisonings are periodically reported throughout the United States and worldwide. Not only are these poisonings almost universally fatal but the fact that they are generally preventable makes this an important topic. It is a concern that should always be with a horse owner or barn manager as they go to the feed room, open their bags of grain or buckets of supplements, start to put food into buckets and possibly begin to poison their horses.

Poisonings in horses associated with dietary causes are generally of two types. There are natural poisons that find their way into the normal diet of the horse and the majority of these are hay and pasture associated. There are man-made poisons and these tend to be most commonly associated with processed grains, bagged feeds, supplements and various food or dietary additives.

It is important to take a moment to define “poison” for the purpose of our discussion. Paracelsus (1493-1541) was a Renaissance physician and botanist and is widely regarded as the “Father of Toxicology”. Paracelsus wrote that “all things are poison and nothing is without poison, only the dose permits something not to be poisonous”. It is important that we remember this as we proceed with a discussion of Ionophores in the horse. Many common and beneficial products, foods and drugs can be poisonous in the wrong situation, wrong species or incorrect dosage. It is unwise to view poisons as only some extremely toxic ingredients that will undoubtedly be found in a bottle with a skull and cross-bones label on it. Poisons are everywhere in our horse’s environment, often doing good work in one area only to become a killer somewhere else. In the food industry we must be able to accept these toxins in the correct dosages and locations and be ever watchful for their presence in other sensitive species and locations.
There are many naturally occurring poisons and many will be discussed at this seminar. The most common natural poisonings in the horse include (in order of highest recorded percentage, though many poisonings are either unreported or a definitive diagnosis is never made so poisoning statistics are somewhat suspect)-

Botulism toxin- commonly found in haylage, silage, alfalfa cubes and any feedstuffs where decomposing animal parts might become mixed into the product.

Mycotoxins- These are secondary metabolites of molds and will be thoroughly discussed in other lectures.

Aflotoxins- Toxins of the mold species Aspergillus are so common and so potent that they are often discussed separately. These toxins occur in hay, vegetation, many field crops and are commonly associated with conditions of high heat and humidity.

Braken fern (Pteridum aquilinum) can be commonly found on any walk through fields and forests throughout parts of the United States and it is poisonous to horses.

Hemlock (Conium maculatum) has a historical significance and most owners know of its poisonous properties but many of these same owners would walk past this plant in the woods and not know of the danger lying so close to their horses.

Tansy Ragwort (Senecio species) is a common weed with wide distribution and is very poisonous to horses.

Locoweed (Astragalus or Oxytropis species) is commonly found in the western United States and, despite its severely toxic effects on horses, it is generally palatable and often consumed.

Oleander (Nerium oleander) is another toxic plant that is well known but still accounts for periodic poisonings in both people and animals.

Red Maple Trees (Acer rubrum) are found commonly throughout pastures in the east. These beautiful trees are very toxic in certain situations and each year equine poisoning as reported.

Water Hemlock (Cicuta species) is another toxic plant that horses have access to and will occasionally consume with deadly results.

Yellow Star Thistle (Centauria species) is another western plant with toxic properties.

Japanese Yew (Taxus species) is an ornamental shrub commonly found around homes and gardens. The leaves are extremely toxic to horses and each year an accidental exposure (and commonly death) occurs when horses gain access to clippings from these plants.

Blister beetles (containing the toxin Cantharidan) are found in cut hay. These particular beetles are found in various areas of the United States (Arizona through Texas and Oklahoma commonly) and they tend to hatch in alfalfa fields. These beetle swarms tend to become baled in the hay and the Cantharidan toxin they contain causes damage to all mucous membranes in the horse with the kidneys being severely affected and often resulting in death of the poisoned horse. Most hay growers have been alerted and educated as to the potential problems with Blister beetles and horse owners have been instructed to purchase only “safe” alfalfa hay and to always check their bales before feeding anyway, yet Blister beetle cases occur yearly (3 horses died in south Georgia in February and a client north of Atlanta had 7 horses affected with 4 deaths last winter) and losses are common.
There are hundreds of other toxic plants, trees and shrubs but these represent the most commonly seen causes of poisonings in the horse.

Man-made causes of dietary poisonings in the horse are generally from Ionophores or other lesser known and lesser used antibacterial agents. Dr. J.O. Hall of the Department of Animal, Dairy and Veterinary Sciences at the Utah State University sums up this type of poisoning by stating “Feed mixing errors and ingestion of feed formulated for other species are the most common means by which poisonings from man-made material occur”. This succinctly and accurately describes the cause of most Ionophore poisonings in the horse. Yet it is important to understand why Ionophores are present in feeds to begin with.

The cow exists in symbiotic harmony with the bacteria present in its rumen. These bacteria digest fibrous plant material via fermentation and make carbohydrates and fatty acids available to the cow. Yet this process is inherently inefficient, 12% of the dietary carbon and energy is converted to methane gas, heat or unusable products and 50% of dietary protein is degraded to ammonia and lost in the urine. Ionophores are components that change the bacterial content of the rumen and change the types of fatty acids produced. They also improve the energy production from the feed consumed which is the prime reason they are included in cattle feeds. (They are also used as coccidiostats in poultry foods). Ionophores increase feed efficiency by 5-10% in cattle and can boost rate of gain by 2-7 %. This is primarily done by increasing carbon and nitrogen retention.

Monensin (crystalline monensin sodium), the most commonly encountered Ionophore, has a combined yearly sales of $150 million and a cost/benefit ratio estimated to save the cattle industry nearly 1 billion yearly. Monensin is a methane inhibitor. It inhibits the production of lactic acid producing bacteria which stabilizes the rumen Ph and monensin reduces protein deamination which decreases nitrogen loss in the urine. These properties combine to increase energy availability, increase nitrogen retention, improve feed efficiency and reduce morbidity and mortality in feedlot cattle by reducing bloat, rumen acidosis and protecting against caustic emphysema.

Though an excellent case can thus be made for the beneficial effects of monensin in cattle feed, the problem now arises because horses are exquisitely sensitive to even minute amounts of this and other ionophores. Toxic doses in the horse can be little as 2-3 mg/kg while cattle can tolerate 10 times this dose. There are many cases documenting Ionophores inadvertently added to horse feeds and supplements. While the dosages may not be high, the extreme sensitivity of the horse makes these situations invariably fatal. There are also reported cases of horses gaining exposure to cattle of poultry feeds with equally poor results. Ionophores alter the transport of ions across cell membranes and therefore disrupt sodium and potassium gradients in the cells of the horse. Cell and tissue failure is associated with mitochondrial damage, decreased ATP formation, myofibril hyper contraction and ultimate necrosis. Tissues highly dependent on energy production are the most severely affected so the myocardium (heart), skeletal muscles and nerves show the earliest and most severe effects of this type of poisoning.

Horses typically show signs of Ionophore poisoning within 12 to 24 hours of ingestion of a toxic dose. The first signs are inappetence or refusal of food. Horses will show signs of colic, muscle weakness and tremors or incoordination. Affected horses will have a high heart rate, difficulty breathing , and will be sweating and possibly passing dark brown to reddish urine (myoglobinuria). There may be pitting edema, jugular pulses and bladder distention/urine retention. These horses will quickly become recumbent and will often die suddenly(within 24 hours). Often there are no observed signs and the owner or barn manager simply finds a dead horse.

When bloodwork can be done, muscle enzyme elevations may be slight and difficult to differentiate from those seen in any recumbent horse. There are no ante-mortem diagnostic tests for the detection of Ionophore toxicity. Thin layer Chromatography can be done on stomach contents in
suspected cases and questionable feed material can also be tested to arrive at a diagnosis. This is important to remember when faced with a case of sudden death and the possibility that Ionophores might be involved. Because many of these cases involve feed processing or supplier errors and often can involve litigation, it is imperative that appropriate samples be taken by licensed individuals and that a “chain of control” is established and maintained for all samples.

Horses surviving acute Ionophore toxicity have evidence of significant heart damage as myocardial tissue is repaired with fibrous tissue which lacks contractibility. Sudden death can be seen in these horses for weeks to months following toxic exposure. These horses are never suited for athletic use and should not be ridden as this myocardial damage is permanent and irreversible.

There are also interesting consequences to low level chronic toxic exposure to Ionophores. Horses that are exposed to such low levels over periods of time may show milder, more subtle signs that can be assigned to any number of other causes. Owners and managers should be aware of these signs, their possible causes and should consider chronic low level Ionophore toxicity when no other causes can be found. Horses affected in this manner may be unthrifty, with poor body condition despite adequate food, appropriate dental care and deworming. They may show poor performance, exercise intolerance and may be associated with a chief complaint of just not doing as well as expected. Owners should be especially concerned and might consider Ionophores if these vague signs are seen in a number of horses at the same facility who may all be exposed to low level ionophores.

Ionophore toxicity is a very real and constant threat to the horse population. Errors in feed production and the accidental exposure of horses to contaminated feed or feed intended for other species are the prime methods of poisoning. There are no treatments for this type of poisoning and no ante-mortem diagnostics available. Ingestion is usually fatal, often without clinical signs. Stomach content of suspicious feed should be tested for a definitive diagnosis and chronic Ionophore poisoning should be investigated in cases of poor performance and unthriftyness if other more common causes have been ruled out. In the case of Ionophores, it is up to the quality control of the companies that make our horse feeds and the watchfulness of the owners and barn managers who have their hands on the feed scoop to insure that what we give our horses will not hurt them.
MYCOTOXIN EFFECTS IN HORSES

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Summary

Mycotoxins are toxins produced by molds and affect horses primarily by consumption of contaminated feed. The most frequent and amply documented mycotoxicoses in horses are caused by fumonisin, aflatoxin, and ergot alkaloids from fescue. Mycotoxins such as deoxynivalenol, zearalenone, T-2 toxin, ochratoxin, and others are also of concern. Research and documentation of mycotoxin effects in horses are limited. Effects may be mild with slight effects on health and performance or effects may be so severe as to cause death. Good management avoids molds and mycotoxins in the feed and environment.

Mold growth

Molds grow and mycotoxins are produced in feedstuffs pre-harvest or post-harvest and during storage, transport, processing or feeding. Mold growth and mycotoxin production are related to plant stress caused by weather extremes, insect damage, inadequate storage practices and faulty feeding conditions. Temperature, water activity and insect activity are primary determinants of mold growth (Coulombe, 1993). A feed can be moldy without the presence of mycotoxins and a feed can contain toxic levels of mycotoxins without being obviously moldy.

The more common toxigenic molds include Aspergillus, Fusarium and Penicillium. Aspergillus species normally grow at lower water activities and at higher temperatures than Fusarium species and therefore, aflatoxin in corn is favored by the heat and humidity, but also the drought stress, associated with warmer climates. The individual Penicillium species have variable growth requirements, but are more likely to grow under post-harvest conditions, in cooler climates, in wet conditions and at a lower pH. Penicillium molds are found in silage, probably because they are acid tolerant. The Fusarium species are important plant pathogens that can proliferate pre-harvest, but continue to grow post-harvest. In corn, Fusarium molds are associated with ear rot and stalk rot, and head blight (scab) in small grains. In wheat, Fusarium is formed with excessive moisture at flowering and early grain-fill stages. In corn, Fusarium graminearum is referred to as a red ear rot and is more commonly formed with a cool, wet growing season and with insect damage. Fusarium ear rots that produce fumonisins are referred to as pink ear rots and vary in their environmental requirements, but are often associated with dry conditions in mid-season followed by wet weather (CAST, 2003). Delayed harvest may also increase mold growth.

Molds can grow in wet spots due to moisture migration when feed moisture contents exceed 12-15%. Field-dried hay which is the basis for many horse diets can be a particular problem for mold growth and mycotoxin formation. Hay with excess moisture because of rain during harvest, baling too wet or improper storage can be a source of mold and mycotoxins. In a review of mold contamination, Yiannikouris and Jouany (2002) indicate that hydrophilic and heat-tolerant species, such as Aspergillus fumigatus and Stachybotrys atrata both predominate in hay harvested and stored in humid conditions. They further confirm that Aspergillus, Penicillium and Fusarium can be contaminants of hay and straw. Scudamore and Livesay (1998) indicate that several other molds may be present in forages.
Molds Cause Disease

Molds (fungi) often from feed, bedding or the housing area can cause infectious disease referred to as a mycosis (Blackford, 1984). Connole, 1990, indicates that the following systemic mycoses have been recorded: adiaspiromycosis, aspergillosis, candidiasis, cryptococcosis, dactylariosis, fusariomycosis, histoplasmosis, miscellaneous mycoses, mycotic abortion and related conditions, zygomycosis, pythiosis, protothecosis and green algal infections. Skin infections caused by fungi such as ringworm may be caused by several different fungi (Connole, 1990). Perhaps the most common agents of systemic mycoses are members of the genus *Aspergillus*. Aspergillosis has been reported most often as occurring in the guttural pouch but it is considered a rare, life-threatening, opportunistic infection (Guillot et al., 1997; Lepage, 2002). *Aspergillus* infections may also occur in the nasal cavity, placental, eye, lung, and chest cavity (Blomme, et al., 1998). A mycosis resulting from other fungi often from the class *Phycomyceta* may be referred to as a phycomycosis, zygomycosis or mucormycosis. According to Fretz and Fischer (1976), a phycomycosis has been described in the skin of the lower limbs and nasofacial area, particularly the nostrils, but also in the nasopharynx and larynx. Austin (1976) indicated that a phycomycosis may also affect the lung, kidney, spleen, liver, intestine and nervous system.

Mycotoxin effects

Mycotoxins are toxic secondary metabolites produced by toxigenic filamentous fungi that cause an undesirable effect (mycotoxicosis) when animals are exposed. Exposure is usually by consumption of contaminated feeds, but may also be by contact or inhalation. Biological effects include liver and kidney toxicity, central nervous system effects, immune suppression and estrogenic effects, to name a few. The primary classes of mycotoxins are aflatoxins, zearalenone, trichothecenes, fumonisins, ochratoxin A and the ergot alkaloids.

Mycotoxins occur frequently at low levels in a variety of feedstuffs and are routinely consumed by animals. Mycotoxins, in large doses, can cause acute health disorders including death. A more likely scenario is to find mycotoxins at lower levels consumed over time and interacting with other stressors to cause chronic problems. Symptoms can be many and variable, making diagnosis difficult. Mycotoxin occurrence and concentrations are variable by year, because of the annual variation in weather conditions and plant stresses known to affect mycotoxin formation (Coulombe, 1993). Worldwide, approximately 25% of crops are affected by mycotoxins annually (CAST, 1989).

The general effects of mycotoxins include 1) reduced intake or feed refusal; 2) reduced nutrient absorption and impaired metabolism; 3) altered endocrine and exocrine systems; 4) suppressed immune function; 5) altered microbial populations in the digestive tract, and 6) cellular death and damage to various organ systems. Horses may exhibit few or many of a variety of symptoms. Young or stressed animals may be most affected; perhaps because their immune systems are already suppressed. Symptoms may include: lethargy, unthriftness, rough hair coat, reduced performance; lower feed consumption; digestive disorders (including colic), intermittent diarrhea (sometimes with bloody or dark manure); increase in incidence or severity of disease, brain damage, hemorrhage, respiratory distress, abortion, and reduced reproductive performance. Symptoms of a mycotoxicosis vary depending on the mycotoxins involved and their interactions with other stress factors.

A diagnosis of a mycotoxicosis is difficult or impossible because of the complex clinical scenario resulting from a cascade of events producing a wide diversity of nonspecific symptoms and which may reflect presence of an opportunistic disease (Schiefer, 1990). The difficulty of diagnosis is increased due to limited research, occurrence of multiple mycotoxins, non-uniform distribution, problems of sampling and analysis and interactions with other stressors. Therefore, diagnosis of a mycotoxin problem becomes a process of elimination and association. Certain basics observations can be helpful (Schiefer, 1990): 1) Mycotoxins should be considered as a possible primary factor resulting in production losses and increased incidence of disease; 2) Documented symptoms in other species can be used as a general guide to
symptoms observed in the field; 3) Systemic effects as well as specific damage to target tissues can be used as a guide to possible causes; 4) Post mortem examinations may indicate no more than gut irritation, edema, or generalized tissue inflammation; 5) Because of the immune suppressing effects of mycotoxins, increased incidence of disease or atypical diseases may be observed; 6) Responses to added dietary adsorbents or dilution of the contaminated feed may help in diagnosis; 7) Feed analyses should be performed, but accurate sampling is a major problem and only a few mycotoxins are commercially analyzed.

Safe levels of mycotoxins

Research and case reports with horses are limited and therefore safe levels of mycotoxins are unknown. Other factors which make it difficult to establish safe mycotoxin levels include, sensitivity differences by animal species, imprecision in sampling and analysis, the large number of potential mycotoxins and interactions with stress factors or other mycotoxins (Schaeffer and Hamilton, 1991). Action, guidance and advisory levels have been established by the FDA primarily to protect public health. Feeds that exceed these levels may be considered by the FDA as adulterated and unfit for use in animal feed (Henry, 2006).

Toxicity of Individual Mycotoxins

**Ergot alkaloids, including fescue toxicity**

Ergot alkaloids are the toxic principle in *Claviceps* toxicity, fescue toxicity, ryegrass staggers and paspalum staggers. Ergotism is one of the earliest recognized mycotoxicoses. In general, ergotism may result in either a gangrenous condition resulting from blood vessel constriction or a nervous condition in animals. With *Claviceps purpurea*, ergot bodies called sclerotia may be visible in small grains or flowering heads of grasses. The sclerotia displace the seed with small black-colored bodies similar in size to the grain. Also ergot producing fungi can grow symbiotically and unseen within the vasculature of the plant. In fescue, *Neotyphodium coenophialum* grows within the plant and produces ergot alkaloids, of which ergovaline and lysergic acid are thought to be the primary toxicants (Strickland et al., 2009). Ryegrass staggers may result from ryegrass infected with the endophyte *Neotyphodium lolii*. Horses in Australia have suffered from ergot toxicity in grasses infected with *Claviceps paspali* (Cawdell-Smith et al., 2010).

There are few reports of *Claviceps purpurea* toxicity in horses. In the U.S., fescue toxicity is the primary ergot toxicity of concern. Symptoms in the horse are noted primarily in late gestation mares and include increased gestation lengths, reduced prolactin, agalactia (little or no milk), increased foal and mare mortality, tough and thickened placentas, weak and dysmature foals, increased sweating during warm weather, reduced serum prolactin and progesterone, and increased serum estradiol levels (Cross, 2009).

**Aflatoxin**

Aflatoxins are a group of extremely toxic, mutagenic, and carcinogenic compounds produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxin metabolites include B1, B2, G1, G2 and several others. Aflatoxin targets the liver causing centrilobular necrosis. Horses affected by aflatoxin show signs of inappetence, depression, fever, tremor, ataxia and cough. Necropsy findings include a yellow–brown liver with centrilobular necrosis, icterus, hemorrhage, tracheal exudates and brown urine (Caloni and Cortinovis 2010). Both Newman and Raymond (2005) and Caloni and Cortinovis (2010) have raised the possibility of an association between aflatoxin and chronic obstructive pulmonary disease. Aflatoxin in naturally contaminated feeds may co-occur with fumonisin, ochratoxin, cyclopiazonic acid, zearalenone and other mycotoxins. Impure aflatoxin produced by culture has been demonstrated to be more toxic than equal amounts of pure aflatoxin (Applebaum et al., 1982). Aflatoxin has been reported toxic over a wide
range of concentrations, 55 ppb to 6,500 ppb (Asquith, 1985). The lowest level of concern may be associated with interactions of stress, other mycotoxins, duration of exposure and other factors. It is generally recommended that horses be exposed to < 20 ppb aflatoxin.

Aflatoxin is more often found in corn, peanuts and cottonseed grown in warm and humid climates and is less likely to be found in forages. The US General Accounting Office has concluded that industry, federal and state programs are effective in detecting and controlling aflatoxin and that it is doubtful that additional programs or limits would reduce the risk of aflatoxin in the food supply. However, aflatoxin problems can sometimes occur. FDA action levels for aflatoxin are presented in table 1 (Henry, 2006). Aflatoxin regulations worldwide have been reviewed by Van Egmond and Jonker (2005).

<table>
<thead>
<tr>
<th>Class of Animal</th>
<th>Feed</th>
<th>Aflatoxin Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finishing beef cattle</td>
<td>Corn and peanut products</td>
<td>300 ppb</td>
</tr>
<tr>
<td>Beef cattle, swine or poultry</td>
<td>Cottonseed meal</td>
<td>300 ppb</td>
</tr>
<tr>
<td>Finishing swine over 100 lb.</td>
<td>Corn and peanut products</td>
<td>200 ppb</td>
</tr>
<tr>
<td>Breeding cattle, breeding swine and mature poultry</td>
<td>Corn and peanut products</td>
<td>100 ppb</td>
</tr>
<tr>
<td>Immature animals</td>
<td>Animal feeds and ingredients, excluding cottonseed meal</td>
<td>20 ppb</td>
</tr>
<tr>
<td>Dairy animals, animals not listed above, or unknown use</td>
<td>Animal feeds and ingredients</td>
<td>20 ppb</td>
</tr>
</tbody>
</table>

**Fumonisin (FB)**

Fumonisin causes equine leukoencephalomalacia (ELEM) in horses, pulmonary edema in swine, and hepatotoxicity in most other animals. For many years, scientists associated ELEM with *Fusarium* mold. After years of intensive study, FB1 was isolated in South Africa from a batch of moldy corn associated with a field outbreak of leukoencephalomalacia in horses (Gelderblom et al. 1988). Fumonisins produced primarily by *F. verticillioides* and *Fusarium proliferatum* occurs in several forms with fumonisin B1 (FB1) being the most prevalent and the one receiving the most focus. FB1 is found primarily in corn and corn by-products. FB1 promotes oxidative stress, causes DNA fragmentation and cell cycle arrest. It is cytotoxic and inhibits both protein and DNA syntheses. It is carcinogenic in rats and mice and may be a promoter of esophageal cancer in humans (Rheeder et al., 1992). Fumonisins are structurally similar to sphingosine, a component of sphingolipids, which are in high concentrations in certain nerve tissues such as myelin. Fumonisin toxicity results from reduced sphingolipid production by inhibiting ceramide biosynthesis and thus degeneration of tissues rich in sphingolipids. While there is a reduction of sphingosine, there is a buildup of intermediates such as sphinganine.

ELEM is a fatal equine disease denoted by the presence of liquefactive necrotic lesions in the white matter of the cerebrum (gray matter may also be affected) (WHO, 2000) as a result of vasogenic cerebral edema (Haschek et al., 2002). First symptoms may include a sudden onset of one or more of the following signs: frenzy, incoordination, aimless circling, head pressing, paresis, ataxia, blindness, depression and hyperexcitability (Ross et al., 1991). Foreman et al., (2004) includes the following as early signs: lethargy, decreased feed intake, hindlimb ataxia, delayed forelimb placing reactions and tongue paresis and followed by convulsions. The clinical course of ELEM is generally short with the acute onset of signs followed by death within hours or days. Mortality is usually high and death may occur without clinical signs. Progression and presentation of the disease may be related to length of exposure, level of contamination, individual animal differences, previous exposure, and pre-existing liver health. Fatal liver
disease as a result of fumonisin has occurred in the absence of any brain lesions. There is an increase in free sphingoid bases in serum followed by an increase in enzyme levels indicative of liver damage. Serum enzyme concentrations may fluctuate, but usually increase immediately near the onset of behavioral changes (WHO, 2000).

A USDA, APHIS survey of 1995 corn from Missouri, Iowa, and Illinois found that 6.9% contained more than 5 ppm fumonisin B1. Fumonisin was prevalent in Midwestern corn from the wet 1993 season as well. Corn screenings can contain about 10 times the fumonisin content of the original corn. The risk of ELEM is not increased at fumonisin levels below 6 ppm. Risk is increased with fumonisin levels > 10 ppm and ELEM has generally been observed with fumonisin levels above 15 ppm. Fumonisin guidance levels from FDA are in table 2, (Henry, 2006).

Table 2. FDA guidance levels for total fumonisins in animal feeds, (Henry, 2006)

<table>
<thead>
<tr>
<th>Class of Animal</th>
<th>Feed Ingredients &amp; Portion of Diet</th>
<th>Levels in Corn &amp; Corn by-products</th>
<th>Levels in Finished Feeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equids and Rabbits</td>
<td>Corn and corn by-products not to exceed 20% of the diet **</td>
<td>5 ppm</td>
<td>1 ppm</td>
</tr>
<tr>
<td>Swine and Catfish</td>
<td>Corn and corn by-products not to exceed 50% of the diet **</td>
<td>20 ppm</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Breeding Ruminants, Breeding Poultry and Breeding Mink*</td>
<td>Corn and corn by-products not to exceed 50% of the diet **</td>
<td>30 ppm</td>
<td>15 ppm</td>
</tr>
<tr>
<td>Ruminants ≥3 Months Old being Raised for Slaughter and Mink being Raised for Pelt Production</td>
<td>Corn and corn by-products not to exceed 50% of the diet **</td>
<td>60 ppm</td>
<td>30 ppm</td>
</tr>
<tr>
<td>Poultry being Raised for Slaughter</td>
<td>Corn and corn by-products not to exceed 50% of the diet **</td>
<td>100 ppm</td>
<td>50 ppm</td>
</tr>
<tr>
<td>All Other Species or Classes of Livestock and Pet Animals</td>
<td>Corn and corn by-products not to exceed 50% of the diet **</td>
<td>10 ppm</td>
<td>5 ppm</td>
</tr>
</tbody>
</table>

* Includes lactating dairy cattle and hens laying eggs for human consumption.
** Dry weight basis.

Zearalenone (ZEA)

Zearalenone is a *Fusarium* produced mycotoxin associated with ear and stalk rots in corn and with scab in wheat. Zearalenone has a chemical structure similar to estrogen and can produce an estrogenic response in animals. In sensitive animals zearalenone has disrupted the female reproductive cycle, conception, ovulation, implantation, caused embryonic death, inhibited fetal development, produced nymphomania, pseudopregnancy, and ovarian atrophy. Zearalenone has caused vaginal and rectal prolapse and swelling of the vulva and mammary glands. In the male, symptoms include reduced testes size, feminization and suppressed libido.

The meager amount of information available concerning the sensitivity of horses to zearalenone, suggests that horses are less sensitive than swine but may be affected by high concentrations of
zearalenone. Gimeno and Quintanilla (1983) reported a case where animals were exposed for about 30 days to feed containing approximately 2.6 ppm of zearalenone from corn screenings. Exposed mares showed symptoms of enlarged edematous vulva, prolapsed vagina, oversized uterus and internal hemorrhage. Males had severe flaccidity of the genitals.

In another study where zearalenone was fed at slightly lower levels (about 1 ppm), and started at 10 days after ovulation in cycling mares, there was no influence on the interovulatory interval, the length of luteal and follicular phase, plasma progesterone concentration or the number of large follicles (>2cm) (Juhasz et al., 2001).

Minervini et al., (2006) investigated the effect zearalenone and its derivatives (alpha and beta zearalenol) on apoptosis and proliferation of cultured granulosa cells from equine ovaries. Zearalenone (and derivatives) induced a simultaneous increase in cell proliferation and an apoptotic process. This most likely indicates that these mycotoxins could be effective in inducing follicular atresia and emphasizes the importance of zearalenone in reproductive disorders in the horse.

In the stallion, in vitro exposure of equine spermatozoa with urine samples containing low concentrations of zearalenone and its derivatives induced instability of the sperm chromatin structure (Filannino et al., 2008). This effect on chromatin structure, strictly related to subfertility in stallions was measured by flow cytometry by using acridine orange.

The FDA has established no guidelines for zearalenone in feed, and deals with any contamination issue on a case by case basis (Henry, 2006).

**Deoxynivalenol (DON) or Vomitoxin**

Deoxynivalenol is a *Fusarium* produced mycotoxin, commonly detected in feed. It is sometimes called vomitoxin because it was associated with vomiting in swine. While known to be a problem for swine, DON is considered much less toxic for horses. Pure DON added to diets, produces less toxicity than does DON from naturally contaminated feeds, perhaps due to the presence of multiple mycotoxins or other toxic factors in naturally contaminated feeds (Foster et al., 1986). Therefore, the presence of DON may serve as a marker, indicating that feed was exposed to a situation conducive for mold growth and that feeds may contain multiple mycotoxins. Multiple mycotoxins may interact to produce symptoms different or more severe than expected. For example, fusaric acid interacts with DON to cause the vomiting effects, which was earlier attributed to DON alone (Smith and MacDonald, 1991).

Johnson et al. (1997), without noting major adverse effects, fed five horses 2.8 lb daily for 40 days of a feed containing 36 to 44 ppm of DON from contaminated barley. The authors concluded that horses are relatively resistant to the adverse effects of DON. Serum IgA and IgG declined. Hematocrit and serum enzyme activities decreased slightly. Serum total protein, globulin, and albumin declined. Grain intake was constant and forage intake was not monitored. Horses were not weighed, but were described as being in good condition and heavier at trial end.

In contrast, Raymond et al. (2003) investigated in horses the effects of mycotoxin on feed intake, serum immunoglobulin (IgA, IgG, IgM) concentrations, serum chemistry, and hematology. Horses were fed an approx. combination of up to 6 lb of concentrates and 11 lb of mixed hay. Grain in the concentrates was naturally contaminated by *Fusarium* mycotoxins resulting in concentrates averaging 15 ppm DON, 0.8 ppm 15-acetyldeoxynivalenol, 9.7 ppm fusaric acid (FA) and 0.2 ppm ZEN. Horses consuming mycotoxin-contaminated feed had lower intake and higher serum c-glutamyltransferase (GGT) activities measured on days 7 and 14 of supplementation but not on day 21. The lack of difference in serum activities of GGT on day 21 may imply an adaptation to the hepatotoxicity caused by *Fusarium* mycotoxins.

In an additional study, (Raymond et al., 2005) investigated the effects of feeding grain naturally contaminated with *Fusarium* mycotoxins on mature, exercised horses. Six horses were fed in a 21 day
experiment. Concentrates containing contaminated grains averaged 11.0 ppm deoxynivalenol, 0.7 ppm 15-acetyldeoxynivalenol, and 0.8 ppm zearalenone (as-fed basis). Feed offered was a combination of up to 7.7 lb of concentrate and 11 lb of hay. Feed intake was decreased in horses fed contaminated grains. Hay intake was not affected. Weight loss from 0 to 21 d was observed in horses fed contaminated grains. No effect of diet was seen on variables used to measure athletic ability.

Bedding material contaminated with DON has resulted in several case reports of toxicity including one from Germany reporting distinct weight loss and elevated hepatic enzymes with recovery after removal of DON contaminated materials (Zeyner et al., 2002).

<p>| Table 3. FDA advisory levels for deoxynivalenol in livestock feed, (FDA, 2010) |</p>
<table>
<thead>
<tr>
<th>Class of Animal</th>
<th>Grains &amp; Grain Byproducts</th>
<th>Other By-products</th>
<th>Total Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruminating beef &amp; feedlot cattle &gt; 4 months age</td>
<td>10 ppm</td>
<td>30 ppm</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Dairy cattle &gt; 4 months age</td>
<td>10 ppm</td>
<td>30 ppm</td>
<td>5 ppm</td>
</tr>
<tr>
<td>Chickens</td>
<td>10 ppm (&lt;50% of the diet)</td>
<td>5 ppm</td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>5 ppm (&lt;20% of the diet)</td>
<td>1 ppm</td>
<td></td>
</tr>
<tr>
<td>All other animals</td>
<td>5 ppm (&lt;40% of the diet)</td>
<td>2 ppm</td>
<td></td>
</tr>
</tbody>
</table>

**T-2 Toxin (T-2) and HT-2**

T-2 toxin is a very potent trichothecene mycotoxin produced by *Fusarium* species. T-2 is rapidly metabolized to HT-2 in animals and thus the toxicity of T-2 and HT-2 may be considered together. Surveys have shown the feed occurrence of T-2 is low (<5%). However, in the drought year of 1988, Russell et al. (1991) found 13% of surveyed Midwestern corn grain was contaminated with T-2 toxin. T-2 is found in both grains and forages.

T-2 is extremely toxic to laboratory animals, to most farm animals, and to humans, but toxicity to horses is unknown. T-2 inhibits protein synthesis, causes dermal irritation, hemorrhaging, reduced immunity, and damage to major organs including the heart, lung and spleen. T-2 was the likely agent of “alimentary toxic aleukia” which caused the death of many humans in Siberia during the 1940’s (Ueno et al., 1972a). T-2 was the likely toxic agent associated with bean-hull poisoning involving horse deaths in Japan, but other mycotoxins (diacetoxyascirpenol and neosolaniol) may have been present (Ueno et al., 1972b). A number of mycotoxin poisonings have been associated with trichothecenes other than T-2 toxin (Ueno, 1977). Davis et al., (1982) reported horse deaths associated with T-2 contaminated hay from Canada. Juhasz et al., (1997) administered T-2 at 7 mg per os daily (equivalent to approximately 1 ppm of the diet) to six Trotter mares for 32 to 42 days. Except for skin lesions observed around the mouth in 3 of the 6 cases, all animals remained in good physical condition with no reproductive effects noted. Mouth lesions are symptomatic of trichothecenes in other species and such lesions were reported in horses exposed to the trichothecenes involved with stachybotryotoxicosis.

T-2 toxin or a combination with zearalenone and DON may induce colic. Barnett et al. (1995) collected feed samples from farms with horses experiencing colic (n = 16) and from control farms (n =
and found an association with mycotoxin presence. DON was found in feed from the both the colic group (100% of farms at levels ranging from 0.20 to 8.3 ppm) and the control group (70% of the farms at concentrations of 0 to 2.5 ppm). Neither T-2 nor zearalenone were found in the control group, but T-2 was present in 31% of the colic group (> 0.5 ppm) and zearalenone was present in 44% of the colic group (> 0.7 ppm).

Guidelines for T-2 toxin are not established, but avoiding levels above 100 ppb may be reasonable. Diacetoxyscirpenol, HT-2 and neosolaniol may occur along with T-2 toxin and cause similar symptoms. The FDA has established no guidelines for T-2 toxin in feedstuffs.

**Ochratoxin A**

The research literature contains little information on ochratoxin poisoning of horses. Ochratoxin A (OTA) is produced by species of *Penicillium* and *Aspergillus* and is a causative agent of kidney disease in pigs that has been referred to as mycotoxin porcine nephropathy (Krogh, 1979). The primary toxic effect of ochratoxin is inhibition of protein synthesis and a nephrotoxic effects in all animal species (Creppy et al., 1984). Ochratoxin induces oxidative damage by enhancing lipid peroxidation, impairs blood coagulation and glucose metabolism, and is immunosuppressive.

The FDA has no guidelines for ochratoxin in feed, and deals with contamination issues on a case by case basis (Henry, 2006).

**Slaframine**

Slaframine is an idolizidine alkaloid produced by *Rhizoctonia leguminicola* associated with “slobbers” syndrome and the unique symptom of excessive salivation. Slaframine has been primarily found in moldy red clover and infrequently in other legumes. It is thought that slaframine may, at times, be accompanied by swainsonine, another mycotoxin produced by the same mold and incriminated in different situations as the cause of locoism. Other symptoms of the “slobber” syndrome have included lacrimation, feed refusal, diarrhea, vomiting, stiff joints, respiratory distress, hypothermia, frequent urination, and abortion. Death has occurred as a result of emphysematous lungs and suffocation. Other effects include collapse of the cardiovascular system, liver necrosis and renal congestion. Toxicity may occur at levels > 50 ppm. Removal of the contaminated hay generally returns animals to normal within days. (Hagler and Croom, 1989).

**Penicillium mycotoxins**

Citrinin and ochratoxin can be produced by *Penicillium* in wheat, barley, corn, rice, rye, oats and other grains. Both mycotoxins have been associated with kidney toxicity CAST, 2003). Silage is particularly prone to *Penicillium* because it grows at a low pH and in a damp condition. Silage contaminated with *Penicillium* may contain mycotoxins such as roquefortone C, mycophenolic acid, PR toxin and patulin. Surveys of grass and corn silage in Europe have found an occurrence of *P. roquefortii* in up to 40% of samples (Auerbach, 2003) and associated with cattle disorders (Boysen et al., 2000).

**Stachybotryotoxicosis**

Stachybotryotoxicosis has never been confirmed in the U.S. In the 1930’s moldy forage was causing economic havoc in Russia due to horse losses (Forgacs, 1972). The disease, stachybotryotoxicosis, was determined to be caused by the trichothecene mycotoxins satratoxin and verrucarin which are produced by *Stachybotrys chartarum*, a black sooty-like mold that grows on moist hay or straw. The same mold has been associated with moldy “sick-house” syndrome. This is a very serious disease that develops over a 2 week to 3 month period but can develop quicker if a large amount of toxin is ingested. Horses first develop fissures at the corners of the mouth which become necrotic over time with swelling of the lips. The entire head may become edematous. Blood leukocyte counts drop and
blood clotting times increase. Body temperatures rise. Appetites disappear and the pulse becomes arrhythmic and weak. Animals become weak, recumbent and die. There were reports of this disease in 1984 in Hungary (Harrach et al., 1987) and in 1985 in France (Servantie et al., 1985).

**Literature Cited**


Summary

Tall fescue is an extensively grown perennial forage grass in the United States. It is vigorous and easily established, having much potential as suitable and nutritious forage for livestock, but its qualities are not without disadvantages. Most tall fescue lives symbiotically with an endophytic fungus that delivers a competitive advantage to the plant but causes fescue toxicity in livestock. The action of the toxins produced by the endophyte are to enhance dopamine and inhibit prolactin secretion from the pituitary, leading to an array of clinical signs in livestock. In horses, fescue toxicity is almost exclusively limited to reproductive problems in mares. Broodmares consuming endophyte-infected fescue often have prolonged gestation with few normal signs of impending parturition, dystocia, retained and thickened placentas, large but weak foals, and agalactia. Death of mares and/or foals is a common factor in dystocia. Septicemia in foals is a common secondary effect, due to agalactia and lack of passive transfer of immunity from colostrum. Early embryonic loss may also occur. Broodmare owners should remove pregnant mares from fescue and feed alternative forage at least 60 days before foaling, or dose daily in the last 20 days of gestation with domperidone, a drug that supports prolactin secretion and reverses symptoms of fescue toxicity. Recently, a novel endophyte placed in tall fescue plants confers advantages to the plants while not producing toxins that affect livestock.

History

The tall fescue story is not a new one. Tall fescue was introduced into North America from Europe in the late 19th century, but it did not become widely used until the 1940s. In 1931, Dr. E.N. Fergus of the University of Kentucky became impressed with a tall fescue ecotype growing on the Suiter farm in Menifee County. He collected seed, planted and tested it in many locations throughout Kentucky, leading to the release of “Kentucky 31” in 1943. Kentucky 31 was widely promoted by UK Extension agronomist W.C. Johnstone and readily accepted by farmers, leading to widespread planting of tall fescue, especially in the Midwest and the South. Today, several ecotypes of tall fescue make it one of the most important cool-season perennial grasses in the United States, covering an estimated 35 million acres, providing pasture for livestock, turf in lawns, “rough” areas for golf courses, and erosion control in road ditches, among other uses.

The Endophyte: Mutualistic Symbiosis, Toxins and Prolactin Response

An endophyte fungus (endo – “inside,” and phyte – “plant”) lives in mutualistic symbiosis within most tall fescue plants. The endophyte grows in leaf stem tissue between the plant cells, and the endophyte mycelium resides in fescue seed. The fungus completes its entire life cycle living within the fescue and does not alter the appearance of the plant, so it must be detected by laboratory analysis. The endophyte is transmitted exclusively through seed. It does not live outside of the plant, nor does it...
transmit from plant to plant. Infected plants can only arise from seed produced by plants carrying the endophyte.

Although endophyte-free tall fescue varieties are available, the symbiosis confers a competitive advantage to tall fescue containing the endophyte (Ball et al., 2007). Compared to endophyte-free fescue, endophyte-infected fescue has higher germination rates and improved insect resistance. Endophyte-infected fescue roots produce compounds that protect the plant from soil acidity and toxic elements. Compared to endophyte-free, endophyte-infected fescue produces a deeper crown that increases drought tolerance and provides protection from overgrazing. Lack of vigor and persistence of endophyte-free tall fescue varieties have made them generally unpopular as pasture plants. Of particular interest to livestock producers has been the use of tall fescue as stockpiled winter grazing, thus reducing supplemental hay feeding.

Ergopeptide alkaloids, predominantly ergovaline, are synthesized by the endophyte and are present in all aboveground parts of infected fescue plants (Lyons et al., 1986). Ergovaline has been considered to be the main toxic agent in endophyte-infected tall fescue. Loline alkaloids and peramine are also present and implicated in symbiotic fungus-host interactions and insect resistance. More recently, lysergic acid has been shown to play an important role. Lysergic acid was excreted in urine and manure of geldings in greater amounts than consumed (Schultz et al., 2006), suggesting that ergovaline or other ergot alkaloids may be metabolized to lysergic acid. A full review of the compounds associated with fescue toxicity is beyond the scope of this paper. Readers interested in a review of alkaloids and toxicology are referred to Strickland et al. (1993).

The mechanism in fescue toxicity is related to the balance between the hormone, prolactin, and the neurotransmitter, dopamine. In normal animals, prolactin is produced by the lactotroph cells of the anterior pituitary. Prolactin plays a role in several life processes, including reproductive cycles, circulation, seasonal changes associated with photoperiod (e.g. shedding of hair coat), gastrointestinal tract efficiency, mammary gland development and lactation. Prolactin release is controlled in part by negative feedback from dopamine, which is produced in the hypothalamus and posterior pituitary. The balance of prolactin and dopamine is necessary in normal animals to regulate and de-regulate life processes as required (Ben-Jonathan et al., 1989).

In fescue toxicity, ergovaline enhances dopamine action and inhibits prolactin release (Strickland et al., 1992). Decreased prolactin deregulates reproductive cycles, constricts circulation, causes abnormal seasonal shedding cycles, decreases gastrointestinal tract efficiency, and decreases mammary gland development and lactation. Different animals are affected in different ways, which accounts for the wide range of symptoms found in fescue toxicity. Decreased circulation in cattle, for example, contributes to over-heating, high rectal temperatures, fat necrosis, and gangrenous extremities, or “fescue foot.” Cattle also exhibit abnormal shedding cycles and retain long winter hair coats well into summer, while horses shed normally.

**Fescue Toxicity in Broodmares**

Pregnant broodmares are particularly susceptible to fescue toxicity, and their reactions to the endophyte are almost exclusively related to reproductive problems. Both prolactin and progesterone were lowered in broodmares grazing endophyte-infected fescue (McCann et al., 1992a). Broodmares with fescue toxicity have longer gestation lengths (+27 to 30 days) and thus larger foals (Monroe et al., 1988; Putnam et al., 1991; Redmond et al., 1994). These mares do not exhibit adequate physiological signaling and readiness of the reproductive tract for birth, so the combination of a large foal without physiological readiness for birth results in severe dystocia. Foals are often rotated 90 to 180 degrees from normal birthing position (Taylor et al., 1985; Monroe et al., 1988). Premature placental separation, or “red bag”
syndrome is common, as are thickened and retained placetas (Taylor et al., 1985; Monroe et al., 1988). Foals are large framed but weak, emaciated and dysmature (Cross et al., 1995). Loss of the foal and/or the mare due to dystocia is common.

Mares may fail to lactate (agalactia) or have reduced milk production. Unlike cows and ewes, which rely on both prolactin as well as lactogen from the placenta to stimulate milk production, mares rely solely on prolactin to stimulate milk production (Forsyth, 1986). As a result, cows and ewes with fescue toxicity have decreased milk production while mares have agalactia. A secondary issue of agalactia is failure of passive transfer of immunity from mares to foals due to lack of colostrum.

In a definitive study at Clemson University (Monroe et al., 1988), two groups of 8 mares each were maintained on endophyte-free or endophyte-infected fescue through foaling. The mares grazing endophyte-infected fescue had longer gestation lengths by 27 days. Of the 8 mares on endophyte-infected fescue, 4 had stillborn foals, 5 had retained placentas, and 7 did not lactate. In a similar study at Auburn University (Putnam et al., 1991), two groups of 11 mares each were maintained on endophyte-free or endophyte-infected fescue through foaling. Of the 11 mares on endophyte-free fescue, all had healthy foals with no foaling problems, and all lactated normally. Of the 11 mares on endophyte-infected fescue, 10 of the 11 had dystocia. Only 4 of the 11 resulting foals were alive at birth, and of those, only two survived. Nine of 11 mares did not lactate. Four mares died as a result of foaling complications.

In rebreeding and early pregnancy, it was suggested that mares grazing endophyte-infected compared to those grazing endophyte-free fescue had a decreased per cycle successful pregnancy rate (45% vs 75%) and had greater early embryonic loss (30% vs 8%), compared to mares grazing endophyte-free fescue (Brendemuehl et al., 1994). These numerical differences are compelling but not statistically significant, so additional work would be needed to verify these conclusions.

Other than pregnant mares, fescue toxicity appears to have little effect on stallions, geldings and non-pregnant mares. While a 57% reduction in average daily gain was noted in yearling horses grazing endophyte-infected fescue without grain supplementation (Aiken et al., 1993), similar average daily gains were reported in studies where yearling horses grazing endophyte-infected fescue were supplemented grain (McCann et al., 1992b; Pendergraft and Arns, 1993). It is possible that these findings are a result of lower palatability and intakes of endophyte-infected pasture and hay and not fescue toxicity. It is also possible that ergovaline affected nutrient absorption in the gastrointestinal tract through reduced circulation and this effect was moderated by grain supplementation. More work in this area is needed to determine if fescue toxicity affects growth in horses.

The Defensive Line-up

Converting endophyte-infected fescue pasture to endophyte-free fescue or other pasture species is challenging at best due to the persistence and competitiveness of the endophyte-infected varieties. Personal experience with eradicating endophyte-infected tall fescue (including herbicide application, planting a winter annual cover crop, repeat herbicide application and planting of other species) can attest that it is expensive and often results in reappearance of endophyte-infected fescue in as few as five years. Thus, it is often more practical for management practices to work with, rather than without, the presence of endophyte-infected fescue.

The most common management practices include either removing pregnant mares from endophyte-infected fescue prior to foaling, or if removal is not possible, therapeutic treatment of mares grazing endophyte-infected fescue with domperidone, which blocks dopamine receptors and increases prolactin.
Pregnant mares should be removed from endophyte-infected fescue pastures 60 to 90 days prior to their expected foaling date in order to avoid effects of fescue toxicity on foaling and milk production (Cross et al., 1995). A study by McCann et al. (1992) indicated that serum prolactin was restored to normal concentrations in as little as 8 days after removal from endophyte-infected pasture, but more time may be needed for the reproductive tract to recover and fully prepare for foaling. Mares removed from endophyte-infected fescue 10 and 20 days before foaling had a higher incidence of agalactia but fewer losses of foals, and mares removed 30 days before foaling were normal except for a higher incidence of retained placenta (Cross et al., 1995). In any case, removal of a late-gestation mare from endophyte-infected fescue closer to foaling than 60 days is better than not removing the mare from fescue at all.

Domperidone, sold under the trade name Equidone® by Equi-Tox Pharma, is a dopamine receptor antagonist, meaning that it blocks the action of dopamine and also prevents the ergot alkaloids from acting like dopamine. Thus, prolactin is allowed to increase normally as expected for a late gestation mare. In a study at Clemson University, mares grazing endophyte-infected fescue and treated with domperidone had shorter, normal gestation lengths and higher mammary gland scores (Redmond et al., 1994). In addition, serum progesterone was higher and estradiol was lower in domperidone-treated than in non-treated mares grazing endophyte-infected fescue. If mares cannot be removed from endophyte-infected pastures, then domperidone should be given daily to mares for the last 15 to 20 days before foaling, until the mare foals. For mares that have been removed from endophyte-infected pastures but are not progressing with proper udder development, domperidone should be given in the last 10 days prior to expected foaling and continuing until foaling (Cross et al., 1995).

The method of harvest and storage of endophyte-infected tall fescue hay and haylage affects toxic ergot alkaloid concentrations. When tall fescue hay is treated with 3% anhydrous ammonia or propionic acid, cattle and sheep showed few symptoms of fescue toxicity (Chestnut et al., 1991; Strickland et al., 1993). The Nutrient Requirements for Horses (NRC, 2007) notes this in their review of endophyte contamination and further notes that “during ensilage, ensuring anaerobic conditions and enhancing a rapid decline in pH immediately post-harvest by the use of various biological inoculants and/or enzyme preparations can help reduce levels of fungal contaminants.” Recent research in our laboratory indicates otherwise. In fact, ensiling fescue doubled ergot alkaloid concentrations and increased ergovaline concentrations from below detection to 400 ppb, certainly high enough to induce fescue toxicity, which was noted in weaned calves (Black et al., 2010). Since fescue toxicity may be induced in mares with ergovaline concentrations as low as 300 ppb, feeding fescue haylage to pregnant mares is absolutely contraindicated.

**Fescue with a Novel Endophyte**

The idea that the endophyte could be engineered to produce symbiotic benefit to the plant while not producing toxins associated with animal issues was first proposed by Bacon and Siegel (1988). The discovery of novel non-toxic endophytes in New Zealand made this idea a reality. Cooperative work between researchers in New Zealand and the University of Georgia followed, leading to the first fescue cultivar containing the novel non-toxic endophyte (Bouton et al., 2002). This was accomplished by removing the wild-type toxic endophyte and inserting the novel non-toxic endophyte. In the first few years, Jesup Max Q™ was the only type of fescue available in the US containing the novel endophyte, but more recently, several different types have become available.

The novel endophyte does what it was intended to do: it confers symbiotic advantages to the plant without producing the ergovaline that causes fescue toxicity in cattle and horses. The persistence of novel endophyte-containing fescue rivals that of fescue with the old endophyte (Bouton et al., 2002). Research at Mississippi State University indicates that mares grazing fescue containing the novel endophyte had no foaling or lactation issues (Ryan et al., 2001).
Recently, a new form of toxicity associated with horses on fescue has been described. This syndrome, equine fescue edema, has clinical signs including subcutaneous edema around the head, neck, chest and abdomen, as well as inappetence, depression, and sometimes death (Bourke et al., 2009). This toxicity has been found only in Australia in horses grazing pastures containing Mediterranean (summer dormant/winter active) tall fescue containing the novel endophyte. It is critical to note that the tall fescue used with the novel endophyte in the US is Continental (summer active) tall fescue, and distinctly different from the Mediterranean type. No problems in horses grazing Continental fescue containing the novel endophyte have been noted in the last ten years of equine research in the US, so perhaps the Mediterranean-novel endophyte pairing creates a different compound that causes equine fescue edema. Further work is needed to better elucidate the mechanism of this syndrome.

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POISONOUS PLANTS IN THE HORSE’S ENVIRONMENT

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Summary

Plant poisoning continues to be a problem for horses wherever they are raised. In some instances poisoning results from horses being exposed to new pastures where native plants and/or invasive weeds are growing, or trees that are poisonous have been planted to provide landscaping and shade. At other times the source of toxic plants is in the hay being fed. It is therefore important for horse owners and pasture managers to recognize the important poisonous plants that are a risk to horses, and the clinical signs of plant poisoning that will help prevention of further poisoning and enable timely treatment.

In north eastern North America plant poisoning of horses is often associated with overgrazing of pastures that encourages hungry horses to eat plants they would not normally eat. Native plants such as yew (*Taxus* spp.), rhododendron (*Rhododendron* spp.), white snake root (*Ageratina altissima*), and water hemlock (*Cicuta* spp.) are notorious toxic plants in the north east. Some trees such as black walnut (*Juglans nigra*), red maple (*Acer rubrum*) are well known for being poisonous to horses. An ever increasing problem is the introduction of invasive noxious weeds that can also be poisonous to animals that graze them. Of importance in the north eastern States are weeds such as poison hemlock (*Conium maculatum*), yellow starthistle (*Centaurea solstitialis*), Russian knapweed (*Acroptilon repens*), hoary alyssum (*Berteroa incana*), and flat weed (*Hypochaeris radicata*).

Yew (*Taxus* spp.)

Yews are common evergreen native shrubs or trees that prefer the acidic soils of the eastern and western states, and are commonly used in landscaping. The most common species in the eastern States are *Taxus canadensis* and *T. cuspidata*. Yews contain several toxic compounds, the most toxic being taxine (Khan and Parveen, 1987). All parts of the plant are toxic except for the ripe red fleshy fruits (arils). The alkaloid taxine acts primarily on the cardiovascular system slowing and stopping atrial and ventricular conduction that results in death (Tekol et al., 1987). The alkaloid taxol has useful antineoplastic properties. Although yews are common in many horse environments relatively few cases of poisoning have been reported in horses (Cope et al, 2004; Tiwary, 2005).

The most common manifestation of yew poisoning is peracute death with little if any clinical signs. If observed early enough in the course of poisoning, affected horse will show cardiac irregularity, weakness, difficulty in breathing and rapid progression to recumbency, cardiac shock and death. At necropsy, pulmonary congestion and hemorrhages on the heart, suggestive of acute circulatory failure may be the only lesions. Microscopically, acute mild multifocal necrosis of papillary muscles and ventricles may be present (Tiwary, 2005).

A diagnosis of yew poisoning can be made based on the signs, the finding of yew leaves in the stomach, and the detection of the taxine alkaloid in the stomach contents (Kite et al., 2000).
Rhododendrons, azaleas (*Rhododendron* spp.)

One of the most common native, evergreen or deciduous shrubs or small trees in the eastern and western states where acidic soils predominate, rhododendrons (azaleas) are frequently planted in landscapes and around horse enclosures for their attractive flowers. There are at least 30 species native to North America, all of which are poisonous to some degree. Other members of the family Ericaceae that are toxic include the laurels (*Kalmia* spp.), fetterbush (*Leucothoe* spp.) and pieris (*Pieris* spp.). The toxicity is due to a variety of diterpenoids (Grayanotoxins) that act on the sodium channels of cardiac cells that result in decreased cardiac activity, and heart arrest (Narahashi and Seyama, 1974).

Rhododendron poisoning is rarely reported in horses, being more commonly seen in other livestock species. The toxic dose is 0.2% by weight of green leaves. None the less the risk is significant! Hypotension, tachycardia, excessive salivation, increased nasal secretions, colic and irregular respirations develop several hours after rhododendron leaves are ingested.

Red Maple (*Acer rubrum*)

Red maple and its hybrids are common in eastern North America but only the red or swamp maple (*Acer rubrum*) and silver maple (*A. saccharinum*) have been shown to be poisonous to horses and zebras (Divers *et al.*, 1982; Weber and Miller, 1997; DeWitt *et al.*, 2004).

Only the wilted or partially dried leaves of the red maple are known to cause oxidation and destruction of equine red blood cells (Alward *et al.*, 2006). Fresh, green maple leaves do not appear to be toxic. The toxin responsible for the oxidation of the hemoglobin in the red blood cells has not been determined (Boyer *et al* 2002). Ingesting as little as a pound or two of wilted leaves can be fatal.

After eating wilted maple leaves, horses become depressed, anorexic and develop dark, red-brown colored urine (Divers *et al.*, 1982; Weber and Miller, 1997). If large quantities of maple leaves are consumed in a short time, depression, cyanosis and death may occur within a 24 hour period. Typically, horses becoming depressed, weak, with increased respiratory and heart rates. Anemia develops rapidly as the damaged red blood cells are destroyed. The hematocrit may drop to 10%, and numerous Heinz bodies may be seen in the red blood cells for several weeks. Methemoglobin levels are usually markedly elevated. Liver enzymes, bilirubin, creatinine levels are usually elevated. Death may occur anywhere up to a week after the ingestion of the maple leaves and is generally due to severe anemia and renal failure. Abortion may occur in pregnant mares consuming red maple leaves (Stair *et al.*, 1993).

Treatment of red maple requires rapid and accurate diagnosis for treatment to be successful. Intravenous fluids and blood transfusions may be necessary to correct the anemia and support renal function. Large doses of ascorbic acid orally (125mg/kg body weight) and intravenously (30 mg/kg body weight) every 12 hours in conjunction with anti-inflammatory drugs and intravenous fluids have been used successfully to counter the oxidant effects of the maple leaves (McConnico and Brownie, 1992).

Blackwalnut (*Juglans nigra*)

Some 20 species of walnut tree are native to Europe, Asia and North America. Most cases of walnut poisoning in horses is associated with horses being bedded on wood shavings containing black walnut. Fresh wood shavings containing as little as 5-20% walnut shavings can induce laminitis (Ralston and Rich, 1983; Thomsen *et al.*, 2000).

Horses must obtain the toxin orally, or possibly from inhalation because fresh walnut shavings applied to directly to the horses hooves experimentally failed to cause laminitis (True, 1980). Weathering of walnut shavings appears to reduce their toxicity.
The toxic component of black walnut responsible for causing laminitis in horses has not been determined, but the compound juglone may play a role in the toxicity. Aqueous extracts from the heart wood of black walnut when administered intravenously to horses does cause laminitis apparently as a result of vasoconstriction of the vessels supplying the sensitive laminae of the hoof (Galey et al., 1990; Galey et al., 1991). The heart wood of black walnut does not contain juglone, while the leaves contain as much as 150 ppm suggesting that other toxic compounds are involved (Coder, 1983).

Horses that have been bedded on fresh walnut shavings may develop elevated body temperature, increased heart and respiratory rates, edema of the legs, marked digital pulse and lameness due to laminitis (True, 1980). Ponies and foals appear to be less affected than adult horses, but signs are quite variable. Signs of laminitis may appear from 1-3 days after exposure to the walnut shavings.

**Hoary alyssum (Berteroa incana)**

Hoary alyssum is annual, biennial, or perennial, introduced weed that has become naturalized in most areas of North America. It causes toxicity primarily in horses when it is grazed or incorporated in hay (Geor et al., 1992; Hovda and Rose, 1993). Since poisoning is characterized by limb edema and laminitis, it should be considered along with black walnut toxicity as a differential diagnosis in laminitis.

Horses fed hay containing 30% hoary alyssum develop pyrexia, increased digital pulse and limb edema within 24 hours. Laminitis with rotation of the third phalanx may occur in severe cases. Red colored urine as a result of red blood cell break-down, and a hemorrhagic diarrhea and late term abortion have been reported in pregnant mares fed alfalfa hay containing hoary alyssum. Death may occur in 1-2% of natural cases of hoary alyssum poisoning.

**Flatweed (Hypochaeris radicata)**

Flat weed is a common, non-native perennial plant that is widespread throughout North America where it invades pastures and disturbed soils. Closely resembling a dandelion, flatweed has basal leaves and yellow flowers produced on branching stems. Horses grazing flatweed over a period of time develop a unique lameness referred to as stringhalt.

The toxin in flatweed is not known. Horses develop degeneration of the long myelinated nerve fibres that results in neurogenic atrophy of the muscles, lameness and eventual recumbency. Usually both hind legs are affected, although one leg will be more affected. The horse develops a peculiar hyperflexion of the hock, and if both legs are affected the horse develops a ‘hopping’ gait (Araujo et al., 2008; Gardner et al., 2005; Cahill et al., 1985).

Affected horses should be removed from the affected pasture and treated with thiamine and phenytoin (Gardner et al., 2005). Most horses recover after 6-12 months.

**White snakeroot (Ageratina altissima)**

White snakeroot is native perennial of North America preferring woodlands in the eastern, south eastern and Great Plains areas. It grows to 1.5m (5 ft.) in height, with erect stems from a woody base and spreading by rhizomatous roots, opposite leaves and has white flowers produced in flat-topped clusters at the end of the branches.

White snakeroot contains a complex mixture of sterols and ketones collectively referred to as Tremetol (Sharma et al., 1998; Beier and Norman, 1990). The primary action of tremetol is associated with enzyme inhibition in the tricarboxylic acid cycle resulting depletion of liver glycogen, hypoglycemia and cellular damage to the liver and heart. Tremetol is excreted in the milk of lactating animals and
therefore poses a risk to the suckling animal. Tremetol concentration is highest in the green plant, with the dried plant being less toxic.

Horses, after eating white snakeroot for several days develop a variety of signs ranging from sweating, rear leg weakness and stumbling, severe depression, red-dark brown urine, and choke. Cardiac arrhythmias and rapid heart rate are often present (Thompson, 1989; Smetzer et al., 1983; Sanders, 1983). There is no specific treatment for white snakeroot poisoning and horses should be prevented access to the plants.

Considerable interest has been shown in ‘grass sickness’ of horses in Europe and more recently in the United States where it was thought that seasonal pasture myopathy in horses in the midwestern United States was due to the ingestion of white snakeroot. However in a series of cases of seasonal pasture myopathy there was no evidence that tremetol was the underlying cause of the syndrome (Finno, 2005).

**Water hemlock (Cicuta spp.)**

Water hemlock (cowbane, beaver poison, musquash root, poison parsley) is a native perennial plant of North America that is highly poisonous to all animals (Burrows and Tyrl, 2001). Cicuta species of which there are 4 in North America are erect, perennial or biennial plants, maturing at 4-5 feet in height, with smooth hollow stems arising from thickened tuberous roots. The hollow stems are partitioned and towards the base are closer together. The stem base adjacent to the root crown is chambered, containing a yellowish pungent liquid. All parts of the plant should be considered poisonous. The fleshy roots contain the highest concentrations of the unsaturated alcohols cicutoxin and cicutol. Cicutoxin is highly toxic to all animals, including man.

Cicutoxin acts primarily on the central nervous system blocking the gamma amino butyric acid (GABA) receptors, and therefore the major inhibitory pathways of the brain (Wittstock et al., 1997). Muscle twitching, followed by seizures, violent chewing movements, respiratory paralysis and death occur within 1-2 hours of eating the plant. Although water hemlock poisoning of horses is rarely reported, it is one of the most toxic plants to avoid in animal pastures (Yuriko, 2007).

**Poison or Spotted Hemlock (Conium maculatum)**

Introduced from Europe, poison hemlock has become widely distributed throughout North America. It is a tall (6 feet), branched biennial plant with hollow smooth stems covered with purple spots particularly towards the base. The plant has a stout taproot. Leaves are alternate, carrot-like, and the white flowers are produced terminally on the branches.

A wide variety of animals including horses, cattle, sheep, goats, elk, pigs, poultry and rabbits have been poisoned by *Conium maculatum* (Panter et al., 1999) Several pyridine alkaloids including the highly toxic coniine, and N-methylconiine are predominantly responsible for the central nervous system depression and teratogenic effects seen in many species of animal eating the roots, leaves, and especially the seeds (Lopez et al., 1999) The alkaloid effects on the central nervous system are poorly understood, but are assumed to be similar to that of nicotine. The alkaloids appear to initially stimulate and then block autonomic ganglia. At high doses, neuromuscular blockade results in death of the animal. Pregnant animals consuming poison hemlock in the first trimester of pregnancy produce fetuses with cleft palates and variable degrees of limb deformities (Bunch et al., 1992; Panter et al., 1985).

Horses that eat poison hemlock may develop muscle tremors, weakness, incoordination, excessive salivation, increased frequency of urination, and colic depending upon the quantity of plant consumed. In high doses, severe depression, progressive paresis leading to recumbency, slow heart rate and respiratory depression may lead to death of the animal.
Yellow Star Thistle (*Centaurea solstitialis*)

Yellow star thistle is an invasive noxious weed that was introduced from the Mediterranean area and has become well established in North America. It is annual, herbaceous weed, with ‘winged’ branches and distinctive yellow flowers surrounded by bracts are tipped with characteristic stiff yellow spines.

Several neurotoxic compounds in yellow star thistle have been identified that specifically destroy the dopaminergic nigrostriatal pathway that has coordinating and inhibiting effects on the cerebral cortex centers that control prehension and chewing of food (cranial nerves V, VII, IX) (Roy *et al.*, 1995). The plant is poisonous only to horses and is toxic in both its green and dried states. Horses must eat from 86-200 percent of their body of the green plant over a period 1-2 months before toxicity develops (Young *et al.*, 1970).

Russian knapweed (*Acroptilon repens*)

Russian knapweed is a noxious weed introduced from Europe that has spread across North America. It is a spreading invasive perennial plant with black horizontal roots, erect, branched stems from 1 to 3 feet height. The young stems are covered with soft gray hair or knap. The flowers are thistle-like, lavender to whitish in color with papery bracts and no spines.

The neurotoxic compound sesquiterpene lactone repin is the primary toxin, causing similar effects as the toxin in yellow star thistle (Roy *et al.*, 1995). Russian knapweed is toxic only to horses and is more toxic than yellow star thistle requiring less plant mass (1.8-2.6 kg/100 kg body weight) and a shorter feeding period (28-35 days) to produce disease in horses (Young *et al.*, 1970).

Both yellow star thistle and Russian cause ‘chewing disease’, that is characterized by increased tonicity and incoordination of the muscles that enable prehension and chewing of food (Young *et al.*, 1970; Cordy, 1954). The hypertonicity of the facial muscles produces a “wooden” expression to the face. Food is often held in the mouth because it cannot be chewed normally, and salivation is often excessive. Even though prehension and mastication are severely affected, swallowing is unaffected. The tongue has increased tone and the horse will often curl the tongue from side to side. Some horses may show more involvement of one side so that the lips, tongue, and head movements are to one side. Circling, head tossing, excessive yawning, weight loss and depression are common. Pneumonia resulting from inhalation of feed is a serious complication. Once affected horses do not recover and will die of starvation if not euthanized.

Hound’s Tongue (*Cynoglossum officinale*)

Imported from Russia, hound’s tongue is a noxious weed that is widespread in North America. It is a biennial, forming a rosette of basal leaves the first year, the second year the plant produces flowering stems up to 4 feet tall. The flowers are reddish purple, followed by four nutlets at maturity, which are covered with hooked barbs facilitating their adherence to clothing and animal hair.

Hound’s tongue contains toxic pyrrolizidine alkaloids (PA) comparable to the levels found in the most toxic *Senecio spp*. The plant is rarely eaten in the green state, but horses find it palatable dried in hay. As little as 15 mg of dried plant/kg body weight fed to horses over a 2-week period induced fatal liver disease (Knight *et al.*, 1984) This equates to about 6 percent of a horse’s daily intake of food if the hound’s tongue is dried in the early blossom stage when the PA content averages 0.08 percent (Stegelmeier *et al.*, 1994).
Alsike clover (Trifolium hybridum)

Alsike clover is a perennial legume that is commonly grown for livestock consumption in northern regions of North America. Two disease syndromes encountered in horses have been associated with the grazing of alsike clover. The first is an irreversible liver disease often accompanied by neurologic disturbances and referred to as alsike clover poisoning (Nation, 1989). The second syndrome is one of photosensitivity without apparent concurrent liver disease and is referred to as trifoliosis (dew poisoning) (Nation, 1989). The specific toxins responsible for either of these syndromes have not been determined. A fungal toxin may be involved because alsike clover poisoning is often a problem in years when there is high rainfall and humidity (Nation, 1991).

A close association exists between liver disease and photosensitivity seen in horses and the grazing of alsike clover (Nation, 1991). Horses develop signs of liver failure including weight loss, jaundice, depression, and neurologic abnormalities. Significant elevations of specific liver enzymes are usually present and are indicative of severe liver disease (Colon et al., 1996). The appearance of photosensitization can be attributed to the accumulation of phylloerythrin in the horse’s circulation as a result of liver failure. The primary lesion found in horses with alsike clover poisoning is a grossly enlarged, fibrotic liver, with marked bile duct proliferation (Nation, 1991).

With both hounds tongue and alsike clover, the clinical presenting signs of toxicity are weight loss and photosensitization of the white skinned areas that result from the underlying liver disease. Especially in the case of pyrrolizidine alkaloid poisoning from plants such as hounds tongue and groundsel (Senecio spp.), the presence of photosensitization is a strong indicator of liver disease and a very poor prognosis for recovery.

Conclusions

Plant poisoning of horses continues to be a problem that requires constant vigilance of the horse owner to ensure their horse is on a good plane of nutrition, and that toxic native plants or noxious weeds do not become an issue. Using certified weed-free hay helps to reduce the problem of toxic plants being fed to horses through their hay. For further information on the plants discussed in this paper and the many other plants that can be a problem to horses, the reader is referred to the author’s web page: http://southcampus.colostate.edu/poisonous_plants.

References:


Appendix

Cultivated Trees and Plants Potentially Poisonous to Horses

The following commonly available trees, shrubs are often sold through plant nurseries, and pose a potential hazard to animals if they are planted in or around animal enclosures. If these plants are found to be desirable for landscaping purposes, it is important to position the plants well away from where animals can reach them. Furthermore it is essential to always provide a balanced nutritious diet to animals at all times so that they are not driven through hunger to eating unusual plant material. It is also important to remember that the careless disposal of tree and plant prunnings into an animal enclosure is a frequent cause of poisoning.
### Trees

- **Black walnut**  
  *Juglans nigra*
- **Red Maple and its hybrids**  
  *Acer rubrum*
- **Oaks**  
  *Quercus spp.*
- **Black locust**  
  *Robinia pseudoacacia*
- **Golden chain tree**  
  *Laburnum anagyroides*
- **Horse chestnut, buckeye**  
  *Aesculus spp.*
- **Choke cherry & other cherry trees**  
  *Prunus spp.*
- **Kentucky coffee tree**  
  *Gymnocladus dioica*
- **Russian Olive**  
  *Elaeagnus angustifolia*
- **Persimmon**  
  *Diospyros virginiana*
- **Chinese tallow tree**  
  *Sapium sebiferum*
- **China berry**  
  *Melia azadarach*
- **Avocado**  
  *Persea Americana*

### Shrubs

- **Yew**  
  *Taxus spp.*
- **Oleander**  
  *Nerium oleander*
- **Yellow oleander**  
  *Thevetia spp.*
- **Privet**  
  *Ligustrum spp.*
- **Hydrangea**  
  *Hydrangea spp.*
- **Rhododendron (azalea)**  
  *Rhododendron spp.*
- **Japanese Pieris**  
  *Pieris japonica*
- **Laurel**  
  *Kalmia spp.*
- **Black laurel**  
  *Leucothoe davisiiae*
- **Boxwood**  
  *Buxus sempervirens*
- **Burning bush**  
  *Euonymus atropurpurens*
- **Lantana**  
  *Lantana camara*
- **Angels Trumpet**  
  *Brugmansia spp.*
- **Mesquite**  
  *Prosopis veluntina*
- **Day/night blooming Jasmine**  
  *Cestrum diurnum, C. nocturnum*
- **Scotch broom**  
  *Cytissus scoparia*
NUTRITION OF THE YOUNG HORSE – RISKS OF OVERFEEDING

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Summary

Sound nutritional management of the young, growing horse involves many factors. Growth is not only the accretion of tissue, but development of the skeletal system as well. Looking beyond average daily gain and feed efficiency industry demands require rapid and consistent growth for early training and competition. While the NRC (2007) provides guidelines for prevention of nutrient deficiency, it does not adequately address needs for optimal growth and skeletal development. Furthermore, the issue of overnutrition has received limited attention. Developmental orthopedic disease (DOD) is a term used to describe a number of disorders including osteochondritis dissecans (OCD), epiphysitis, and flexural and angular limb deformities (McIlwraith, 2001). This multifaceted problem affects the entire equine industry and can be influenced by nutrition, exercise, and genetics.

Research into the role of nutrition in DOD has concentrated in three primary areas: digestible energy intake, protein intake, and mineral intake/balance. Many industry professionals believe that excessive digestible energy and crude protein intake, and the associated rapid growth, increase the risk of DOD in foals. Excessive intake of DE has been implicated in DOD, particularly when diets high in non-structural carbohydrates (NSC) are fed to horses with impaired glucose tolerance. This role of nutrition and metabolism in skeletal development requires further investigation. A common belief in the equine industry is that diets high in crude protein contribute to DOD. In response many producers have fed diets deficient in protein which may be detrimental to skeletal development (Glade, 1986). Finally, the role of mineral intake and imbalance may also play a role in DOD development. In particular, calcium, phosphorus, copper, and zinc all play roles in the development of bone and soft tissue. Therefore, disruptions in dietary concentrations, both deficient and excessive, may increase risk of DOD.

Introduction

In order to more closely meet industry demands, the nutrient requirements of young, growing horses must be more thoroughly investigated. At this time little is known about amino acids requirements, energetic needs and nutrition partitioning, and mineral requirements for optimal skeletal development. Current recommendations are based on data primarily from horses 6 to 18 months of age and of Thoroughbred breeding (NRC, 2007). There is limited data dealing with true nutrient deficiency, and even less information about excessive nutrient intake. In addition, many deficiency/excessive studies are confounded by alterations in other nutrients. For example, many researchers alter the concentrate: forage to increase digestible energy intake, but this also alters consumption of every other nutrient. Finally, many studies with young, growing horses provide poor estimations of forage intake and quality. Forage is often offered in the form of pasture with changing content with season, or hay is group fed with no individual intakes recorded.

It is common industry opinion that rapid growth contributes to increased risk of developmental orthopedic disease (DOD). While many factors have been implicated in this disease, including genetics...
and early exercise, it appears that nutrition does play a role. However, it does not appear that one nutrient or imbalance is to blame. It is clear that rapid growth does not increase risk of DOD if horses are managed properly through this time. This includes dietary management, exercise, and health.

Sound feeding management includes a balanced nutrient supply. Evaluating diets for more than just nutrient content, but the balance between (nutrient: calorie). High energy diets with nutrient imbalances may increase the risk. Growth is a combination of weight gain and skeletal development. Because horses reach 90% of mature height by 12 months of age this time period is important for skeletal development and nutritional management during this time is critical.

**Nutrition and Developmental Orthopedic Disease**

Developmental orthopedic disease (DOD) is a multifaceted problem and includes disorders such as osteochondritis dissecans (OCD), epiphysitis, and flexural and angular limb deformities (Jeffcott, 1991; McIlwraith et al., 1991; McIlwraith, 2001). Jeffcott (1996) described OCD as one of the largest problems in the horse industry, regardless of discipline or breed, and a significant loss of revenue for the equine industry. There is no clear cause for DOD, and it appears that genetics, nutrition, and exercise may all play a role (Jeffcott, 1996).

Incidence of OCD has often been linked to rapid rates of growth (Stromberg, 1979). However, it appears that rapid rate alone is not sufficient to induce the disorder, and growth rate is controlled by a variety of factors (Hintz, 1987; Hintz et al., 1979). Rapid and often inconsistent growth coupled with imbalanced nutrition in breeds predisposed to the disorder appears to have an increased incidence (Jeffcott, 1991). Several nutritional factors have been associated with OCD including minerals, protein, and energy in the form of soluble carbohydrates (Ralston, 1999). In particular, deficiencies and imbalances of calcium, phosphorus, copper, and zinc have all been linked to DOD.

**Energy**

Current dietary recommendations for energy describe digestible energy (DE) needs for both growth and maintenance as a function of age and body weight. At this time there is not sufficient data to make more specific recommendations based on breed, frame size, etc. In particular, requirements for gain are based on limited data and do not take into account nutrient partitioning, gender, or dietary components (forage vs. concentrate). While the goal of most nutrient recommendations is to prevent deficiency, the subject of overnutrition in the growing horses has received little attention (NRC, 2007).

Overnutrition has been explored as a possible cause of DOD and more specifically OCD. Glade and Belling (1986) reported increased body weight, but not height or heart girth, in weanlings fed 130% of NRC requirements for 8 months. The excessive plane of nutrition also resulted in increased cartilage DNA content and lowered levels of hydroxyproline and hexosamine content similar to horses fed 70% of NRC requirements. Additionally, other studies found increased incidence of OCD when young horses were fed at 128% of NRC requirements for digestible energy (DE) and crude protein. Foals fed only high crude protein levels and the control diet (100% NRC) had no radiographic evidence of OCD (Savage et al., 1993a and 1993b). When further investigating the influence of elevated DE and/or crude protein on the incidence of OCD in young horses, Glade et al. (1984) observed when high energy and protein diets (160% NRC requirements) were fed to young horses that serum cortisol levels were elevated, and that serum insulin peaked more rapidly resulting in a more rapid glucose decline post-meal. The authors proposed that this large insulin secretion may result in a conversion of thyroxin (T4) to triiodothyronine (T3).

Because collagen and proteoglycan synthesis are related to thyroid hormone concentrations it appears that DE, particularly soluble carbohydrate, may impact joint health (Glade et al., 1984). High intake of NSC, but not crude protein, causes blood T4 to peak before glucose, insulin, or T3. Glade et al. (1984) suggested that because postprandial rise in insulin and T4 does not occur simultaneously, cartilage maturation could be suppressed.
The interaction of nutrition and the endocrine system in the pathogenesis of OCD appear to be strongly related. Growth rate and skeletal development is regulated in part by the somatotropic axis composed of insulin, growth hormone (GH), and insulin-like growth factor I (IGF-I). In particular, IGF-I has been identified in the regulation of skeletal development. These endocrine factors help link nutrient intake to metabolism, thus regulating growth and skeletal development (Yakar et al., 2002).

Young, growing horses are often fed diets high in DE, with high energy diets achieved through products high in non-structural carbohydrate (starch). The use of high grain diets stimulates insulin secretion post-meal. Insulin secretion stimulates clearance of T₄ and the corresponding increase in T₃. It appears that hyperglycemia and hyperinsulinemia may play a role in OCD (Glade et al., 1984). In particular, foals that exhibit prolonged increases in circulating glucose and insulin in response to carbohydrate intake may be predisposed to OCD (Ralston, 1999). In vitro data suggests than insulin suppresses differentiation of chondrocytes (Henson et al., 1997). Research at Rutgers University showed a relationship between foals with exaggerated glycemic responses to an oral glucose challenge and the development of OCD (Ralston, 1999). A field study conducted with 218 Thoroughbred weanlings from 6 Kentucky farms observed an increased incidence of OCD lesions in foals that had an elevated glucose and insulin response to a single meal. This study suggested that a farm’s choice of feed and its non-structural carbohydrate (NSC) content may contribute to the incidence of OCD, particularly in individual horses that may be glucose intolerant (Pagan et al., 2001). In contrast, in two controlled feeding experiments weanlings were offered varying levels of dietary starch (0.0% to 34.7%) with no influence on rate of gain, body measurements, or bone mineral deposition. In addition, concentrations of T₄ were unaffected (Ott et al., 2005). It appears that the influence of dietary starch on incidence of OCD may be a multifaceted problem and may be more problematic on individuals with exaggerated glycemic response.

In response to previous studies that demonstrated a possible influence of dietary starch on skeletal development, further research was conducted to determine whether source of dietary calories (fat or starch) impacted growth, skeletal development, and the endocrine system. The use of high grain diets and the associated concerns with hyperinsulinemia have led to the increased use of fat as a DE source in equine diets. Fat supplementation as a replacement for NSC has been used in growing horses with no negative effects on growth or bone development (Scott et al., 1987; Davison et al., 1991; Ott et al., 2005). When fat was used to substitute NSC in weanling Quarter Horse diets, there were no changes in circulating levels of IGF-1, but glucose and insulin levels did decline when fat was increased from 2.21% to 10.3% of the concentrate and starch was reduced from 33.9% to 24.0% (Ropp et al., 2003). When growing Thoroughbreds were fed diets composed of starch and sugar compared to fat and fiber, there was no influence of diet on IGF-I concentrations. However, the authors noted a relationship between average daily gain and IGF-I, indicating interactions between age and environment that need to be explored further (Staniar et al., 2007).

**Protein**

When considering the protein requirements of young horses, it is important to consider the digestibility of dietary protein and the location of digestion. Young horses have reduced capability for fermentation in the large intestine compared to a mature horse and rely mainly on the upper tract for digestion of protein; therefore, the amino acid profile of the concentrate portion of the diet has more influence than the forage source (Gibbs and Potter, 2002). Young horses respond to amino acid supplementation are sensitive to both protein quantity and quality in the diet, allowing reductions in dietary crude protein when amino acid profiles are improved (Ott and Kivipelto, 2002).

While the dietary requirement for lysine has been established at 4.3% of the horse’s crude protein requirement (Ott et al., 1981), there is little information regarding the remaining essential amino acid requirements of the young growing horse (NRC, 2007). While lysine is considered the first limiting amino acid in equine diets; threonine has been suggested to be the second limiting amino acid, but the requirement has not been established (Graham et al., 1994; Staniar et al., 2001). Methionine may be
limiting due to its important roles in methyl group donation, free radical defense, and other crucial portions of protein structure and folding pathways (Brosnan and Brosnan, 2006). Methionine is the second limiting amino acid in other monogastric species at various stages of growth (Mavromichalis et al., 1998). However, recent work in horses did not determine a requirement (Winsco et al., 2009). In general crude protein requirements and needs for essential amino acids should be more closely investigated, particularly related to phases of growth.

Industry concerns about high dietary protein and increased incidence of OCD appear to be unfounded. Most likely it was imbalances of digestible energy and crude protein that led to previous reports of excessive protein and DOD in the 1970’s. In response breeders began to limit protein content of diets to restrict growth rate (Frape, 1989). Reductions in dietary protein decreased the rate of growth and were detrimental to bone development in young horses (Glade, 1986). Protein deficiency often restricts skeletal development; with foals receiving deficient diets having reduced gains of third metacarpal circumference with little influence on wither height (Schryver et al., 1987). It is important to consider the balance of DE, crude protein, and minerals when evaluating diets for young horses, particularly when producers begin supplementing nutrients on top of balanced commercial rations.

Minerals

While many minerals are critical to proper growth and development of young horses, it appears that calcium, phosphorus, copper, and zinc play important roles in skeletal development. Mineral imbalances have been implicated in the incidence of OCD. Research determining the influence of mineral imbalances on DOD has concentrated on calcium, phosphorus, copper, and zinc.

Dietary calcium has been fed at three times the NRC recommended levels with no increase in the incidence of OCD (Savage et al., 1993b). Likewise, Grace et al. (2003) determined that increasing calcium intake over a 3 month period had little influence on bone growth and development. However, Thompson et al. (1988) observed that when a diet high in DE (150% NRC) and low in Ca (35% NRC) was fed to weanlings that third metacarpal length and radiographic bone density were decreased. While it appears that overfeeding calcium has little impact on skeletal development, traditional diets such as whole oats, often contain low dietary calcium and excessive phosphorus (ratios lower than the recommended 1.5:1) due to intake of cereal grains without supplemental calcium (Frape, 1989). This illustrates the importance of balanced diets, particularly the balance of nutrients to calories, and relationships of minerals to each.

Cymbaluk and Christison (1989) fed high forage and high concentrate diets supplemented with various levels of P (0.24 to 1.06%) exhibited an 88% incidence of DOD. While signs of DOD coincided with normal ages of incidence, dietary treatment had no influence on rate of DOD in the herd and was not able to mitigate severity. The authors felt that poor mineral status pre-trial combined with the feeding regime of the study allowed for rapid weight gains.

Deficiencies in dietary copper have also been linked to increased incidence of OCD. Bridges and Harris (1988) were able to experimentally induce OCD in foals with low copper diets (1.7 ppm). These authors hypothesized that low copper status reduced collagen cross linkages via lysyl oxidase. Deficiencies in copper may reduce structural strength through cartilage defects rather than disruption of calcification. Another study also suggested that foals fed low copper diets (7 ppm) had greater incidences of OCD over 6 months when compared to foals fed high copper (25 ppm) diets (Hurtig et al., 1990). While copper supplementation of dams and foals appears to decrease incidence of OCD, it does not seem to remove the risk of DOD entirely. Mineral supplementation, particularly in balance with DE, may decrease the risk of DOD, but it does not appear to be the only factor in this multifactorial disease. In response to research on copper supplementation, it has become increasingly popular to supplement copper to young horse. However, overemphasizing copper in equine rations goes against sound nutritional management. Zinc acts as an antagonist to copper and the ratio of copper to zinc must be considered, and
excessive zinc intake increased the incidence of equine OCD in one intoxication case study (Messer, 1981). Recommendations for proper ratios of zinc: copper are currently 4:1 or 5:1.

**Conclusion**

Proper nutrition of young, growing horses involves both balanced diets and effective management. While overfeeding young horses seems to increase the incidence of DOD, imbalanced diets may also contribute to this disorder. When planning feeding programs for young horses it is important to recognize their high nutrient requirements and offer high quality ingredients that are digestible and nutrient dense. This digestive can not yet fully utilize forage to a large extent, so producers should avoid bulky, low quality ingredients. Young horses require diets that are calorically dense and respond well to the use of fat as an energy source. In horses with exaggerated glycemic responses fat may be an alternative to NSC as fuel for growth. Restricting crude protein in diets has been suggested as a method to decrease OCD, however it is not recommended. Diets low in crude protein decrease both growth and skeletal development. Careful consideration of dietary protein quantity and quality is important in the young horse. Mineral content and balance of diets must be carefully considered. Sound growth can be achieved by utilizing balanced diets that support steady growth while maintaining young horses in moderate body condition.

**References**


UPDATE OF NUTRIENT MANAGEMENT REGULATIONS AND THE EQUINE INDUSTRY

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Summary:

State and federal agencies are implementing stronger regulatory and accountability programs to control agricultural runoff. Equine operations are now beginning to be included in these regulations. Many state’s laws have regulated equine farms requiring farm managers to incorporate conservation practices. All horse farms are covered under the Federal Animal Feeding Operation (AFO/CAFO) these laws are regulated through the Environmental Protection Agency (EPA). Nutrient management planning is mandatory in many instances, record keeping and developed by a certified planner. The federal CAFO regulations require a comprehensive nutrient management plan (CNMP) be filed through the proper state agency. Equine operations with at least 500 head of horses (not 500 au) that are in confinement for 45 days (nonconsecutive) over a 12 month period are considered a CAFO. Some horse farms may be eligible for federal financial incentive programs; concentrated animal feeding operations (CAFO) must comply with the conditions in their nutrient management plan to avoid noncompliance. State requirements may be more stringent than the federal requirement. A specific farm operation's requirements are spelled out in their permit and it is against those requirements that the state and EPA will inspect an operation and evaluate compliance. A major goal of developing a CNMP is creating a whole farm nutrient balance on the farm. To meet this goal Best Management Practice (BMPs) are used to minimize nutrient and sediment from equine operations.

In some states a horse farm may be regulated by more than one agency. Each horse farm should have a plan for managing manure, pastures and mud. Know your state's law(s)!

Introduction

Proper management of equine operations requires the adoption of Best Management Practices (BMPs) strategies to balance nutrient production with nutrient utilization. States located in the Northeast region have reported that horses are the fastest growing segment of the livestock industry. Nationwide, equine has increased by 77% since 1997; and it is reported there are 9.5 million horses in the United States (American Horse Council, 2005). Governmental agencies are concerned about non-point sources of pollution and have focused on agriculture as a major contributor to water quality issues. Equine operations are now beginning to be included in these regulations. Many state’s laws have regulated equine farms requiring farm managers to incorporate conservation practices.

Recently, state’s Conservation Districts, Departments of Environmental Protection (DEP), USDA Natural Resources Conservation Service (NRCS), U.S. Environmental Protection Agency (EPA) and several other federal agencies are working together to implement stronger regulatory and accountability programs to control agricultural runoff. Their plans are to minimize nutrient and sediment from not only large agricultural operations but also small acreage animal farms. These government agencies are
targeting all small farm livestock species but are focusing on equine because of the large number of high
density horse operations in nearly all U.S. states. These agencies have identified areas of concern that
needed to be addressed, and highlighted the need to provide assistance and educational programs for
horse and small farm managers/owners. The object for all industry providers, extension educators and
agency personal will be identify Best Management Practice (BMPs) that would minimize nutrient and
sediment from equine and small acreage animal farms.

History

This “Nutrient Management Issues” is nothing new. The U.S. Congress recognized the
importance of meeting the challenge of continued growth and animal agriculture in the coastal zones by
passing the Coastal Zone Management Act (CZMA) in 1972. The Act, administered by NOAA's Office of
Ocean and Coastal Resource Management (OCRM), provides for management of the nation's coastal
resources, including the Great Lakes, and balances economic development with environmental
conservation. The CZMA outlines two national programs, the National Coastal Zone Management
Program and the National Estuarine Research Reserve System. The 34 coastal programs aim to balance
competing land and water issues in the coastal zone, while estuarine reserves serve as field laboratories to
provide a greater understanding of estuaries and how humans impact them. According to the United
States Department of Commerce (2007), the overall program objectives of CZMA remains to balance
"preserve, protect, develop, and where possible, to restore or enhance the resources of the nation's coastal
zone."

Concentrated Animal Feeding Operations (CAFO) Regulations

USDA and EPA held public listening sessions on the draft Unified National Strategy for Animal
Feeding Operations in 1998 and released the final Unified National Strategy for Animal Feeding
Operations in 1999. EPA conducted nine public meetings on the 2001 proposed CAFO regulations. The
purpose of the meetings was to enhance public understanding of the 2001 proposed regulations for
CAFOs. The meetings were not a mechanism for submitting formal comments on the proposal. They
involved a brief presentation by EPA officials on the proposed regulations followed by a question and
answer session. In 2003, revisions were released to the Environmental Protection Agency's (EPA)
regulatory requirements for concentrated animal feeding operations (CAFOs) under the Clean Water Act.
This final rule ensured that CAFOs take appropriate actions to manage manure effectively in order to
protect the nation's water quality. According to the U.S.DEP (2010), the rule revises two sections of the
Code of Federal Regulations (CFR), the National Pollutant Discharge Elimination System (NPDES)
permitting requirements for CAFOs (Sec. 122) and the Effluent Limitations Guidelines (ELGs) for
CAFOs (Sec. 412) (EPA-821-03-001).

Farms defined as Concentrated Animal Feeding Operations (CAFOs) and Concentrated Animal
Operations (CAOs) are required to develop written plans (Figure 1), but nutrient management plans for
these higher intensity animal operations follow a detailed process and must be developed by a Certified
Nutrient Management Specialist that is registered and certified by their specific state laws.
Current and History of the Chesapeake Bay Program

Clean Water Act passed in the US in the 1970’s and the USEPA designated the Bay as being impaired soon after. The Chesapeake Bay Program is a unique regional partnership that has coordinated the restoration of the Chesapeake Bay and its watershed since 1983. Bay Program partners include the states of Delaware, Maryland, New York, Pennsylvania, Virginia and West Virginia; the District of Columbia; the Chesapeake Bay Commission, a tri-state legislative body; EPA, representing the federal government; and participating citizen advisory groups.

In 1987, the District of Columbia, Maryland, Virginia, and Pennsylvania and the Federal Government signed an agreement to reduce the amount of nitrogen and phosphorus entering the Chesapeake Bay. The States included the control of runoff from farmland and manure in order to reduce nutrient-pollution. To date, limited studies have been conducted to look at horse management practices (and until recently horses have been exempt from the state CAFO regulations).

Recently, President Obama implemented a Chesapeake Bay Executive Order that is publishing a first-annual Action Plan. The plan is working through the U.S. Environmental Protection Agency (EPA). The U.S. EPA has established a "pollution diet," formally known as a Total Maximum Daily Load (TMDL) this identifies the amount of pollution that needs to be reduced to restore the Chesapeake Bay and its streams, creeks and rivers. The TMDL is based on cleanup plans developed by the six Bay states and the District of Columbia. The allocations that make up the “Draft TMDL” are based on a version of the Chesapeake Bay watershed model (5.3) that has only been functional since June 2010 (The White House, Press, 2010). Parts of this model update were made available for public review in June 2010. See http://ches.communitymodeling.org/models/CBPhase5/index.php

Federal agencies working together to implement President Obama’s Chesapeake Bay Executive Order have published a first-annual Action Plan that details $491 million in fiscal year 2011 funding and activities dedicated to restoration and protection of the Chesapeake Bay and its watershed, including meeting the specific goals set forth in the Executive Order strategy.

“The Action Plan for FY 2011 reflects a deep commitment and unprecedented coordination among federal agencies and the Obama Administration to improve our results in protecting and restoring the Chesapeake Bay and its watershed,” said Pete Silva, EPA Associate Administrator for Water. “The proposed funding and planned activities will help support state and local efforts, as well as be an investment in countless communities and local economies throughout the region.”
Allocations are based on funding proposed in the President’s Budget that is directly attributable to implementing the Executive Order strategy by the FLC agencies. This includes direct budgets for Chesapeake Bay activities, allocations of agency base funding toward the Executive Order strategy and shares of national programs that can be attributed to supporting the Executive Order strategy in the Chesapeake watershed. Among the restoration projects and programs identified for FY 2011: $72 million in financial and technical assistance targeted to help farmers implement voluntary conservation practices in high-priority areas; over $20 million directly to the states and the District to implement stronger regulatory and accountability programs to control urban, suburban, and agricultural runoff; and $30 million dollars for land protection (The White House, Press, 2009).

American Farm Bureau believes that EPS’s rule is unlawfully "micromanages" state actions and the activities of farmers, homeowners and businesses within the six-state Chesapeake Bay watershed. EPA’s plan imposes specific pollutant allocations on activities such as farming and home-building, sometimes down to the level of individual farms. The Clean Water Act, the AFBF action contends, requires a process that allows states to decide how to improve water quality and take into account the economic and social impacts on businesses and communities in the states (Southeast Press, 2011).

AFBF asserts that EPA violated the requirement that agencies allow meaningful public participation on new rules. The suit alleges that EPA failed to provide the public with critical information about the basis for the TMDL and allowed insufficient time (45 days) for the public to comment on the incomplete, but highly technical, information that EPA did provide (Southeast Press, 2011). This battle continues today.

Introduction to Manure Management: What the Agencies want Animal Operators To Do

Manure, when properly applied can provide plant nutrients for plant growth and improve the tillage, aeration and water holding capacity of soils. Applying manure in excess of plant needs, or at the wrong time, or handling it improperly, may release nutrients into water, where they can become pollutants. Leaching of N through the soil can raise groundwater nitrate levels above the EPA drinking water limit, which can adversely affect the health of young children and livestock. Surface movement of nitrogen and phosphorus in runoff increases levels of these nutrients in surface waters. There are consequences of surface water pollution associated with poor manure application practices which can lead to eutrophication and death of fish or other aquatic life. (PA DEP, 2001, Manure Manual). Nutrient management generally involves decision-making about a wide range of farm operations. These decisions include day-to-day details of farm operations, such as spreading manure on a specific field on a particular day, or deal with the long-range future of an entire farm, such as building a manure storage handling system. Nutrient management is an ongoing process including assessment, option selection, planning and implementation. This decision process is repeated as necessary, but at least annually or when conditions change.

The nutrient management options can be specific practices, such as incorporating field-applied manure soon after application, identifying other landowners who may be interested in having manure spread on their fields. The assessment and the options selected can be the basis for many decisions that will be made in the development of a farm manure management plan to allocate the manure and to determine any supplemental commercial fertilizer requirements.

Implementation of a manure management plan involves the actual activities called for in the plan plus the appropriate recording of those activities so that the effectiveness of plan implementation can be assessed. Because of factors beyond the farmer's control, such as the weather, and because of changes in management, even the best manure management plan may not be implemented exactly as prepared. Thus record keeping and assessment to evaluate where the implementation deviated from the plan are critical for improving the plan for the following year.
Plant nutrient management decisions deal largely with the flow of plant nutrients to, from and within farms. The organization of farms will lead to different patterns of materials, such as crops, fertilizers and manure, to be moved in the managed pathways of the farm operation. Understanding the various types of farm organization can be helpful in practically all activities associated with the nutrient management process for crop production and environmental protection. This is especially true in evaluating the nutrient management situation on farms. A major goal of nutrient management for crop production and for protecting the environment is to balance agronomic crop requirements and the supply of nutrients from all sources. Based on the movement of farm materials, and the nutrients contained in those materials, nutrient management should be sensitive to specific farm situations and existing strategies of farm management. (PA DEP, 2001, Manure Manual).

**Whole Farm Nutrient Balance Example**

A major goal of developing a comprehensive nutrient management plan (CNMP) is creating a whole farm nutrient balance on the farm. Assume that we have a farm boundary as illustrated with the dotted line (Figure 2). In a farming operation there are nutrient inputs such as feed, possibly irrigation water, fertilizer, possibly animals brought on the farm. Managed nutrient outputs include meat, milk, eggs, crops, and possibly manure that can be exported from the farm. The difference between inputs and outputs represents the amount of nutrients that must be assimilated on the farm. If more nutrients are generated in this difference than can be properly assimilated by the cropping system used on the farm a nutrient imbalance occurs. Excess nutrients will lead to nutrient losses through runoff, leaching and possibly gaseous emissions. What we really want is all these nutrient inputs and outputs are in balance. If not properly managed, nutrient imbalances can occur with buildup of nutrients on the farm to the level of potentially creating water and air (for nitrogen) pollution problems. The key reason for strategic planning is to determine if a farm has an adequate land base for long-term sustainability from a nutrient utilization perspective.

![Figure 2. Whole Farm Nutrient Balance Model with Inputs, Outputs and Nutrient Imbalances](extenson-animal-manure-site-2011)

Some outcomes of strategic planning are to select nutrient management strategies and evaluate different options or alternatives and determine the greatest benefit and success for a whole nutrient mass balance on the operation. A plan may define procedures to implement some strategies including the annual pasture plan, conservation plan, manure export plan and feed management plan, as needed to achieve whole farm nutrient mass balance.
Any successful nutrient management planning process should begin with the following fundamental questions: “Is my operation concentrating nutrients on the farm?” and “What is the underlying cause of nutrient concentrations?” Most nutrient related problems are associated with either a poor distribution of nutrients and or an excess of imported nutrients compared to exported nutrients.

**Some States are Requiring More Regulations**

Many state’s laws are beginning to regulate equine operations requiring farm managers to develop plans and incorporate conservation practices. Pennsylvania is a state that has more than one regulation. Later this year (2011), any farm that houses animals in the state of Pennsylvania will have to have a written Manure Management Plan, meeting the guidelines provided in the PA Department of Environmental Protection’s Manure Management Manual. According to the PA, DEP (2010), the plan simply needs to be kept on file at the farm and doesn’t need to be approved, unless the farm is a Concentrated Animal Operation (CAO) or Concentrated Animal Feeding Operation (CAFO).

Manure management requirements (PA DEP, 2010) are under revision, and the current proposed draft requires determining manure application rates and setbacks by using one of the following three options:

1. Using “book values” from a Manure Application Rate Chart based on the crop and manure type, with setbacks of 100 feet from water bodies, and 100 feet of sinkholes or drinking water sources;
2. Calculations using the Nitrogen or Phosphorus Balance Worksheets, with setbacks of 150 feet from streams and other water bodies, and 100 feet from sinkholes or drinking water sources; or
3. A Manure Management Plan developed by a certified planner using the Phosphorus Index and setbacks of 100 feet (or vegetated 35-foot buffers) from streams or other water bodies or sinkholes, and 100 feet from drinking water sources (PA, DEP, 2010).

The proposed regulation found in the “Chapter 91, PA Manure Management Manual” would also require the winter application maximum rates of 5,000 gallons liquid or 20 tons solid manure, and restricted manure spreading to fields with at least 40% cover crop and slopes less than 15%. Farm managers will need to keep records of manure applications, inspections of facilities, repairs, and other practices. They will need to manage animal concentration areas (such as barnyards, feedlots, loafing areas) to prevent manure runoff to water bodies. In addition, pastures within 100 feet of waters (unless there is a 50-foot non-grazed permanent vegetative buffer) to minimize manure runoff, with stocking rates and other measures will need to be managed. Manure stockpiles or stacks will need to be managed so rainwater does not transport manure (PA, DEP, 2010).

Manure Management Plans can be prepared by the farmer although assistance is also available from a variety of sources including certified nutrient management specialists, certified manure brokers, county conservation districts, Natural Resource Conservation Service (NRCS) staff and farm organizations.

Keep in mind, that even though, there are national guidelines for Nutrient Management Plans (NMP), they need to be cognizant of state specific requirements. Sometimes there may be state regulations to be followed, state specific P indexes that may indicate whether manure can be N or P-based, winter spreading restrictions, nutrient standards, etc. There may also be producers who are fulfilling more than one requirement, such as seeking NRCS cost share requirements and state CAFO permits that may have similar but different requirements.
Some farmers may have operations that are Concentrated Animal Operations under the Nutrient Management Act Regulations, or Concentrated Animal Feeding Operations under the state’s strategy for meeting federal requirements. These farmers would follow requirements in addition to those found in this manual. Farm managers who do not follow these requirements and the practices listed in the Pa Manure Manual publication will be required to obtain DEP approval or a water quality permit.

The PA Environmental Quality Board (Board), through the DEP, amended Chapter 102 (relating to erosion and sediment control and storm water management). This is part of the Federal Clean Water Act "Phase II" National Pollutant Discharge Elimination System (NPDES) permit requirements for storm water discharges associated with construction activities, codifies post construction storm water management (PCSM) requirements, including BMPs. The Chapter 102 order was adopted May 17, 2010 and took effect November 19, 2010. This same board is reviewing the “PA Manure Manual, Chapter 91” order and is expected to be adopted spring 2011.

Other State’s Nutrient Management Regulations

Concentrated animal feeding operations (CAFO) must comply with the conditions in their nutrient management plan to avoid noncompliance. State requirements may be more stringent than the federal requirement. A farm operation's requirements are spelled out in their permit and it is against those requirements that the state and EPA will inspect an operation and evaluate compliance. Some state’s laws require the development of P-Index based manure or nutrient management plans and use of RUSLE2. The Mid-Atlantic region has had environmental policies in place in several states beginning as early as the 1990’s (Table 1.).

<table>
<thead>
<tr>
<th>Environmental Policy Development in the Mid-Atlantic Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennsylvania Nutrient Management Act (1993-97)</td>
</tr>
<tr>
<td>Maryland Water Quality Improvement Act (1998)</td>
</tr>
<tr>
<td>North Carolina Moratorium (New Swine Farms) (1998-?)</td>
</tr>
<tr>
<td>Virginia Poultry Waste Management Act (1999)</td>
</tr>
<tr>
<td>Delaware’s TMDLs (1997) and NMP Act (1999)</td>
</tr>
<tr>
<td>USDA-USEPA Unified AFO Strategy (1999)</td>
</tr>
<tr>
<td>USEPA CAFO Rule (2002)</td>
</tr>
</tbody>
</table>

Table 1. Mid-Atlantic Region’s Environmental Policy Dates Regulations were Adopted.

Nutrient Management Planning for Arkansas

Federal Animal Feeding Operation (AFO/CAFO) Regulation are regulated through the Environmental Protection Agency (EPA). Nutrient management planning is mandatory in many instances, record keeping and developed by a certified planner. In Arkansas, a farm has to have at least 500 horses,
must be in confinement for 45 days (nonconsecutive) over a 12 month period. Some horse farms may be eligible for federal financial incentive programs, Environmental Quality Incentives Program (EQIP). Farms located in identified nutrient-sensitive areas of the state may be required to do more regulation due to Arkansas Acts 1059 and 1061, which took effective in 2004.

**Nutrient Management Planning for Virginia**

The Virginia Department of Conservation and Recreation works to manage both urban and agricultural nutrients found in fertilizers, manure, municipal sewage sludge and other sources. Regulations focus on manure management, BMPs, P-Index management, and VA has a “no discharge” permit requirement. The state regulation animal feeding operation and defines them as 150 horses, 300 slaughter steers or 200 dairy cattle; and are kept in confinement for 45 days, over a 12 month period.

**Nutrient Management Planning for New Jersey**

The New Jersey Department of Agriculture coordinates the Animal Waste Management Regulations that require facilities with as few as 8 animals to develop animal waste management plans. Agricultural management professionals in New Jersey assist livestock operations in preparing environmentally responsible animal waste management plans. The rule has a tiered approach with only larger farms (200-300 animal units) having to complete a fully certified nutrient management plan. The farms have more than 7-10 animal units will complete a self-certified plan. The smallest farms (less than 7-10 animal units) will not be required (but encouraged) to complete any plan at all.

**Nutrient Management Planning for Delaware**

Delaware's law dictates that anyone with eight animal units have a waste management plan, which provides specific guidelines about where and how to store manure and document what is done with it. In Delaware, one horse equals 1.25 animal units, so only properties with seven or more horses must comply. Also, if fertilizer is applied to 10 or more acres, one must have a nutrient management plan. (DE, DOA, 2011)

**Nutrient Management Planning for West Virginia**

Regulations took effect in 2010. At this time there are two CAFO racetracks under NPDES requirements. The West Virginia Department of Agriculture (WVDA) has established the nutrient management certification program.

**Nutrient Management Planning for Maryland**

In 1998 the Water Quality Improvement Act, took effect and horses were included July of 2004. The regulatory agency is the Maryland Department of Environment (MDE). However, in addition Maryland will also be regulated under the EPA Chesapeake Bay TMDL.

Under the Water Quality Improvement Act, horse operation that make $2,500 gross annual income or house 8 AU's (MDE) are required to file NMP. Farms are required to update their nutrient management plans once every three years and more if operation changed.
**Nutrient Management Planning for North Carolina**

North Carolina 1993 developed its own water quality permitting program through the N.C. Division of Water Quality (DWQ), Department of Environmental & Natural Resources (DENR). Facilities are subject to state permits if they include 75 head of horse (or 100 confined cattle) in the operation.

**Nutrient Management Planning for Kentucky**

Water Quality Act 1998 requires a plan be developed for any operation with land base of 14 contiguous acres.

Twenty-five states administer a state NPDES CAFO Program with some other state permit, license, or authorization program. In most cases, this additional state authorization is a construction or operating permit.

The bottom line is that all farms must have proper soil conservation and manure management planning. The plan must be implemented. Manure needs to be applied at a proper rate, time, location and method to ensure it does not pollute. State programs provide guidance on these topics. A benefit of following these state approved practices is that most states will help defend (in court) a farm manager if there is a problem. Help is available on soil conservation and manure management practices. Farmers can use the help of private consultants, conservation districts, cooperative extension and NRCS in developing conservation plans.

**Best Management Practices for Equine Operations**

Each farm should have a plan for managing manure, pastures and mud. Construct sacrifice areas (hard porous surfaces, holding lots) to keep horses off pastures and maintain grass cover year around. These sacrifice lots help with mud/dust management. According to a Maryland equine study, 39% of the state’s horse farms do not utilize sacrifice lots to rest pastures (Fiorellino et al., 2009). A similar Pennsylvania study reported 54% did not use sacrifice lots (Swinker et al., 2011). Pasture stocking rates should be managed for over and under grazing. Horse farms should have a pasture management plan that includes some rotational grazing or hourly limited turn-out, permitting pasture grass to recover. When asked most horse farms (78% Maryland, 65% Pennsylvania) do use some form of rotational grazing on their operations.

Store manure in a dry, level, location free from storm-water runoff. Actively compost manure and bedding; utilize or dispose of manure properly. Most Pennsylvania horse farms (52.3%) reported storing manure on unprepared sites. In addition to manure management, order/dust management should be included in the CNMP plan. Some state added requires for order management into their regulations.

Keep clean surface water clean. Fence horses out of stream and riparian areas. Manage storm-water to prevent manure contamination of water and eliminate runoff. Place gutters and down spouts on all buildings and channel the rain water away from high density animal areas. Most (76.3%) horse farms reported having some rainwater runoff systems to divert runoff or for a collection systems on buildings (Fiorellino et al., 2010 and Swinker et al., 2011). Equine shower stalls and brown waste water should be filtered using grassy catch buffers.

Precision feeding is a successful nutrient management tool it requires formulating feed rations to meet the horses NRC requirements. Precision feeding programs are significant keys to successful nutrient
management any operations. Nutritional technologies are available to reduce both nutrient excretion and limit land requirements for nutrient application. Up-to-date research information will impact the animal's diet, nutrients lost in the storage of manure, the application of those nutrients to the land, and the amount and proportion of nutrients available to plants. State NRCS has some cost share programs available to farm managers that qualify (USDA, NRCS, 2010).

Conclusion

Incorporating BMPs should be the goal for all animal agricultural operations and developing a comprehensive nutrient management plan (CNMP) helps create whole farm nutrient balance. Only a few horse farms are utilizing BMPs to help reduce environmental impact (Fiorellino et al., 2010 and Swinker et al., 2011). More educational programming and cost share funding is needed to target specific BMPs underutilized by the equine industry. Each horse farm should have a conservation plan to manage manure, pastures and mud. State requirements may be more stringent than the federal requirement. Know your state’s nutrient management law(s)!

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Pennsylvania Department of Environmental Protection, November 15, 2001, Manure Management for Environmental Protection, Document MM1, 361-0300-001.
USDA, Natural Resource Conservation Service (NRCS), November 2010, Feed Management, Increases Profitability and Decreases Nutrient Levels, 11-10.
THE EFFECT OF EUROPEAN UNION BROILER AND LAYER DIRECTIVES ON THE COMPOSITION OF POULTRY FEEDS

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Summary

Increased focus on animal welfare in the European Union has resulted in directives for poultry husbandry. This results in changes from housing in cages to alternative housing systems for laying hens, pressure on maximum stocking densities in broiler chickens and on increased focus on welfare in restricted fed broiler breeders.

Alternative housing systems for laying hens can result in an increased feed intake and higher mortality due to pecking / cannibalism. Dietary balanced crude protein levels can be decreased to some extend due to the increased feed intake, but dietary calcium levels can not be lowered on the basis of the limited amount of research data that is available. Due to positive effects of low-energy or high non-starch polysaccharides (NSP) diets on feather pecking behaviour, more NSP-rich feeds are used during the rearing and laying period in order to prevent cannibalism.

In broiler breeders, low-density, fibre rich feeds have a limited effect on the reduction of hunger feeling. Because of the effects of these feeds on breeder and offspring performance, low-density diets are applied in practice. A further reduction of hunger feeling might have to be realised by higher feed allowances in the rearing period. Regarding broiler chickens, maximum stocking densities can be increased when criteria for mortality and foot pad lesions are met. Therefore, there is increased pressure on maintaining a good (gut) health and litter quality. This is realised by formulating on minimum crude protein and mineral levels, adjusting feed composition during periods with a high incidence of intestinal diseases and use of specific feed additives.

Introduction

Animal welfare and food safety are important issues in the European Union (E.U.). Basic guidelines for bird welfare have been provided for the EU in directive 1998/58/EU for animal husbandry in general, in 1999/74/EU for laying hens and in directive 2007/43/EU for broiler chickens. In addition to this, individual countries can add own directives, like the directive welfare standards broiler breeders 2003 of the Dutch Product Board for Poultry and Eggs. These directives include rules for supply of drinking water and feed, stocking density, ventilation and heating, light intensity and day length, litter material, cleaning and monitoring of bird health. The E.U. directives for animal welfare can have consequences for the feed composition for poultry. This paper will focus on these effects. Effects of E.U. food safety directives, like the use of GMO feedstuffs and reduction of Salmonella spp., on poultry feed composition will not be discussed here. Up to now, these directives have had less impact on feed composition than more welfare related directives.
Implications of E.U. Directives on Layer Feed Composition

The E.U. directives for laying hens imply that from next year on, all hens have to be housed in alternative systems, like aviaries, floor housing or enriched cages. In laying hens housed in alternative systems, feed intake is in general higher than in cage housed layers (Enting and Pérez de Ayala, 2007; Table 1). If nutrient requirements, like for amino acids and minerals, are not affected by housing system, these required nutrient levels might be lower in feeds for hens housed in alternative systems than for hens housed in cages.

Table 1. Relative Production of Laying Hens Housed in Different Housing Systems in Practice (Enting and Pérez de Ayala, 2007; parameters of white hens in cages are set at 100)

<table>
<thead>
<tr>
<th>Housing System</th>
<th>Feed intake</th>
<th>% Lay</th>
<th>Egg weight</th>
<th>Egg mass</th>
<th>FCR</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage housed white hens</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Cage housed brown hens</td>
<td>102.0</td>
<td>97.4</td>
<td>102.7</td>
<td>99.1</td>
<td>102.9</td>
<td>110.3</td>
</tr>
<tr>
<td>Floor housed hens</td>
<td>111.2</td>
<td>97.6</td>
<td>101.6</td>
<td>98.5</td>
<td>112.7</td>
<td>126.5</td>
</tr>
<tr>
<td>Free range hens</td>
<td>112.6</td>
<td>97.1</td>
<td>101.8</td>
<td>98.7</td>
<td>114.3</td>
<td>138.2</td>
</tr>
</tbody>
</table>

Eits et al. (2005) studied the effect of dietary balanced protein on laying hen performance depending on the housing system (Table 2 and 3). The response to increased dietary balanced protein levels was dependent on housing system. Significant interactions between housing system and dietary balanced protein levels were observed for all performance parameters with exception of feed conversion ratio. Figure 1 is illustrating this for egg mass. The average difference in feed intake between the two housing systems was 8.7 %. The estimated differences in requirements for laying percentage and egg mass, expressed as g/kg of feed, were 9.3 and 8.7 % respectively. Therefore, the difference in feed intake between the housing systems corresponds with the requirements for laying percentage and egg mass. This indicates that if the requirements are expressed as intake per day, the requirements are not affected by housing system for these parameters. The requirements for egg weight and feed conversion ratio, expressed as g/kg, did not differ between housing systems. Therefore, the difference in balanced crude protein levels in feeds for hens housed in cages and hens housed in alternative systems is in practice smaller than the relative difference in feed intake between these housing systems.

Figure 1. Effect of Housing System on Egg Mass with Increased levels of Digestible Lysine (Eits et al., 2005)
Stress can have a negative effect on performance apart from a reduction in feed intake (Emery et al., 1984; Star et al., 2008), which might affect dietary amino acid to energy ratios. This is another reason that in practice balanced protein levels in feeds for hens in alternative systems are not decreased in proportion with the difference in feed intake between hens housed in cages and housed in alternative systems. Moreover, Ambrosen and Petersen (1997) showed that low dietary protein levels have a negative effect on feather condition and mortality rate due to cannibalism. Also this explains that dietary balanced crude protein levels in practice are not decreased that much in diets for hens housed in alternative systems as compared with hens housed in cages.

As for dietary protein levels, also dietary calcium and available phosphorus levels might be different for hens housed in alternative systems compared with hens housed in cages. This might be due to differences in feed intake, calcium metabolism due to a more active bone mineralization process in hens that are more active (Arafa and Harms, 1987) and to recycling of litter material. Den Hertog et al. (unpublished) studied the effect of feeds which differed in calcium and digestible phosphorus levels in caged and floor housed laying hens: 25 g/kg Ca with 2.2 g/kg dP, 30 g/kg Ca with 2.6 g/kg dP, 35 g/kg Ca with 3.0 g/kg dP and 40 g/kg Ca with 3.4 g/kg dP (Table 4).

Performance parameters were not clearly affected by housing system, which might have been due to the fact there was no clear difference in feed intake between the two housing systems. No significant interactions between calcium level and housing system were observed for performance parameters. Eggshell characteristics were significantly affected by calcium level, housing system and the interaction between calcium level and housing. Although feed intake and calcium intake did not differ clearly between housing systems, eggshell parameters were impaired at the lowest calcium level in floor housed laying hens. Therefore, it seems that for floor housing a higher safety margin is required for dietary calcium levels than for cages.

Table 3. Linear and Quadratic Models Describing Egg Production and Feed Conversion Responses to Dietary Balanced Protein Content in Caged and Floor-housed Laying Hens (week 23-34; Elts et al., 2005)

<table>
<thead>
<tr>
<th>Housing system</th>
<th>% Lay</th>
<th>Linear R²</th>
<th>Quadratic R²</th>
<th>Balanced protein requirement (CP), g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Lay</td>
<td>Cage</td>
<td>0.38</td>
<td>0.51</td>
<td>164</td>
</tr>
<tr>
<td>% Lay</td>
<td>Floor</td>
<td>-0.01</td>
<td>0.14</td>
<td>150</td>
</tr>
<tr>
<td>Egg weight, g</td>
<td>Cage</td>
<td>0.76</td>
<td>0.75</td>
<td>≥ 175</td>
</tr>
<tr>
<td>Egg weight, g</td>
<td>Floor</td>
<td>0.75</td>
<td>0.78</td>
<td>≥ 175</td>
</tr>
<tr>
<td>Egg mass, g/day</td>
<td>Cage</td>
<td>0.80</td>
<td>0.83</td>
<td>≥ 175</td>
</tr>
<tr>
<td>Egg mass, g/day</td>
<td>Floor</td>
<td>0.32</td>
<td>0.51</td>
<td>161</td>
</tr>
<tr>
<td>FCR</td>
<td>Cage</td>
<td>0.72</td>
<td>0.71</td>
<td>≥ 175</td>
</tr>
<tr>
<td>FCR</td>
<td>Floor</td>
<td>0.80</td>
<td>0.83</td>
<td>≥ 175</td>
</tr>
</tbody>
</table>

1 Balanced protein requirement to maximise egg production or minimise feed conversion ratio, calculated from the quadratic model. In case of a linear model, the balanced protein requirement is set at ≥ 175 g/kg
Table 2. Effect of Housing System (H) and Dietary Balanced Protein Level (P) on Performance in Week 23-34 (n=46, where n is the number of experimental units analysed (Eits et al., 2005))

| Housing | Crude protein, g/kg | 130 | 145 | 160 | 175 | 130 | 145 | 160 | 175 | H       | P       | H*P    |
|---------|---------------------|-----|-----|-----|-----|-----|-----|-----|-----|------|--------|--------|--------|
| Feed intake, g/day | 134.5 | 132.8 | 125.6 | 122.1 | 114.0 | 121.2 | 124.0 | 121.5 | <0.001 | 0.002 | <0.001 |
| % Lay | 93.2 | 94.6 | 94.5 | 92.1 | 88.1 | 93.6 | 96.5 | 95.6 | 0.882 | 0.001 | 0.002 |
| Egg weight, g | 58.5 | 60.0 | 61.6 | 61.9 | 57.5 | 60.0 | 61.5 | 64.1 | 0.403 | <0.001 | 0.007 |
| Egg mass, g/day | 55.4 | 58.1 | 59.3 | 58.5 | 51.2 | 56.9 | 60.3 | 62.6 | 0.899 | <0.001 | <0.001 |
| FCR | 2.43 | 2.29 | 2.12 | 2.09 | 2.23 | 2.13 | 2.06 | 1.94 | <0.001 | <0.001 | 0.116 |

Table 4. Effect of Different Dietary Calcium and Digestible Phosphorus Levels on Performance and Eggshell Characteristics in the Period from 22-27 Weeks of Age in Hens Housed in Cages and on the Floor (Den Hertog et al., unpublished)

<table>
<thead>
<tr>
<th>Calcium, g/kg</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>P-value</th>
<th>C</th>
<th>H</th>
<th>C*H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake, g/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 22-24</td>
<td>119.0</td>
<td>118.7</td>
<td>119.9</td>
<td>122.2</td>
<td>116.5</td>
<td>117.2</td>
<td>121.1</td>
<td>124.2</td>
<td>0.001</td>
<td>0.165</td>
<td>0.167</td>
<td></td>
</tr>
<tr>
<td>Week 25-27</td>
<td>115.2</td>
<td>116.2</td>
<td>114.9</td>
<td>117.1</td>
<td>123.4</td>
<td>116.7</td>
<td>114.1</td>
<td>119.5</td>
<td>0.404</td>
<td>0.069</td>
<td>0.127</td>
<td></td>
</tr>
<tr>
<td>% Lay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 22-24</td>
<td>0.95</td>
<td>0.94</td>
<td>0.93</td>
<td>0.95</td>
<td>0.91</td>
<td>0.95</td>
<td>0.94</td>
<td>0.94</td>
<td>0.499</td>
<td>0.192</td>
<td>0.230</td>
<td></td>
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<tr>
<td>Week 25-27</td>
<td>0.95</td>
<td>0.97</td>
<td>0.96</td>
<td>0.98</td>
<td>0.94</td>
<td>0.93</td>
<td>0.89</td>
<td>0.97</td>
<td>0.087</td>
<td>0.894</td>
<td>0.461</td>
<td></td>
</tr>
<tr>
<td>Egg weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 22-24</td>
<td>53.3</td>
<td>54.3</td>
<td>54.6</td>
<td>54.8</td>
<td>52.0</td>
<td>53.6</td>
<td>54.6</td>
<td>54.1</td>
<td>0.001</td>
<td>0.288</td>
<td>0.437</td>
<td></td>
</tr>
<tr>
<td>Week 25-27</td>
<td>57.4</td>
<td>57.8</td>
<td>58.2</td>
<td>57.9</td>
<td>56.6</td>
<td>57.0</td>
<td>57.7</td>
<td>57.7</td>
<td>0.003</td>
<td>0.078</td>
<td>0.186</td>
<td></td>
</tr>
<tr>
<td>FCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 22-24</td>
<td>2.35</td>
<td>2.33</td>
<td>2.35</td>
<td>2.36</td>
<td>2.43</td>
<td>2.30</td>
<td>2.30</td>
<td>2.48</td>
<td>0.579</td>
<td>0.920</td>
<td>0.808</td>
<td></td>
</tr>
<tr>
<td>Week 25-27</td>
<td>2.13</td>
<td>2.07</td>
<td>2.04</td>
<td>2.05</td>
<td>2.28</td>
<td>2.17</td>
<td>2.15</td>
<td>2.12</td>
<td>0.001</td>
<td>0.094</td>
<td>0.329</td>
<td></td>
</tr>
<tr>
<td>Eggshell thickness, mm</td>
<td>0.363</td>
<td>0.397</td>
<td>0.420</td>
<td>0.384</td>
<td>0.378</td>
<td>0.377</td>
<td>0.391</td>
<td>0.387</td>
<td>&lt;0.0001</td>
<td>0.066</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td>% Shell</td>
<td>9.19</td>
<td>9.91</td>
<td>10.02</td>
<td>10.04</td>
<td>10.02</td>
<td>9.85</td>
<td>10.17</td>
<td>10.08</td>
<td>&lt;0.0001</td>
<td>0.0004</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>SWUSA1, mg/cm2</td>
<td>90.45</td>
<td>98.86</td>
<td>99.34</td>
<td>99.37</td>
<td>97.96</td>
<td>96.25</td>
<td>99.92</td>
<td>98.62</td>
<td>&lt;0.0001</td>
<td>0.0006</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

1 Specific weight per unit surface area
Compared to hens housed in cages, hens housed in alternative housing systems show a higher mortality rate (Enting and Pérez de Ayala, 2007). This increased mortality rate is partly due to an increase in feather pecking and cannibalism. In order to reduce feather pecking behaviour and cannibalism, different diets have been tested. Van Krimpen et al. (2008) concluded that hens that were fed low-energy feeds or feeds high in, coarsely ground, non-starch polysaccharides (NSP) spend more time on feed intake in comparison with hens that were provided a control feed. Due to this, hens showed less feather-pecking behaviour. The same authors also found that hens fed standard-NSP diets had more feather damage compared with hens fed high-NSP feeds (Van Krimpen et al., 2009). They observed an effect of rearing diets on behaviour during the laying period and hypothesized that pullets were increasingly imprinted on feed as pecking substrate if dietary dilution level increased. This explained that feather pecking was decreased and feather condition was improved at 49 weeks of age the rearing diet was diluted. Hartini et al. (2002) and Hetland and Choct (2003) also found a reduced incidence of feather pecking and an increased feed consumption time and on increased dietary levels of insoluble fibre, indicating an increased satiety. Therefore, there is a tendency towards more NSP-rich feeds during the rearing and laying period of hens housed in alternative systems in order to reduce feather pecking and cannibalism.

**Implications of E.U. Directives on Broiler Breeder Feed Composition**

There are no specific E.U. directives that focus on current levels of feed restriction in broiler breeder. However, there has been increased attention to welfare associated effects of feed restriction, because there are several indications for impaired broiler breeder welfare at current feed restriction levels. Changes in behaviour, like an increase in stereotypic behaviour and hyperactivity, increased plasma corticosterone levels, heterophil to lymphocyte ratios and feed intake motivation have been reported (Gross and Siegel, 1983; Savory and Moros, 1993; Hocking et al., 1993; 1996; Savory et al., 1996; De Jong et al., 2002; 2003). These changes seem to be related to hunger feeling and may be associated with chronic stress.

Increased dietary insoluble fibre contents of different origins (cellulose, methylcellulose or rice hulls) resulted in an increased feed passage rate in broiler chickens and broiler breeders (Hennig and Jeroch, 1965; Savory, 1980; Alvares and Sanz, 1984; Leeson et al., 1991). Savory (1980), who reduced the energy content of quail diets with 40 % cellulose, found an increased feed intake, an increased feed consumption time and shorter intervals between meals. Based on these findings, Savory (1980) concluded that nutritional satiety is not substantially improved with diluted diets. However, Zuidhof et al. (1995) and De Jong et al. (2005) indicated that a low-density diet may have a positive effect on broiler breeder welfare. Behavioural data and the results of the feed intake motivation test indicated that birds experience less frustration and hunger on low-density diets as compared with birds provided normal diets. Positive effects were only found during the first half of the rearing period. Birds still performed stereotypic object pecking during a large part of the observation time, indicating that birds still suffered from hunger and frustration of the feeding motivation (Savory and Moros, 1993; Hocking et al., 1996; Savory and Kostal, 1996).

Results of research indicated that with the restriction levels that are applied in broiler breeders, it is difficult to alleviate hunger feeling by feeding low-density diets when the total energy supplied to the birds is not changed. De Jong et al. (2005) concluded that in the short term broiler breeder welfare during rearing may benefit from diet dilution, but for a more substantial improvement of broiler breeder welfare during rearing other solutions should be studied. Changes in feed allowance during the rearing period, as was studied by Bruggeman et al. (1999) in combination with low-density feeds might be one option to further improve broiler breeder welfare. Due to the positive effects of low-density feeds on breeder and offspring performance and its potential positive effect on bird uniformity (Enting et al., 2007a; b), low-density feeds are applied in practice mainly for these reasons.
Implications of E.U. Directives on Broiler Chicken Feed Composition

E.U. directive 2007/43/EU indicates that the stocking density of broiler chickens should not exceed 33 kg/m$^2$. The directive leaves room to increase this to 39 or 42 kg/m$^2$ when criteria for mortality are met. New additional criteria will also include foot pad lesion incidence, which put a high pressure on litter quality since these seem to related with each other (Francesch and Brufau, 2003; Mayne, 2005, Shepherd and Fairchild, 2010). The pressure on maintaining a good litter quality is also increased due to the fact that use of antibiotics in drinking water has to be decreased and that no growth promoters are allowed in feeds; wet litter is frequently associated with intestinal imbalances, which then results in the use of antibiotics to improve intestinal health and thereby litter quality.

Due to the pressure to maintain a good litter quality, feeds are formulated on the minimum requirements for minerals, since higher levels results in an increased water intake and hence higher water content of excreta (Pesti et al., 1999; Smith et al., 2000a; b). Also a narrow calcium to digestible phosphorus ratio is applied in feeds for growing birds, since wide calcium to digestible phosphorus levels can result in wet litter (Pos et al., 2003). The relative effect of minerals on litter quality is provided in Table 5 (Enting et al., 2009). Effects of sodium, potassium and magnesium increase with age, while chloride has a less clear effect. The negative effect of magnesium on litter quality is more profound when dietary levels exceed 3 g/kg. The effects of calcium and phosphorus on litter quality are stronger in younger birds (Enting et al., 2009).

Besides on minimum mineral levels, also dietary amino acid and fermentable crude protein levels are as low as possible in practice in order to maintain a good litter quality. Increased dietary crude protein levels result in increased water consumption (Marks and Pesti, 1984; Larbier and Leclercq, 1992) and hence increase the risk of wet litter. Moreover, Nagaraj et al. (2007) demonstrated that incidence and severity of foot pad lesions was significantly affected by protein level. In practice, feeds for growing birds are formulated on the basis of minimum levels for digestible lysine, methionine, methionine+cystine, threonine, tryptophan, arginine, valine, isoleucine and glycine+serine, while feeds for laying birds are formulated on the basis of digestible lysine, methionine, methionine+cystine, threonine, tryptophan, valine, isoleucine and total digestible amino acids (or in some case on the basis of total non-essential amino acids) in order to reduce protein levels without negative effects on performance. In addition to this, the amount of fermentable crude protein is minimized in order to prevent wet litter. The amount of fermentable crude protein is hereby defined as the amount of non digestible crude protein which is subtracted by the amount of protein that is normally not fermented by bacteria (as determined in the rumen of cows; Trouw Nutrition, unpublished).

Because Adams et al. (1996a; b) found that in particular the fat digestion is impaired during a coccidiosis infection, dietary fat levels are reduced and starch levels are increased when intestinal infections occur in practice. This helps to maintain a better litter quality, since less undigested fat is present in excreta. Also levels of carbohydrases are increased above recommended levels in order to control litter quality in the case digestion disorders occur. Water insoluble cell walls, that are primarily non-viscous, non-fermentable carbohydrates, have a positive effect on excreta structure and excreta moisture loss (Carre et al., 1994; Enting et al., 1999) and are therefore increased in order to prevent wet litter problems, especially in young broiler chickens and laying hens.

In order to replace anti microbial growth promoters in poultry feeds and to control intestinal microflora, medium chain fatty acids, short chain fatty acids and their salts, especially butyrates, herb extracts, including saponins and tannins, and essential oils are used (Den Hartog et al., 2005; Barug et al., 2006; Gutiérrez del Alamo et al., 2007). It seems that positive effects on performance of these products do not have to coincide with improved litter quality or reverse (Nutreco Poultry and Rabbit Research Centre, unpublished results). Therefore, feeds are more directed towards a good litter quality than to
maximum growth rate when wet litter problems occur in practice. After periods with litter problems and a
sub-optimal growth rate, feed compositions are changed as well in order to maximize compensatory
growth (Enting et al., 2005).

In order to reduce foot pad lesions, there is a tendency to go to higher dietary biotin levels,
especially in the starter period, although some studies indicate that higher dietary levels do not reduce the
incidence or severity of foot pad lesions (Mayne et al., 2007). Also complexed zinc levels are increased in
especially starter feeds, as this can reduce incidence and severity of foot pad dermatitis (Hess et al., 2001;
Saenmahayak et al., 2010).

Table 5. Relative Effect of Minerals on Litter Quality (derived from Enting et al., 2009)

<table>
<thead>
<tr>
<th>Change in diet</th>
<th>% Change in visual litter score day 21-28</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 1 g/kg Na</td>
<td>-7.7</td>
</tr>
<tr>
<td>+ 1 g/kg K</td>
<td>-12.1</td>
</tr>
<tr>
<td>+ 1 g/kg Cl</td>
<td>0.0</td>
</tr>
<tr>
<td>+ 1 g/kg Mg</td>
<td>-22.9</td>
</tr>
<tr>
<td>+ 1 g/kg Ca</td>
<td>-6.3</td>
</tr>
<tr>
<td>+ 1 g/kg dP</td>
<td>+12.9</td>
</tr>
<tr>
<td>+ 1 Ca to dP ratio</td>
<td>-16.7</td>
</tr>
</tbody>
</table>

1 a higher score indicates better litter
2 effect is dependent on anion that is replacing Cl
3 when digestible P levels are lower than the requirement for optimal growth rate

Conclusions

E.U. directives for poultry husbandry have effect on poultry feed compositions. In laying hens,
feed compositions are adjusted to requirements in alternative housing systems. Besides a slight decrease
in dietary protein content, there is a shift towards more NSP-rich diets during the rearing and laying
period in order to prevent cannibalism. Low-density feeds are applied in broiler breeders, but this is
mainly due to the positive effect of these feeds in breeder and offspring performance than on the
reduction of hunger feeling. In broiler chickens, feeds focus on maintaining a good litter quality and
prevention of foot pad lesions, since stocking densities can be increased when mortality and foot pad
lesion incidence are low.

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TRACE MINERAL NUTRITION IN POULTRY –
COPPER, ZINC AND MANGANESE

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Summary

Copper (Cu), zinc (Zn), and manganese (Mn) are metal cations with numerous functions in the body, predominantly as structural or catalytical component of enzymes. In this capacity, Cu, Zn and Mn function in bone development and antioxidative functions, among other functions. Deficiency of these nutrients has negative effects on performance, via reductions in intake and gain, and bone and immune impairment. Supplemental sources of these minerals include salts (e.g., sulfates, oxides), hydroxy minerals (e.g., tribasic copper chloride), and chelated minerals (e.g., zinc methionine). Research has repeatedly demonstrated higher relative bioavailability and efficacy of molecules such as hydroxy- and chelated minerals as compared to more reactive, easily dissociated salts. This is likely due to the stability of the molecule in the feed and GI tract, and resulting changes in the form of mineral found in various portions of the GI tract. Historical data support feeding of moderately high Cu levels to optimize performance of growing birds, and recent data support feeding higher levels of Zn and Mn in growing birds. More research needs to be conducted to better understand how to utilize newer sources of trace minerals to optimize performance, well-being and environmental considerations for growing, laying and breeding poultry.

Trace mineral nutrition in poultry is a complicated subject, as it encompasses a broad range of elements that are required in vastly different quantities, are involved in numerous processes, and have many interactions. Macrominerals, those required in quantities great enough to be expressed as a percentage of the diet, include calcium, phosphorus, sodium, potassium, magnesium and chloride. Microminerals, or trace elements, are those required in smaller amounts, and include copper, zinc, manganese, iodine, iron and selenium. This review will focus on the current knowledge of copper, zinc and manganese in poultry.

Copper (Cu), zinc (Zn), and manganese (Mn) are metal cations with numerous functions in the body. Their essentiality is well-described, and excess of these minerals has significant physiological consequences, given the reactive nature of metal ions. The predominant role of Cu, Zn and Mn is as a structural or catalytical component of enzymes. Copper functions in this capacity due to its ability to participate in single electron reactions, and is essential for ATP production (cytochrome c oxidase), connective tissue formation (lysyl oxidase), defense against free radicals (Cu/Zn superoxide dismutase), pigment formation (tyrosinase), iron transport and metabolism (ceruloplasmin), and hormone and neurotransmitter synthesis (dopamine β-monooxygenase) (Cater and Mercer, 2005). Zinc has structural and catalytic functions in the DNA-binding domains of proteins, including transcription factors and hormone receptors, and at the active site of over 300 enzymes including those involved in bone synthesis, resorption and remodeling (Vallee et al., 1991; Ford, 2004). Additionally, Zn is a component of the mineralized portion of bone (reviewed by Beattie and Avenall, 1992), and functions in the initial stages of bone plate growth (reviewed by Dibner et al., 2007). As a component of metallothionein (MT), Zn has a role in intracellular metal metabolism and storage, metal donation to zinc finger proteins and enzymes, metal detoxification, and protection against oxidants and electrophiles (reviewed by (Davis and Cousins, 2000; Cousins et al., 2006). Zinc also functions in the immune system to modulate inflammatory cytokine production (e.g., Peterson et al., 2008) likely via effects on NF-kB activation (reviewed by Rink and
Haase, 2007), in addition to modulation of the development of oral tolerance (Finamore et al., 2003), primary immune organ development and resulting lymphocyte function, and phagocytic and killing functions of granulocytes, monocytes and macrophages and natural killer cells (reviewed by Rink and Haase, 2007). Manganese provides antioxidant protection in the mitochondrial matrix as a component of Mn-containing super oxide dismutase (MnSOD) (de Rosa et al., 1980), and is a cofactor for many transferases, hydrolases, lyases, glutamine synthetase and integrins (reviewed by Au et al., 2008). Like Zn and Cu, Mn functions in bone metabolism as a cofactor for glycosyl transferase, which is essential for the formation of chondroitin sulfate in the epiphyseal cartilage and bone matrix (reviewed by Beattie and Avenall, 1992).

As a result of the vast number of functions of the various enzymes that these minerals are involved with, signs of deficiency can be varied. In general, Cu deficiency in poultry is normally associated with bone abnormalities, impaired immune responses and anemia (Savage et al., 1966). Deficiency of Zn generally results in reduced feed intake, body weight gains, hatchability, and feather growth (Keinholz et al., 1975), as well as pancreatic insufficiency (McCormick, 1984). Manganese deficiency reduces the activity of MnSOD (Li et al., 2010), although clinical signs are generally related to perosis or lameness (Luo et al., 2007), due to decreased glycosyl transferase activity (reviewed by Finley and Davis, 1999). In poultry, Mn deficiency increases abdominal fat deposition (Lu et al., 2007).

Due to their highly reactive nature, gross excess of Cu, Zn and Mn can cause significant pathologies. Toxicity of Cu is less of a concern in poultry than in other species such as sheep, although toxicity can be seen when feeding extremely high (>500 ppm) Cu, and is manifested as reductions in feed intake and body weight gains (Miles et al., 1998; Luo et al., 2005). This response is source-specific and Cu sources that have more stable bonds appear to have a higher threshold for toxicity, presumably due to slower release in the GI tract and more uniform exposure to the absorptive area of the small intestine. This hypothesis is supported by increased metallothionein (MT) expression in the duodenum of chicks fed Cu sulfate versus a more stably bound Cu source (Naziripour and Klasing, 2010). Increased intestinal MT expression indicates a mechanism to prevent systemic Cu absorption in that portion of the GI tract (Bauerly et al., 2005). Toxicity of Zn can irritate the GI tract and cause reduced absorption of other minerals, and systemically will disrupt the functions of proteins, enzymes and DNA (reviewed by NRC, 2005). High levels of Zn (500 ppm from Zn oxide) in poultry reduced the activity of major pancreatic digestive enzymes (Lu et al., 1990), and reduced tissue vitamin E levels most likely due to reduced intestinal lipase activity (Lu and Combs, 1988). However, high levels of Zn (particularly ZnO) are often fed transiently to swine as growth-permitting feed additive, for which the mechanism may be related to regulating bacterial growth, preventing mucosal damage or enhancing mucosal repair (reviewed by Roselli et al., 2003), induction of intestinal IGF-1 and improvement of intestinal enterocyte surface area (Li et al., 2006), and improvement in the redox state and prevention of apoptosis in the small intestine (Wang et al., 2009). High levels of Mn (3000 ppm) reduces feed intake in chicks (Black et al., 1985), and can also lead to progressive neurological deterioration (Finley and Davis, 1999) and impaired hemoglobin formation (Hartman et al., 1955).

There are numerous sources of Cu, Zn and Mn, including animal and plant products, mineral salts (e.g., sulfates, oxides), hydroxy minerals (e.g., tribasic copper chloride), and chelated minerals (e.g., zinc methionine). The relative bioavailability (RBV) of these sources, defined as the degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilized in metabolism by the animal relative to a reference source, has been determined for many forms of minerals (Littell et al., 1997). RBV is different from nutrient absorption, which can be very difficult to define with trace minerals due to their primary route of excretion via bile or pancreatic secretions, thus confounding measures of absorption. RBV can be useful for comparing sources of a mineral, but also has limitations due to variability in the standard mineral source to which the test source is compared, the choice of response variable, the type of diet used, and the level of mineral tested. For example, using tissue concentrations of
a mineral to determine bioavailability may only reflect storage pools (e.g., liver minerals) and thus factors such as stress, excretion level and level of other minerals can play a role in RBV (Richards, 2010). Diet type can also affect RBV responses (Wedekind et al., 1992); purified or semi-purified diets reduce the number of dietary antagonists such as phytic acid which can bind trace minerals in the GI tract, and also are generally associated with lower food intake than traditional diets. In contrast, use of traditional diets results in high variability in the mineral content of the basal diet. Finally, there has been considerable discussion about the appropriate level of mineral needed to determine RBV (Spears and Hansen, 2008).

Use of high concentrations of trace minerals measures the accumulation of mineral in a storage tissue, and allows for use of practical diets and shorter duration trials. However, this methodology can overwhelm normal physiological processes which regulate mineral accumulation (Ammerman et al., 1998; Ji et al., 2006), and so alternative methodology utilizes mineral-depleted animals fed very low levels of the mineral in question. This practice often requires longer trial duration and more animal numbers due to lower magnitude of differences between sources, but may better represent relevant physiology of animals exposed to different mineral sources. Regardless of methodology, research has generally shown that animal-based sources of minerals have higher RBV than plant-based sources, due to the presence of complexing compounds such as phytate, oxalate and tannins in the latter (Davies and Nightingale, 1975; Halpin and Baker, 1987; Lonnerdal, 2000). Interestingly, only hexa- and penta-phosphates of phytic acid appear to inhibit Zn absorption (Lonnerdal, 2000), implying that the more routine use of phytase in poultry diets will allow greater Zn absorption from plant materials. In regards to supplemental mineral sources, chelated and hydroxy minerals generally have higher RBV than mineral salts, and this can be explained by the chemical properties of ionic-bound sulfated minerals, which dissociate very readily in the presence of moisture. In contrast, covalently bonded minerals like chelated and hydroxy minerals, will have more stability in feed and the GI tract, but due to solubility in the GI environment, will be available at appropriate locations for mineral absorption (Linder 1991; Guo et al., 2001). For example, when broiler chicks were fed covalently bound tribasic copper chloride compared to ionically bound copper sulfate, different proportions of extractable and non-extractable copper were measured in the GI tract (Naziripour and Klasing, 2010). On the other end of the spectrum are very strongly covalently bound minerals such as copper oxide, which have very low dissociation in the GI tract and thus very low availability.

Regulation of Cu, Zn and Mn status occurs at the level of the GI tract, where absorption occurs, and via excretion in the bile or via pancreatic secretions. Cu is absorbed via Cu-specific transporters (e.g., CTR1, Nose et al., 2006) and metal-specific transporters (DMT1, Arredondo et al., 2003). However, there is still debate as to whether Cu can be absorbed as a complex with amino acids or dipeptides, and comprehensive studies examining the site and mechanism of transport in response to various Cu sources have not been conducted. Cu absorption can be impaired by iron, Zn (reviewed by Ao et al., 2009), phytate, and ascorbic acid (reviewed by Wapnir 1998). Zn is absorbed via Zn-specific (proximal intestine) and diffusive (distal intestine) mechanisms (Yu et al., 2008). Similar to Cu, some debate exists about the potential for absorption of intact Zn-amino acid chelates or Zn-proteinate chelates (Ashmead, 1991). In vitro work suggests that Zn methionine (met) is absorbed as Zn$^{2+}$ and met, separately, as the uptake of Zn was 1000x higher than that of met (Beutler et al., 1998), but other work using ex vivo gut tissues showed differences in the uptake of Zn from ZnCl$_2$ as compared to Zn-met, suggesting that different absorption mechanisms may exist (Hill et al., 1987). The latter trial also demonstrated that transport of Zn to the serosa was similar between the sources, suggesting that Zn was transported out of the enterocyte without being complexed to met. Zn-histidine has been shown to be transported by the same translocational mechanism of histidine alone (Wapnir et al., 1983), but this was not the case with glutamate, glycine or tryptophan, suggesting specificity of histidine for potential transport along with Zn. Manganese absorption has been shown to occur primarily in the ileum based on ligated loop methodology (Ji et al., 2006), although as with most minerals, multiple sources have not been examined to elucidate whether sites of absorption vary with mineral source.
Once absorbed, Cu can be used for synthesis of Cu-containing proteins (Linder et al., 1998), stored in the liver bound to MT (reviewed by Bremner and Mills, 1981), or excreted via bile (Owen, 1964). The fate of Zn is similar; Zn can be incorporated into proteins and DNA, bound in tissues to MT (reviewed by Davis and Cousins, 2000), or excreted via the pancreas (reviewed by Cousins et al., 2006). Additionally, Zn can remain in the enterocyte bound to MT, which likely prevents excessive systemic Zn uptake (reviewed by Sandoval et al., 1999). Manganese can also be deposited into tissues (primarily liver, kidney and bone) (Finley and Davis, 1999; Apines et al., 2003). Excretion of Mn occurs via the bile (Papavasiliou et al., 1966), but there also may be via transport across the enterocyte back into the gut lumen (Finley and Davis, 1999).

The effect of feeding different levels and sources of minerals in poultry has received some attention, although there are many questions remaining to be answered. Feeding moderately high copper levels (125-250 ppm) stimulates growth and feed efficiency in swine and poultry (Arias and Koutsos 2006; Minon-Huesca et al., 2006). The mechanism is likely multifactorial, and includes antimicrobial activity in the GI tract (e.g., Naziripour and Klasing, 2010), antimicrobial and antifungal effects in the litter (Johnson et al., 1985), improvements in fat digestibility (in pigs, Dove, 1995), altered immune function (e.g., Arias and Koutsos, 2005), stimulation of feed intake (in pigs, Zhou et al., 1994), and induction of growth hormone (in pigs, Zhou et al., 1994). Zinc level and source have been shown to affect bone and tissue Zn status (Ao et al., 2009), and broiler breeders have shown some improvement in egg numbers and egg Zn content in response to feeding chelated Zn (Hudson et al., 2004). The effects of higher egg Zn are not well established, but poultis from turkey breeder hens fed supplemental Zn-met tended to have higher primary immune organ weights and leukocyte Zn content compared to poultis from hens fed Zn sulfate (Kidd et al., 2000). The NRC recommendation for Zn for growing broilers is 40 ppm (NRC 1994), but higher levels may modulate immunity; broilers fed 80 ppm Zn had higher antibody responses to SRBC (Gajula et al., 2011). Similarly, higher levels of Mn than established to prevent deficiency may modulate physiology. The NRC recommendation for Mn in broiler diets is 60 ppm (NRC, 1994), but recent research suggests that a higher level (120-130 ppm) may be needed (Li et al., 2010) based on increased heart MnSOD mRNA levels (Li et al., 2011), and delayed type hypersensitivity responses (Gajula et al., 2011).

Recent research in mineral absorption and regulation of transport has broadened our understanding of the mechanisms regulating trace mineral metabolism. Recent research in poultry has suggested that higher mineral requirements of broiler chicks may be needed, which may be due to the method of determination of mineral requirements (e.g., use of purified diets in the past) and/or higher needs of new genetic strains of birds. Further research needs to be conducted in many areas, notably 1) to clarify the mechanism of absorption of chelated Cu, Zn and Mn, 2) to determine the site of absorption of various sources of minerals; 3) to determine the optimal levels of Cu, Zn and Mn (in combination) for performance, health and environmental management, 4) to expand our knowledge of the effects of these minerals in growing birds to laying and breeding poultry, and 5) to better understand the interactions between feed components and mineral availability. New technologies in mineral production afford great opportunities to optimize management of birds for optimal performance, well-being and environmental considerations.

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DIET AMINO ACID DENSITY AND POULTRY PRODUCT COSTS

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Summary

Formulating protein in poultry first has evolved from formulating for minimum crude protein (CP) concentrations to formulating based on minimum amino acid (AA) requirements using total or more recently digestible AA and ratios of essential AA to lysine (Lys) within the ideal protein concept. Since feeding represents the largest cost component of broiler production, least cost formulation has been used for a long time. Broilers have been actively selected for growth rate and breast meat yields and thus the modern broiler is more responsive to protein and balanced AA diets than in the past. Therefore, protein and AA requirements are now more important as part of an economic decision in terms of amounts of harvested meat per bird than as a singular physiological response that can be fitted to a broken line or quadratic models. Current day broilers have a larger demand of Lys because its body composition has changed to higher breast meat content. Ideal AA profiles, however, have to be used in order to maximize body protein accrual, otherwise AA other than Lys will become limiting for growth. Many studies have been conducted in the past ten years with diets differing in AA densities at different ages. Increasing Lys and other AA at the beginning of the bird’s life has been demonstrated to have some carry over effect on later production phases; however, increasing Lys and essential AA at the end sometimes leads to breast meat yield improvements because breast muscle continues to grow at rapid rates for longer periods than other muscles. In general, feeding high AA density diets as compared to typical commercial US diets leads to improvements in feed conversion and breast meat yields, but ultimately may not results in the best income.

Introduction

The progress experimented by the broiler industry in the last decades is marked by impressive improvements in all areas of management, health and nutrition but it is genetics that is responsible for most of the improvements in broiler live performance and meat yields. At least 85% of the improvements obtained in live performance between 1957 and 2001 can be attributable to the selection for growth, feed efficiency and meat yields, which continues to improve at rates between 2 and 3% per year (Havenstein et al., 2003). Comparisons made between non selected chickens since 1940 and a present commercial broiler grown in 2009 showed increases of 72% in body weight at 34 days of age and 3.4 times more breast meat (Schmidt et al., 2009). Two major changes that affect the physiology of animal growth have noticeable changed in the 2009 bird: breast muscles maintained their allometric growth after 34 days of...
age which translates into a higher requirement of amino acids (AA), especially lysine (Lys); and an increase of 20% in intestinal surface which could partially explains the extra improvements obtained in feed efficiency.

The high growth rate of the current broilers results in increased demands for AA and energy, even though these demands are not increased in the same proportion. Requirements for AA increase proportionately faster than does the energy requirement, thus a higher AA to energy ratio is required in faster growing strains of broiler (Gous, 2010). Morris and Njurü (1990) fed diets of increasing protein content to broilers and to laying-type cockerels and observed that maximum body weight gain and carcass protein content in the cockerels was obtained with diets having considerably less protein than with the broilers. In that study, the broilers continued to benefit from the additional dietary protein to later ages, and this was likely due to the continued tissue growth, especially of the breast muscles.

It has been demonstrated that providing dietary protein above levels considered adequate by the industry leads to improved feed conversion ratios (FCR) and breast meat yields. Dietary AA levels should match needs for maintenance and skeletal muscle accretion to effectively accrete white meat (Kidd et al., 2004) and therefore, calculations of the requirements have to take both responses into account in order to determine the minimum dietary protein for broilers. In comparison to usual industry levels, benefits of increased AA density diets have been shown early in life as well as in finisher diets. Feeding diets containing high AA levels may result in greater economic return if implemented during periods when the birds’ feed intake is relatively low and growth rate is high (Kidd et al., 2004) and also because at least part of the benefits obtained earlier can be carried through market ages (Pophal et al., 2004). Increasing Lys and other essential AA only in the finisher diets has also been shown to improve FCR and breast meat yields (Dozier et al., 2007). This last impact of high Lys and other essential AA in finisher diets possibly has its impact based on the higher proportional growth rate of the breast muscles compared to other body tissues at later ages.

Feed Formulation Approaches to Provide AA

Linear least cost feed formulation software has been used widely to provide nutrient solutions based on minimums for specific required nutrients. Major differences, however, exist between the approach of nutritionist on the protein provision in the feeds that can affect the final delivery of AA for the birds and potentially limit their full impact on growth. Some of these differences are related to how AA requirements are defined; total or digestible AA. As diets become more complex in terms of feedstuffs used using digestible AA becomes more important. Further differences exist between expressions of digestible AA either on the basis of true or apparent digestibility, with the first providing higher numeric values. Maintaining ingredient matrices within the same system (true vs. apparent, or total vs. digestible) will reduce the spread between the expected formulated AA and the actual delivery in the feed. Regardless of the AA system used, many nutritionists establish a restriction in crude protein (CP) using a minimum value for CP feed formulation, thus providing a safety margin in terms of limiting essential AA beyond those that can be supplemented in the diet as well as for total nitrogen (N). Formulating diets with the use of ideal protein ratios (AA to Lys) allows for a more precise way to deal with AA needs in a balance way to ensure optimum performance. This last formulation approach has been increasing used among nutritionists in the last few years and has the objective to the reduce AA which are in relative excess of needs compared to the first limiting AA thus reducing the AA that would need to be oxidized and decreases N excretion and potentially costs. With the used of the ideal protein concept it is possible to maintain an AA balance in practical situations throughout bird’s life since the ratios between these AA remain fairly stable.

Feeding Regimens with High AA Densities: Economic Evaluations

General nutrient recommendations for broilers are supposed to allow for maximum growth. However, optimum dietary AA levels change with the production goal and that includes the optimization
of growth to feed conversion and breast meat yield. For instance, optimum total sulfur AA (TSAA) levels for breast meat production have been shown to be higher compared to those for whole carcasses growth or weight gain and are affected by broiler genetics (Moran and Bilgili, 1990; Schutte and Pack, 1995; Vieira et al., 2004). While breast meat is presently the main commercial target for broiler meat production, AA supplementation into diets is frequently influenced by factors such as desired market weight, product mix, broiler live cost and genetics. Diets formulated to low AA density to minimize feed cost can limit broiler meat accretion and not allow for maximizing profits, especially when consideration is given to breast meat yield and breast meat prices (Dozier et al., 2008).

Interest exists in studying the benefits of the use of higher AA density diets when compared to the average industry levels. In order for appropriate comparisons, studies with increased AA densities need similar feed formulation standards. For example, increases in Lys have to be coupled with increases in TSAA and threonine (Thr) and possibly CP. In other words, the impact reported in many trials may be limited not by the Lys level but because other essential AA are not at the correct optimal ratios.

Economic evaluations of the bird responses obtained with the use of high AA density diets are complex since product mixes change between industries. However, by looking at the feed cost associated with feed conversion and breast meat yields one could, in a simplified manner, check if the improved performance pays for the higher diet costs. Coneglian et al. (2010) formulated diets using the Brazilian industry average levels for Lys (Moderate), a High (moderate + 12%) AND Low (Moderate – 12%). The diets used were all vegetable corn and soybean meal based and had 1.25%, 1.19%, 1.09% and 1.05% digestible Lys in the Moderate starter, grower, finisher, and withdrawal diets respectively. Ratios of TSAA to Lys were maintained at 73 and 75% from 1 to 21 and 21 to 40 days of age, respectively. The ratio of Thr to Lys was 65% throughout the 40 days of the trial. Both Cobb 500 and Ross 308 broilers responded favorably to the high AA ratio diets in terms of FCR and breast meat yields when birds were processed at 34 and 40 days (Table 1). The feed cost associated with the animal response showed that the Moderate diets provided the best return at 34 days and 40 days.

In a study using Cobb 500 fast feathering broilers, Corzo et al. (2010) also did an evaluation of the effects of high AA density diets on live performance, breast meat yields and on the associated feed costs. They used a Moderate (M) AA diet in a feeding program with 3 diets (1 to 14, 14 to 28 and 28 to 42 days) having 1.16%, 1.03% and 0.97% digestible Lys and minimum ratios of digestible TSAA and Thr of 78% and 62%, respectively throughout all phases. These authors decreased or increased the Lys concentration by around 8% to achieve the Low (L) and High (H) diets, respectively. Diets were exchanged in different phases to provide diverse combinations of H, M and L diets. A summary of their results is presented in Table 2 and shows that BW cost was minimized with the L – L – M feeding program, FC was minimized with the L – L – L feeding program and breast meat was minimized with the H – H – H.

Conclusions

The modern broiler has consistently increased its potential to convert feed into growth and breast meat primarily because of genetic selection. Diets utilized by the poultry industry in general do not provide enough AA to allow the full expression of this potential. In general, breast meat yield is more responsive to higher AA densities than FCR. Therefore, nutritionists have to be careful in assessing the costs of increasing AA density in their feed formulations since underfeeding AA would restrict white meat accretion and overfeeding would represent a waste of expensive resources. Calculating the associated feed cost with actual FCR and breast meat yields is a simplistic tool that could be used as a first approach to support the nutritionist decision with regards to what is the best AA density to be used.
**Table 1. Performance and associated feed cost of male broilers fed diets with graded increases in their ideally balanced protein profile (Coniglione et al., 2010)**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>BW, g</th>
<th>FC</th>
<th>Breast Meat, %</th>
<th>Feed Cost, US/kg&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–21 d</td>
<td>21–40 d</td>
<td>1-34 d</td>
<td>1-40 d</td>
</tr>
<tr>
<td>High</td>
<td>34 d</td>
<td>40 d</td>
<td>2.258 a</td>
<td>2.907 a</td>
</tr>
<tr>
<td>High</td>
<td>2.191 b</td>
<td>2.779 bc</td>
<td>1.55 c</td>
<td>1.65 c</td>
</tr>
<tr>
<td>Moderate</td>
<td>2.178 bc</td>
<td>2.813 b</td>
<td>1.51 b</td>
<td>1.60 b</td>
</tr>
<tr>
<td>Low</td>
<td>2.153 bc</td>
<td>2.741 c</td>
<td>1.50 b</td>
<td>1.59</td>
</tr>
<tr>
<td>Low</td>
<td>2.137 c</td>
<td>2.699 d</td>
<td>1.58 c</td>
<td>1.68 c</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data is averaged for feather-sexable Cobb 500 and Ross 308 broilers.

<sup>2</sup>Feed cost = (US/ton) × feed consumed in each feed phase/ 1 kg of breast meat.

**Table 2. Live performance at 42 d of age of Cobb 500 male broilers fed different amino acid density regimens (Corzo et al., 2010)**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>BW, g</th>
<th>FC</th>
<th>Breast Meat, g</th>
<th>Feed Cost, US/kg&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High – High – High</td>
<td>2,665</td>
<td>1.629</td>
<td>649</td>
<td>0.367</td>
</tr>
<tr>
<td>Low – Low – Moderate</td>
<td>2,465</td>
<td>1.674</td>
<td>554</td>
<td>0.355</td>
</tr>
<tr>
<td>Low – Low – Low</td>
<td>2,381</td>
<td>1.721</td>
<td>537</td>
<td>0.356</td>
</tr>
</tbody>
</table>

<sup>1</sup>Feeding program with 3 diets (1 to 14, 14 to 28 and 28 to 42 days) having 1.16%, 1.03% and 0.97% digestible Lys for Moderate diet with 8% reduction and increase in Low and High diets and minimum ratios of digestible TSAA and Thr of 78% and 62%, respectively.

<sup>2</sup>Values were calculated as follows: feed cost ($/metric ton) × feed consumed in each feed phase/weight (live, carcass, or breast meat).
References


POULTRY PRODUCTION AND ENVIRONMENTAL SUSTAINABILITY

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BY-PRODUCT /CO-PRODUCTS FEEDSTUFFS FROM BIO-FUEL PRODUCTION FOR POULTRY

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Summary

Feeding by-products from the biofuels industry is not new today. Poultry producers have been feeding distillers dried grains with solubles (DDGS) for years. However, the products are changing and many new by-products are becoming available. Ethanol producers are modifying their facilities either in “front-end” fractionation and “back-end” de-oiling, all these technology changes in the plant have lead to significant changes in the nutritional composition of the resulting DDG products. Other new feedstocks are being used and tested to produce biofuels, such as camelina, duckweed, and algae. Camelina meal the by-product from Camelina may the most practical new by-product that is being produced today, and it has been feed successfully to poultry commercially. Duckweed/soy protein concentrate and algae meal are also by-products from new technologies to produce biofuels, these by-products may have some benefits as new feed ingredients however, little is now about the nutritional and feeding quality, and high variation has been observed. Glycerin the by-product biodiesel production had been suggested as a potential energy course for poultry, but the variation between samples is still extremely high. Many of the by-products produced by the biofuels industry can be used successful in poultry diets to save money and maybe even improve performance. However, there is still an extreme amount of variation within the by-products from the biofuels industry thus it is crucial that confirmatory analysis be conducted on any by-product of the biofuels industry.

Introduction

A number of reasons are due to the rise in feed costs in the last several years, especially corn and fat prices. One reason is the diversion of feed ingredients to the biofuels industry. The increase in the feed costs has lead nutritionists to evaluate the feasibility of using cost-effective alternative ingredients, specifically the by-products of the biofuels industry. The main by-product of the biofuels industry, distillers dried grains with solubles (DDGS) has been fed to poultry for years with success. However there are still a number of issues related to the feeding of DDGS that have not been resolved. The composition of DDG(s) has also been changing over the last few years as new technologies have been added to ethanol plants to increase products produced. Some new feedstocks for the biofuels industry have been coming on strong the last few years. These by-products from new feedstocks include camelina meal, duckweed/soy protein concentrate, and algae. Glycerin is also a by-product of the biofuels industry and although it is not a new by-product it still has not been used successful as a energy source in poultry diets. Nutritionist need to have a good understanding of the nutritional composition and feeding value of these by-products to be able to feed them with confidence and success.

Distillers Dried Grains with Solubles

Distillers dried grains with soubles (DDGS) from the ethanol industry it no longer a new or alternative ingredient. Many poultry producers have been feeding DDGS for years now and feel relatively comfortable with the feeding DDGS. Research has shown that DDGS can be fed to successful to broilers,
turkeys, laying hens and breeders without significantly affecting performance and meat and egg quality. However there are still some issues that remain unresolved. Table 1 gives the average nutrient composition of DDGS over the last eight years. Table 2 provides the nutrient composition, table 3 provides the mineral composition, and table 4 provides the AME values determined in 35 day old broilers of 20 DDGS from plants across the US and produced in 2010.

**Table 1. Average Nutrient Composition of DDGS for Poultry**

<table>
<thead>
<tr>
<th>Component (%)</th>
<th>Mean</th>
<th>Range</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TME (kcal/kg)</td>
<td>2,863</td>
<td>2607-3054</td>
<td>3.6</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.78</td>
<td>0.59-0.89</td>
<td>11.6</td>
</tr>
<tr>
<td>Lysine Dig.</td>
<td>68</td>
<td>46-84</td>
<td>11.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.49</td>
<td>0.41-0.60</td>
<td>9.7</td>
</tr>
<tr>
<td>Methionine Dig.</td>
<td>88</td>
<td>85-92</td>
<td>1.9</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.98</td>
<td>0.85-1.14</td>
<td>6.0</td>
</tr>
<tr>
<td>Threonine Dig.</td>
<td>76</td>
<td>69-83</td>
<td>4.8</td>
</tr>
<tr>
<td>Fat</td>
<td>8</td>
<td>4-16</td>
<td>4.8</td>
</tr>
<tr>
<td>CA</td>
<td>0.03</td>
<td>0.02-0.04</td>
<td>38.4</td>
</tr>
<tr>
<td>P</td>
<td>0.73</td>
<td>0.62-0.77</td>
<td>5.3</td>
</tr>
<tr>
<td>P Avail. Coefficient</td>
<td>79</td>
<td>64-100</td>
<td>32.8</td>
</tr>
<tr>
<td>Na</td>
<td>0.25</td>
<td>0.05-0.45</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Average Nutrient Composition of 20 DDGS samples (As-fed basis) (University of Georgia)**

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Fiber</th>
<th>Ash</th>
<th>Starch</th>
<th>Lignin</th>
<th>ADF</th>
<th>NDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>10.3</td>
<td>25.6</td>
<td>9.9</td>
<td>7.1</td>
<td>4.1</td>
<td>5.9</td>
<td>3.5</td>
<td>12.7</td>
</tr>
<tr>
<td>Min</td>
<td>8.0</td>
<td>23.0</td>
<td>7.8</td>
<td>5.6</td>
<td>1.5</td>
<td>3.7</td>
<td>1.7</td>
<td>9.4</td>
</tr>
<tr>
<td>Max</td>
<td>12.4</td>
<td>35.0</td>
<td>12.8</td>
<td>7.9</td>
<td>5.7</td>
<td>10.8</td>
<td>8.0</td>
<td>18.5</td>
</tr>
</tbody>
</table>

**Table 3. Average Mineral Composition of 20 DDGS samples (As-fed basis) (University of Georgia)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Average</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium, ppm</td>
<td>251.9</td>
<td>87.9</td>
<td>673.4</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.70</td>
<td>0.40</td>
<td>0.90</td>
</tr>
<tr>
<td>Phytate Phosphorus, mg/kg</td>
<td>1634.7</td>
<td>328.8</td>
<td>2627.3</td>
</tr>
<tr>
<td>Sodium, ppm</td>
<td>1850.1</td>
<td>528.5</td>
<td>4489.0</td>
</tr>
<tr>
<td>Potassium, ppm</td>
<td>9578</td>
<td>2755</td>
<td>11170</td>
</tr>
<tr>
<td>Copper, ppm</td>
<td>5.2</td>
<td>4.0</td>
<td>7.7</td>
</tr>
<tr>
<td>Zinc, ppm</td>
<td>52.3</td>
<td>37.3</td>
<td>64.8</td>
</tr>
<tr>
<td>Manganese, ppm</td>
<td>14.4</td>
<td>4.3</td>
<td>40.7</td>
</tr>
<tr>
<td>Iron, ppm</td>
<td>86.5</td>
<td>52.1</td>
<td>155.9</td>
</tr>
<tr>
<td>Magnesium, ppm</td>
<td>2795.1</td>
<td>879.2</td>
<td>3417.0</td>
</tr>
<tr>
<td>Sulfur, %</td>
<td>0.6</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Selenium, ppm</td>
<td>0.3</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Choline chloride, mg/kg</td>
<td>180.4</td>
<td>98.7</td>
<td>308.0</td>
</tr>
</tbody>
</table>
Table 4. Average AME\textsubscript{N} of 20 DDGS samples (As-fed basis) (University of Georgia)

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>AME\textsubscript{N} (kcal/kg), as fed basis</td>
<td>3,319</td>
<td>2,917</td>
<td>3,645</td>
</tr>
<tr>
<td>Gross energy, kcal/kg</td>
<td>4,652</td>
<td>4,399</td>
<td>4,933</td>
</tr>
<tr>
<td>AME as percent of GE</td>
<td>64</td>
<td>56</td>
<td>68</td>
</tr>
</tbody>
</table>

\textsuperscript{1}AME\textsubscript{N} of 35 day old broilers.

One of the big issues with DDGS (as with many ingredients) is estimating the ME value. Producers want an equation based of standard parameters in which they can accurately estimate the ME value. This has been extremely difficult and to today few equations have had $r^2$ values better than about 0.5. Prediction equations were established based on the 20 DDGS producers in 2010, all of these samples were from traditional ethanol plants, thus there was no samples from a plant that has “front-end” fraction technology or “back-end” de-oiling equipment. More time needs to be spent to find the best prediction equation based on the least and most practical parameters, but Table 5 gives you and idea of the best indicators, Lignin and Dispersible Protein.

Table 5. Prediction equations for AME\textsubscript{N} of distiller’s dried grains with solubles based on various variables (DM basis)

<table>
<thead>
<tr>
<th>Variable(s), %</th>
<th>Prediction Equation\textsuperscript{1}</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>$\text{AME}_\text{N} = 1731.5 - 87.47 \text{ (lignin)}$</td>
<td>0.58</td>
</tr>
<tr>
<td>Fat, fiber, protein, ash</td>
<td>$\text{AME}_\text{N} = 1110.6 + 5.66 \text{ (fat) + 4.39 (protein) + 46.7 (fiber) – 25.52(ash)}$</td>
<td>0.29</td>
</tr>
<tr>
<td>Fat, fiber, protein, ash, GE</td>
<td>$\text{AME}_\text{N} = 2797.48 + 59.69 \text{ (fat) + 17.60 (protein) + 77.10 (fiber) – 56.58(ash) – 0.570 (GE)}$</td>
<td>0.39</td>
</tr>
<tr>
<td>Fat, ADF, NDF, Fiber</td>
<td>$\text{AME}_\text{N} = 600.38 + 23.66 \text{ (fat) – 48.26 (ADF) +24.71 (NDF) +74.04 (fiber)}$</td>
<td>0.37</td>
</tr>
<tr>
<td>Lignin, fiber, fat, protein, ash</td>
<td>$\text{AME}_\text{N} = 1542.9 – 105.83 \text{ (lignin) + 33.51 (fiber) - 12.96 (fat) + 0.10(protein) + 30.67(ash)}$</td>
<td>0.71</td>
</tr>
<tr>
<td>NPN, Lignin, protein fiber, ash, GE</td>
<td>$\text{AME}_\text{N} = 2171.8 + 2.45 (NPN) -123.71 \text{(Lignin) +4.43 (protein) +36.93 (fiber) + 26.08 (ash) – 0.19 (GE)}$</td>
<td>0.75</td>
</tr>
<tr>
<td>Dispersible Protein (DISPRT)</td>
<td>$\text{AME}_\text{N} = 1726.23 – 21.52 \text{ (DISPRT)}$</td>
<td>0.59</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Predictions equations are based of the values from 20 samples of distiller’s dried grains with solubles (DDGS) and AME\textsubscript{N} values determined in 35 day old broilers.

One of the other issues is the quality of fat in DDGS. Is this fat rancid or does oxidation occur during drying? Ethanol plants from around the US were surveyed and 15 plants were selected that had various drying temperatures, times, processing methods, etc; the fat in DDGS was evaluated and compared to the fat in the original corn. The results clearly indicated that oxidation stability index (OSI) was the most sensitive indicator to measure fat quality and correlated extremely well with the active oxygen method (AOM) ($r^2 = 0.79$). The OSI values for DDGS were approximately 2 times higher than the values in the original corn, which suggests that the fat in DDGS is more stable than that in the original corn. The antioxidant capacity (FRAP total Phenol) of the lipid from DDGS was very high and is 4 to 6 times higher than that found in the original corn. This suggests that lipid in DDGS would be much more stable and more resistance to oxidation as compared to the fat in the original corn. However, the work is continuing in this area, the research needs to correlate with performance studies.
Until recently, the majority of the dry-grind ethanol plants used unmodified corn (or other cereal grains) to produce ethanol and some type of distillers dried grains (DDG). There is growing interest by many ethanol producers to modify the technology to get better ethanol yield from the feedstock which will result in by-products that may have superior or inferior nutritional value for poultry. The use of these new manufacturing processes will result, and has resulted in by-products that have markedly different nutritional composition.

**High Protein Distillers Dried Grains**

Currently, many plants are implementing a modified dry milling process as the first step in the ethanol facility in which they are recovering the nonfermentables (germ and fiber) prior to the dry-grind process. The whole corn is milled into three fractions; corn germ, bran and the endosperm which is used for ethanol fermentation. The products from the fermentation of the endosperm are ethanol, syrup, CO$_2$ and high protein distiller dried grains (HP-DDG), which does not have the syrup or solubles added back. When considering the potential use of a feed ingredient such as HP-DDG primary emphasis is placed on obtaining accurate information regarding metabolizable energy, phosphorus availability and amino acid composition and digestibility. The average nutritional composition of HP-DDGS is shown in Table 6. As one would expect from the name of this product it does contain a considerable amount of crude protein, however it is much lower in fat and phosphorus than DDGS because the syrup is not added back on DDG (the syrup contains the high levels of fat and phosphorus) and the germ fraction of the corn is removed. High protein distillers dried grains surprisingly has a similar TME as compared to DDGS, which is likely due to the large increase in crude protein, as the fat concentration is notable lower. The total amino acids concentration is higher and the amino acid digestibility coefficients appear to be similar to what is seen with DDGS.

### Table 6. Average Composition of High Protein Distillers Dried Grains

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TME (kcal/kg)</td>
<td>2500-2850</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>40- 44</td>
</tr>
<tr>
<td>Lysine (Dig. Coefficient)</td>
<td>1.12-1.35 (70-73)</td>
</tr>
<tr>
<td>Methionine (Dig. Coefficient)</td>
<td>0.92-1.10 (85-89)</td>
</tr>
<tr>
<td>Crude fat</td>
<td>3.0-5.0</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>7.2-8.5</td>
</tr>
<tr>
<td>Phosphorus (Avail. Coefficient)</td>
<td>0.32-0.48 (56)</td>
</tr>
</tbody>
</table>

Most of the work conducted to date with products from new processing techniques in the ethanol plant has been done to look at the effects on the nutritional value of HP-DDG. HP-DDG is an acceptable feed ingredient for broiler diets however as levels increase over 6% special attention must be paid to amino acid levels when using HP-DDG in order to prevent deficiencies (especially lysine). High protein distillers dried grains can be incorporated up to 12% in laying hens diet without negatively affecting performance. Applegate et al., 2008 also found that the dietary replace of up to 50% of the SBM inclusion with HP-DDG had no effect on bird performance through 42 days of age or breast meat yield at 42 days of age.
Corn Germ

Corn germ is also a by-product of “front-end” fractionation ethanol plants. Corn germ is not a new ingredient it has been fed for years. However, corn germ from “front-end” fractionation ethanol plants is relatively new. Corn germ is a good source of energy, amino acids, which are highly digestible and is high in total phosphorus (1.25%, 25% bioavailability coefficient). The nutritional and amino acid (AA) composition of corn germ are show in table 7 and 8. Corn germ has commercial been fed successfully to poultry for years.

Table 7. Nutrient Composition (as-fed basis) of Corn Germ Samples (University of Georgia)

<table>
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<tr>
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<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>TME, kcal/kg</td>
<td>3,253</td>
<td>3,689</td>
<td>2,911</td>
<td>3,019</td>
<td>3,203</td>
<td>2,617</td>
<td>3,106</td>
<td>1,625</td>
<td>3,023</td>
<td>3,346</td>
<td>3,321</td>
<td>1,433</td>
<td>2,912</td>
</tr>
<tr>
<td>GE, kcal/kg</td>
<td>4,389</td>
<td>4,441</td>
<td>4,798</td>
<td>4,463</td>
<td>4,834</td>
<td>4,025</td>
<td>4,657</td>
<td>4,616</td>
<td>4,575</td>
<td>4,822</td>
<td>4,606</td>
<td>4,607</td>
<td>4,204</td>
</tr>
<tr>
<td>Protein, %</td>
<td>12.3</td>
<td>12.2</td>
<td>15.6</td>
<td>15.5</td>
<td>14.4</td>
<td>15.7</td>
<td>13.1</td>
<td>13.2</td>
<td>13.2</td>
<td>14.4</td>
<td>13.6</td>
<td>14.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Fat, %</td>
<td>9.59</td>
<td>9.62</td>
<td>18.2</td>
<td>16.8</td>
<td>17.6</td>
<td>15.7</td>
<td>14.4</td>
<td>16.8</td>
<td>17.6</td>
<td>17.6</td>
<td>17.6</td>
<td>17.6</td>
<td>7.18</td>
</tr>
<tr>
<td>Fiber, %</td>
<td>6.81</td>
<td>3.17</td>
<td>6.22</td>
<td>6.25</td>
<td>4.96</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.91</td>
</tr>
<tr>
<td>Ash, %</td>
<td>5.51</td>
<td>3.80</td>
<td>6.09</td>
<td>5.70</td>
<td>5.40</td>
<td>3.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.53</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>10.4</td>
<td>8.4</td>
<td>8.2</td>
<td>8.3</td>
<td>10.0</td>
<td>9.2</td>
<td>9.8</td>
<td>11.4</td>
<td>8.2</td>
<td>7.9</td>
<td>5.8</td>
<td>13.8</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Table 8. Amino Acid (AA) Composition (as-fed basis) of Corn Germ Samples (University of Georgia)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
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<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>0.81(84)</td>
<td>0.88(81)</td>
<td>0.83(87)</td>
<td>0.70(80)</td>
<td>0.62(95)</td>
<td>0.80(82)</td>
<td>0.67(96)</td>
<td>0.68(96)</td>
<td>0.68(96)</td>
<td>0.71(94)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met</td>
<td>0.25(78)</td>
<td>0.27(82)</td>
<td>0.26(85)</td>
<td>0.24(90)</td>
<td>0.19(95)</td>
<td>0.25(83)</td>
<td>0.21(94)</td>
<td>0.22(90)</td>
<td>0.21(94)</td>
<td>0.22(94)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSAA</td>
<td>0.52(72)</td>
<td>0.56(78)</td>
<td>0.55(80)</td>
<td>0.51(91)</td>
<td>0.41(94)</td>
<td>0.53(81)</td>
<td>0.43(97)</td>
<td>0.46(93)</td>
<td>0.46(95)</td>
<td>0.48(97)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>0.52(63)</td>
<td>0.55(72)</td>
<td>0.53(75)</td>
<td>0.56(62)</td>
<td>0.47(96)</td>
<td>0.53(72)</td>
<td>0.49(94)</td>
<td>0.49(89)</td>
<td>0.50(94)</td>
<td>0.51(95)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trp</td>
<td>--</td>
<td>0.17(90)</td>
<td>0.15(91)</td>
<td>0.20(68)</td>
<td>0.16(90)</td>
<td>0.14(91)</td>
<td>0.17(88)</td>
<td>0.18(87)</td>
<td>0.17(91)</td>
<td>0.19(90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>0.71(75)</td>
<td>0.76(80)</td>
<td>0.74(81)</td>
<td>0.71(73)</td>
<td>0.58(93)</td>
<td>0.73(82)</td>
<td>0.61(90)</td>
<td>0.62(87)</td>
<td>0.62(89)</td>
<td>0.65(92)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Amino acid digestibility coefficient.

Corn Germ Meal

Many ethanol plants that have or are installing “front-end” fractionation technology are also installing equipment to extract the oil from the corn germ fraction to retrieve the corn oil. This allows them to not only sell ethanol and DDG but also corn oil. Corn germ meal is much lower in fat and energy than corn germ but higher in protein as the other components in corn germ are concentrated when the oil is removed (table 9). Although corn germ meal is lower in energy is has been fed commercial to poultry for years.

Table 9. Nutrient Composition (as-fed basis) of Corn Germ Meal Samples (University of Georgia)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
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<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>TME, kcal/kg</td>
<td>2,415</td>
<td>2,252</td>
<td>2,592</td>
<td>2,076</td>
<td>2,097</td>
<td>1,640</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE, kcal/kg</td>
<td>4,140</td>
<td>4,217</td>
<td>4,226</td>
<td>4,121</td>
<td>4,147</td>
<td>4,122</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein, %</td>
<td>23.5</td>
<td>21.8</td>
<td>21.6</td>
<td>22.5</td>
<td>20.4</td>
<td>22.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td>2.63</td>
<td>2.25</td>
<td>2.67</td>
<td>1.42</td>
<td>2.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fiber, %</td>
<td>8.20</td>
<td>8.40</td>
<td>8.55</td>
<td>7.90</td>
<td>8.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash, %</td>
<td>2.82</td>
<td>1.92</td>
<td>2.68</td>
<td>2.57</td>
<td>1.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture, %</td>
<td>12.2</td>
<td>11.7</td>
<td>12.5</td>
<td>12.0</td>
<td>11.50</td>
<td>12.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADF, %</td>
<td>11.1</td>
<td>11.1</td>
<td>11.0</td>
<td>10.8</td>
<td>10.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADF, %</td>
<td>28.7</td>
<td>29.7</td>
<td>28.2</td>
<td>28.1</td>
<td>31.1</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
The processing method or technology used in the ethanol facility can greatly influence the nutritional composition and value of by-products from ethanol facilities. Varying the amount of solubles in DDGS and using new or modified processing technologies to remove fiber, germ, or both can influence the protein-amino acid and P content of the by-products from ethanol plants (Martínez-Amezcua et al. 2007). New processing technologies to remove fiber and germ will result in even greater variation in the nutritional composition and value of the resulting by-product (DDGS, DDG, HP-DDG, etc). Thus, confirmatory analyses should be conducted prior to utilizing these new co-products in poultry diets.

“Back end” de-oiled; Moderate protein Distillers Dried Grains with Solubles that is low in fat

Modifications are also being made to the “back-end” of ethanol plants. This process involves centrifugation of the thin stillage and then whole stillage after the thin stillage (syrup) has been added back. This product is in general lower in energy and higher in protein than traditional DDGS, but lower in protein than HP-DDG. Fifty percent of the plants today may have this “back-end” de-oil technology or are planning to add this technology to the their plants. It appears that a lot of feed mills are currently receiving DDGS from a plant that has “back-end” de-oiling equipment without a clear indication that the product is different than traditional DDGS. Nutrient evaluation and digestible measurements are currently being done on samples from plants that have “back-end” de-oiling equipment.

Camelina/Camelina Meal

There has been increased interest in finding alternative feedstocks, specifically ones with high oil contents such as oilseed crops to produce biofuels. Camelina sativa is an oilseed crop of the Brassica (Cruciferae) family. Camelina sativa is a fast-growing, short-season crop that have a very low seeing rate and is considered the most economical crop to produce because of minimal input requirements needed (Pilgeram et al., 2007). Camelina sativa has gained increased popularity as a biofuel source because of it high oil content (approximately 40%) (Cherian et al., 2009). Camelina meal is the by-product after oil extraction from the camelina seeds. Below is the tentative AAFCO definition:

“Tentative AAFCO Definition; Camelina meal, extracted, is the product obtained from high-pressure crushing of seed, or from a pre-press solvent extraction process, which removes the oil from the whole see of the species Camelina sativa. The meal may be heated. The meal is the material which remains after most of the oil has been removed. It must not contain less than 30% crude protein, a maximum 12% crude fiber, and typically contains 15% or less residual oil. The meal contains less than 30 micromoles of any mixture of 9-Methylsulfinylnonyl glucosinolate, 10-Methylsulfinylnonyl glucosinolate, and 11-Methylsulfinylnonyl glucosinolate per gram of dry oil free solid. It is used in the dies of broilers and cattle fed in confinement for slaughter at an inclusion of no more than 10% of the diet. (Adopted 2010)”.

Preliminary analysis on several CM samples at The University of Georgia has demonstrated that the meal contains 4-11% moisture, 32-39% crude protein, 10-10% fat, and 3058-4038 kcal/kg TME N (true metabolizable energy). Table 10 gives the nutritional composition and TME N of 9 different camelina meal samples. Based on our evaluation, amino acid digestibility of CM is comparable to soybean meal (SBM). Camelina meal is rich in protein, lipids and essential n-3 and n-6 fatty acids and could be incorporated into poultry diets as a source of energy, protein and essential n-3 and n-6 (Cherian et al. 2009). Thus, CM has a lot of potential as an alternative feed ingredient for poultry. The meal contains rather high concentration of omega-3 fatty acids (30% α-linolenic acid) and can thus be an ingredient to help add value to poultry products. Camelina meal has been deemed safe at the level of up to 10% in the broiler and layer diets. However, the anti-nutritional factors in CM (namely NSP and glucosinolate) restrain feeding at higher levels (Ryhänen et al., 2007). Frame et al. (2007) reported that camelina meal may be a potential useful minor ingredient in turkey diets if economically feasible, but caution should be exercised in using camelina meal above 5% of finished feed in a poultry starter diet. The inclusion of more than 10% camelina meal in hen’s diets may affect egg lipid quality characteristic. Thus, when
feeding high levels of camelina meal it is essential measures are taken to minimize lipid peroxidation to ensure egg quality and lipid stability (Cherian et al. 2009). Aziza et al., (2010) reported that camelina meal can be included in broiler diets up to 10% without compromising bird performance while increasing the n-3 fatty acid content of the meat. However, more studies still need to be conducted to have a complete understanding of the feeding value of camelina meal.

| Table 10. Nutritional Composition of Camelina Meal (as-fed basis) (University of Georgia) |
|------------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| TME, kcal/kg                            | 3,675     | 3,627     | 3,104     | 3,105     | 3,158     | 3,698     | 2,911     | 2,499     |
| Protein, %                              | 32.8      | 33.8      | 35.5      | 34.2      | 35.2      | 27.0      | 35.0      | 38.7      |
| Fat, %                                  |           |           |           |           |           |           |           |           |
| Fiber, %                                 | 17.8      | 13.5      | 16.1      | 11.5      | 8.95      |           |           |           |
| Ash, %                                   |           |           |           |           |           | 5.70      | 5.54      |           |
| Moisture, %                              |           |           |           |           |           |           |           |           |
| Gross Energy, kcal/kg                    | 4,988     | 4,955     | 4,632     | 5,143     | 6,110     | 4,606     | 4,726     |           |

Amino Acid

| Lysine                                  | 1.49 (93) | 1.57 (89) | 1.64 (91) | 1.56 (85) |
| Threonine                               | 1.26 (88) | 1.30 (81) | 1.38 (84) | 1.33 (81) |
| Methionine                              | 0.60 (96) | 0.62 (93) | 0.63 (94) | 0.61 (90) |
| Cysteine                                | 0.63 (87) | 0.67 (81) | 0.69 (89) | 0.70 (85) |
| Arginine                                | 2.47 (96) | 2.66 (94) | 2.73 (95) | 2.72 (93) |
| Valine                                  | 1.51 (92) | 1.58 (86) | 1.72 (88) | --         |
| Tryptophan                              | 0.37 (94) | 0.37 (92) | 0.32 (87) | --         |

Glycerin

Increased government pressure for biofuels has led to a significant increase in biodiesel production resulting in increased cost for fat. Demethylated crude glycerin, a by-product of biodiesel production, may be used as an alternate source of fat in broiler diets. The general expected proximate analysis for crude glycerin is given in Table 11. The proximate analysis, TME, mineral content, and fatty acids of 9 glycerin samples are shown in table 12. Glycerin can vary considerably from plant to plant especially in regards to TME, calcium, potassium, salts, and methanol. Dozier et al. (2008) found the average AME of the glycerin samples that they measured to be 3,434 kcal/kg and that the energy supplied by glycerin is used efficiently by broiler chickens. The TME value can safely and conservatively be considered to be at least 80% of the gross energy. Based on studies conducted at the University of Georgia inclusion of glycerin up to 10% can be used as a partial fat replacement in broiler starter diets and up to 10% in all periods. Glycerin with levels used up to 10% with 3.1% methanol did not impact broiler performance, suggesting that broilers may be less susceptible to methanol toxicity. Glycerin may be used at low levels (2.5 to 5%) as a partial fat replacement in broiler diets without any negative effect on performance. However, studies conducted out of Park Waldroup’s laboratory (University of Arkansas) revealed that from up to 10% demethylated glycerin (<0.05% methanol) could be included in broiler diets from 1 to 16 d of age without adversely affected performance. Based on a second study Dr. Waldroup’s laboratory demonstrated that diets with 5% demethylated glycerin supported performance to 42 d of age equal to that of a positive control (with no added glycerin). However, diets with 10% glycerol did not flow well in the tube feeders and inhibited feed intake, resulting in slower growth and poor feed conversion. Also they found the litter from the pens of broilers fed diets with 10% glycerin was visibly wetter and contained about 0.15% higher K levels as a results of residual K in the glycerin. Demethylated glycerin may have some promise as a feed ingredient (energy...
source) for poultry diets however issues with product consistency, methanol, sodium or potassium, and moisture levels and feed flow, handling and manufacturing need to be better understood and it is critical that confirmatory analysis be conducted on every glycerin sample.

Table 11. Average Composition of Crude Glycerin (?)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerin</td>
<td>78-86</td>
</tr>
<tr>
<td>Water</td>
<td>8-15</td>
</tr>
<tr>
<td>NaCL or KCL</td>
<td>2-10</td>
</tr>
<tr>
<td>Methanol¹</td>
<td>&lt;0.05 ?</td>
</tr>
<tr>
<td>ME²</td>
<td>=&gt; 95% of gross energy?</td>
</tr>
</tbody>
</table>

¹ Salts are a byproduct of the chemical used as a catalyst, and will be a formulation concern.
² Methanol is extremely toxic and will be a formulation concern. The level of methanol is extremely variable from plant to plant.
³ ME being 95% of the gross energy of the product needs to be used carefully as the ME varies considerable from plant to plant.

Table 12. The composition of 9 samples (University of Georgia)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nutrient</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TME (kcal/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3,252</td>
<td>3,143</td>
<td>6,549</td>
<td>6,711</td>
<td>3,390</td>
<td>4,427</td>
<td>3,450</td>
<td>5,525</td>
<td>4,827</td>
</tr>
<tr>
<td>2</td>
<td>(3,902)</td>
<td>(3,842)</td>
<td>(6,724)</td>
<td>(9,474)</td>
<td>(3,680)</td>
<td>(4,785)</td>
<td>(4,298)</td>
<td>(5,640)</td>
<td>(9,573)</td>
</tr>
<tr>
<td>3</td>
<td>Glycerol, %</td>
<td>85.7</td>
<td>86.1</td>
<td>57.4</td>
<td>44.6</td>
<td>84.1</td>
<td>66.6</td>
<td>58.3</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>Moisture, %</td>
<td>11.30</td>
<td>9.96</td>
<td>12.23</td>
<td>7.85</td>
<td>14.28</td>
<td>28.40</td>
<td>34.9</td>
<td>32.3</td>
</tr>
<tr>
<td>5</td>
<td>Protein, %</td>
<td>0.15</td>
<td>0.21</td>
<td>0.85</td>
<td>0.58</td>
<td>0.01</td>
<td>1.43</td>
<td>1.91</td>
<td>0.18</td>
</tr>
<tr>
<td>6</td>
<td>Crude Fat, %</td>
<td>0.24</td>
<td>0.12</td>
<td>17.71</td>
<td>13.18</td>
<td>0.00</td>
<td>1.10</td>
<td>16.1</td>
<td>18.3</td>
</tr>
<tr>
<td>7</td>
<td>Fiber, %</td>
<td>0.06</td>
<td>0.33</td>
<td>0.72</td>
<td>1.51</td>
<td>0.01</td>
<td>0.03</td>
<td>0.17</td>
<td>0.09</td>
</tr>
<tr>
<td>8</td>
<td>Ash, %</td>
<td>4.64</td>
<td>4.54</td>
<td>4.18</td>
<td>4.55</td>
<td>4.55</td>
<td>3.12</td>
<td>3.66</td>
<td>3.56</td>
</tr>
<tr>
<td>9</td>
<td>Methanol, %</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>1.79</td>
<td>1.51</td>
<td>&lt;0.01</td>
<td>1.40</td>
<td>2.23</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Calcium, %</td>
<td>78</td>
<td>64</td>
<td>185</td>
<td>136</td>
<td>28</td>
<td>268</td>
<td>0.15</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Free Fatty Acids, %</td>
<td>0.90</td>
<td>4.4</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.80</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Potassium, ppm</td>
<td>788</td>
<td>801</td>
<td>930</td>
<td>444</td>
<td>120</td>
<td>822</td>
<td>766</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Salt, %</td>
<td>4.14</td>
<td>4.11</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>3.08</td>
<td>3.02</td>
<td>2.57</td>
<td>--</td>
</tr>
</tbody>
</table>

Unusual By-Products

New by-products from the U.S. biofuels industry are emerging daily. Companies are looking at developing new feedstocks that are more efficient for the use in producing biofuels. Duckweed and algae are currently being used as a feedstock to produce biofuels thus results in an algae meal and a duckweed meal which is often called high protein concentrate.

Duckweed/Protein Concentrate

The high protein concentrate from duckweed could be a good source of protein and energy (and maybe even phosphorus) for poultry. Based on the nutritional information presented in Table13 the high protein concentrate from duckweed may be as valuable as soybean meal. However, this does not take into any issues due to anti-nutritional factors, under or over-processing, product variability, palatability, spoilage, availability, storage, logistics and handling, these conclusions are simply based on the analytical
nutrient composition. This also does not take into account any benefits of the high protein concentrate from duckweed in poultry diets, such as high xanthophyll content. If the xanthophyll content is high in duckweed, as it has been reported this would add additional value to the duckweed. At the University of Georgia a 21 day broiler feeding trial was conducted in which duckweed/high protein concentrate were formulated into the diet based on the nutritional analysis at 0, 2, 4, and 8% of the diet. Broilers can be fed diets with duckweed/high protein concentrate up to 2% without affecting weight gain and up to 4% without affecting feed efficiency. Broilers fed dies with 2% duckweed/protein concentrate gained more weight and had a lower feed efficiency as compared to the birds fed the control diet with 0% duckweed.

Table 13. Nutrient Composition of high protein concentrate from duckweed

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High Protein Concentrate</th>
<th>High Protein Concentrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross energy, kcal/lb</td>
<td>2,077**</td>
<td>2,371</td>
</tr>
<tr>
<td>TME¹, kcal/lb</td>
<td>--</td>
<td>1,544</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>5.6</td>
<td>11.1</td>
</tr>
<tr>
<td>Dry Matter, %</td>
<td>94.4</td>
<td>88.9</td>
</tr>
<tr>
<td>Crude Fat, %</td>
<td>18.4</td>
<td>--</td>
</tr>
<tr>
<td>Crude Fiber, %</td>
<td>0.11</td>
<td>--</td>
</tr>
<tr>
<td>Ash, %</td>
<td>2.85</td>
<td>--</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.29</td>
<td>--</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.85</td>
<td>--</td>
</tr>
<tr>
<td>Avail. Phosphorus, %</td>
<td>0.43</td>
<td>--</td>
</tr>
<tr>
<td>Crude Protein, %</td>
<td>59.7</td>
<td>51.8</td>
</tr>
</tbody>
</table>

Total Amino Acid Concentration, % (amino acid digestibility coefficients)³

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Concentrate</th>
<th>Digestibility Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>3.22</td>
<td>2.70 (88.3)³</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.38</td>
<td>1.05 (88.5)</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.62</td>
<td>0.52 (67.3)</td>
</tr>
<tr>
<td>TSAA</td>
<td>2.00</td>
<td>1.57 (81.5)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.86</td>
<td>0.73 (98.4)</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.38</td>
<td>1.86 (83.4)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.12</td>
<td>2.55 (86.3)</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.61</td>
<td>1.12 (88.0)</td>
</tr>
<tr>
<td>Valine</td>
<td>3.68</td>
<td>3.21 (85.6)</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.66</td>
<td>4.46 (86.9)</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.69</td>
<td>2.64 (90.1)</td>
</tr>
</tbody>
</table>

** Calculated from proximate data.
³ Amino acid digestibility values were determined at the University of Georgia using the Precision-fed cecetomzied rooster assay.

Algae

Algae is also being used to produce biofuels results in the by-product algae meal. The oil from algae is being used to make biodiesel and what is left after extraction is being termed algae meal. The algae meals that have been studied at the University of Georgia have varied dramatically, which appears to be dependent on the species of algae, extraction processes, washing, drying, etc. The TME values have ranged from 1,690 to 3,472 kcal/lb. This may be expected since the fat was also highly variable; it ranged from 0.34 to 10.68%. The crude protein content can range from 11% to as high as 66% and total lysine and methionine ranged from 0.4 to 4.4 and 0.18 to 1.42, respectively. The true amino acid digestibility coefficient range from 65 to 97%. Because of this variation it is almost impossible to give an average nutrient composition. Feeding studies are being conducted to evaluate the effect of whole algae
and algae meal in poultry diets. Whole algae and algae meal may be a good source of protein and energy however, as this new by-product continues to emerge it is extremely important to get nutrient analysis on the specific algae being used in feeds.

Conclusions

The by-products from the biofuels industry continues to change as the industry matures. The main by-product of ethanol production, DDGS also continues to change as various technologies are added to ethanol plants to increase products produced. Many of the by-products produced by the biofuels industry can be used successful in poultry diets to save money and maybe even improve performance. However, there is still an extreme amount of variation within the by-products from the biofuels industry thus it is crucial that confirmatory analysis be conducted on any by-product of the biofuels industry.

References (more references available on request)


CORN: SUPPLY, USES AND FUTURE

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Summary:

Thanks to technology focused corn growers in the United States there is and will be plenty of corn to eat, make ethanol, feed poultry and other species of livestock and even export to key buyers around the world.

The 2010-11 crop and marketing year has and is proving to be a most unusual year as far as crop reporting. On February 9, 2010 the USDA made a bigger than expected cut to the U. S. corn ending stocks while leaving soybeans and wheat unchanged. USDA has 2010-11 U. S. corn carryout at 675 million bushels, down 70 million on the month following upward adjustments to the food, seed and industrial, with most of the increase in ethanol production. If we see no major changes in USDA numbers the average 2010-11 farm gate price is estimated to be $5.05-$5.75/bushel, compared to the 2009-10 average of $3.55/bushel.

The early reports are all suggesting very good quality from the crop from a mycotoxin view. In fact there are reports from some ethanol plants that ethanol yields are better than expected from the same amount of starch. What we do see and have seen in the past is continued reduction in Crude Protein. We have, as well seen increased starch levels (~75% DM of the kernel). This is being driven by the continued increase in yield (bushels/acre). This increase in yield is certainly driven by advancements in yield and stress technology.

As global feed, fuel and fuel increases, so does the need for corn that can be efficiently and safely produced. Breakthrough technologies include key information on the genetics or “genomes” and traditional breeding methods. Traditional plant breeding focused on the selection of the best plant lines based on phenotypes and the complete genome of corn.

Introduction:

Corn (Zea mays L) has been known only as a cultivated species and could not survive without human intervention. The center of origin was likely Mexico or Guatemala with domestication some 5000 BC. The native inhabitants of the Americas domesticated and improved corn after realizing its potential for food, feed and fuel (Pollack, 1995).

Over the past many decades we have continued to find new and novel uses for corn, from feed to feed to biodegradable plastics to new carbohydrate polymers to sweeteners and fuel and for the most part our production has kept up. Based on a tremendous amount of research in using corn for processing we now have over 3,038 uses of corn (Weigel). In the mid 2008 we saw and old but very hungry customer, ethanol, surface and during the same time period we had flooding in the Midwest. We also started hearing a new argument. If this new customer is going to use more corn, other customers are going to have to make a choice between corn for food or fuel, the perception that there was not enough for both. Thus the new term of “food v. fuel”. Words that went around the world.
In my opinion this is a very bogus argument and very false. Why is this so? Easy, American farmers produced more corn due to improved and advanced technology, like global positioning and much larger planting and harvesting machinery. Literally less than a century ago farmers harvested corn on the ear by hand and used small horse drawn wagons to small storage facilities or put on the ground. But new technology like 18 row combines with thousand fold bushel trucks and grain carts to take corn to multi-million bushel storage facilities while maintaining quality. In fact we have seen 5 fold increases in average yields over the past 5-6 decades.

We also have produced a cleaner environment and continue to demonstrate reductions in environment impact:

- Less pesticide use, due to increased insect resistances
- Growing 5 x corn on 20% less acreage
- Increased use in reduced or no till management systems, reducing soil loss
- ~70% more corn/pound of fertilizer
- This will continue

**Supply:**

U. S. corn ending stocks for the 2010-11 are projected 70 million bushels lower from the February 9, 2011 report with higher ethanol use. Ending stocks is projected at 675 million bushels and is considered to be only supply line requirements. This month's projections lower the stocks-to-use ratio to 5%, the same as what we witnessed in 1995-96, the last time ending stocks fell to multi-year levels. What is most interesting is that we have seen a decline in ending stocks eight out of the past 9 monthly reports.


<table>
<thead>
<tr>
<th>Item</th>
<th>2009-10</th>
<th>2010-11 Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planted Acres (million)</td>
<td>86.4</td>
<td>88.2</td>
</tr>
<tr>
<td>Yield (bu.per acre (bpa)</td>
<td>164.7</td>
<td>152.8</td>
</tr>
<tr>
<td>Beginning Stocks (million bu's)</td>
<td>1,673</td>
<td>1,708</td>
</tr>
<tr>
<td>Production (million bu's)</td>
<td>13,092</td>
<td>12,447</td>
</tr>
<tr>
<td>Feed Use (million bu's)</td>
<td>5.14</td>
<td>5.20</td>
</tr>
<tr>
<td>F.S.I (million bu's)</td>
<td>1,371</td>
<td>1,400</td>
</tr>
<tr>
<td>Fuel Ethanol (million bu's)</td>
<td>4,568</td>
<td>4,950</td>
</tr>
<tr>
<td>Exports (million bu's)</td>
<td>1,987</td>
<td>1,950</td>
</tr>
<tr>
<td>Ending Stocks (million bu's)</td>
<td>1,708</td>
<td>675</td>
</tr>
</tbody>
</table>

It is apparent that corn consumption is progressing as projected. Our corn used for ethanol is running at about 8% greater than last year with growth of ethanol marketing at 15% greater clearly demonstrating the better than average yield conversion from corn to ethanol. It appears based on Census Bureau export estimates were tracking on projections (D. Good, personal communication).

As of the first of January, 2011 we had 204 fuel distillation plants with plate capacity of 15,000.0 mgpy and actual production of about 13,500.0 mgpy. There are some under construction or being refurbished. Such a high capacity utilization rate (~90%) suggests limited upside room for this production figure and plenty of room for production potential, with further reductions in profit margins. It is
important to remember that one of the reasons ethanol producers are over-looking the stocks buildup is the traditional action of fuel blenders and refiners to rebuild inventory toward the end of the year following draw-downs over the prior months.

It is understood that it will take several months for the logistics and labeling complications regarding E15 to be resolved. But all ethanol producers are very confident of the ethanol production increasing.

The rapid pace of distiller's grain production should continue to slow corn use for feed. This maybe a bit over-shadowed by the 5% increase in cattle placements and we are not seeing reductions in dairy cow numbers. The industry is increasing sales and marketing of distiller's internationally with China as a focal point. It is important to remember the surging price of corn have shunted the unit price of ethanol into the red for the first time on record and inventories of the alternative fuel have recently moved to a mid year high.

**Plant Biotechnology:**

In agriculture, desirable crop characteristic are known as traits. One of the most important traits is yield. Improving crop yield can be accomplished through breeding and biotechnology, but more important both put together. Breeding allows plant breeders to improve elite germplasm, for example to develop seeds with the optimum mix of characteristics to deliver the best possible yield for the soil and climatic conditions where they will be grown. Today, plant breeders use a mix of traditional and novel and intellectually property protected (I.P.'ed). One of these methods include *marker assisted breeding* which allows breeders to use the plant genome to select the most elite germplasm with the most desirable characteristics.

There are three categories of biotechnology:

- **Agronomic** - herbicide tolerance (HT) and insect resistance (IR) or traits for crop production
- **Nutritional** - enhanced nutritional characteristics directed toward end-users needs
- **Functional** - improved processing for food, feed or fuel

Many biotechnology companies have referred to increasing corn trend lines (average corn production per acre over the total number of corn acres harvested) of 1.5 to 2.0 fold increases or 150 bpa to 300 bpa over the next 20 years or fruition in 2030. This will be driven by specific genes inserted into the most elite germplasm with the most genetic diversity over the entire United States. These genes will be focused around;

- Drought tolerance
- Heat stress
- Soil differences
- Water use efficiency
- Nitrogen use efficiency

**Nutritional Characteristics:**

Variations in corn nutritional composition are created by genetic, agronomic practices, climatic conditions and even managerial factors. In the past nutritionists within the animal sector have relied on “book values” for feed formulation. Work conducted by Weigel (Jerry Weigel) in evaluating nutritional composition of corn within cultivars, production location, and fertilization found definite differences in
starch and protein (Crude), with much smaller differences in amino acids. There was some differences in oil as well.

One of the concerns I have is how starch is deposited into kernel and the composition of that starch. We know that the prolamin proteins called zein are the primary protein in the starch protein matrix. Prolamin-zein, defines a class of hydrophobic proteins synthesized on the rough endoplasmic reticulum of the amyloplast (starch producing organelle) consisting of four sub zein classes. Because this occurs within the amyloplast without the presence of transit genes on the rough endoplasmic reticulum the zein protein is not intrinsic, but only located in the surface or the exterior portion of the starch granule. It is my opinion that this should be a major focus to enzyme manufacturers to improve the energy value of corn.

In following with the above comments physiological characterization of corn kernel development indicates that starch accumulates in the kernel 12-35 days after pollination (DAP) while storage proteins, mainly zeins, begin 10-15 DAP and continues until maturity Manicacci et al., 2009). As one further understands how pyruvate orthophosphate dikinase (PPDK) plays in the critical role in protein versus starch matrix within the kernel. Much more work needs to be done on this as it relates to animal responses.

As we increase the yield of corn many are asking what will the nutritional profile of corn that yielded 300 bpa as compared to 150 bpa. It is well accepted that yield is driven by elite germplasm that uses starch to enhance yield, as starch is the energy needed by the kernel to fill. We know that starch levels on traditional corn yields ranges between 70.00% - 72.00% (Dry Matter (DM)). We have seen crude protein levels on comparable starch and yields of 7.50% - 8.50% DM. (Jerry Weigel, unpublished data). It must also be noted that there are regional differences, it should be lifted up the corn in the corn growing regions of central Indiana and even southern Indiana has as much as 1% point increase in protein (Weigel). Dr. Paul Scott (Scott et al., 2006) does a very profound overview on how the nutritional characteristics of corn has changed over time. His review follows what we have seen in our lab. The real question will be the differences in the plant genetics from harvested grain and environmental conditions. There also needs to be more research with corn, as with wheat, on the effect of nitrogen (N) fertilizer to crude protein and even protein quality (lysine).

Based on formulation experiments (Weigel) that for 0.1 point increase in crude protein could reflect an increase in corn value of $0.012 or $0.12/point of protein.

It is very important that formulating Nutritionists regularly monitor protein levels in corn for the most accurately depict formulation.

What other traits are being investigated?

- Reductions in anti nutritional factors, low phytate
- Improved amino acid concentrations
- Oil quantity and quality
- Energy concentrations
- Pigmentation
- Aflatoxin/mycotoxin resistance
- Improved corn ensilage
- Vehicle for consumption of edible vaccines
- “Think about more”
Summary:

We are living in very exciting times. We will see new and novel feeding ingredients evolving on a regular basis. The major biotechnology companies are focusing on input traits, as our price discovery system is based on yield, not quality. The industry must push hard to these biotechnology companies the need for quality, especially nutritional quality for feed use. It certainly is very important that these traits are on the most elite germplasm for yield parity.

The problem in the past is very simple; the trait or set of traits did not have the value that the end-user would realize and the providing biotechnology companies did not understand this value. There were also major reductions in yield over historical averages within that growing zone. There has to be enough “money on the table” for everyone in the value chain to make money. In the feed industry this value chain could be;

Biotech Company-->Seed Company-->Farmer-->Elevator-->Feed Mill-->End-User

In some cases there maybe similarities within each part of the chain. One thing that should be considered by the end-user is offering direct grower contracts with the large progressive growers.

In closing, I again want to lift up a statement I made many years ago: "you are only limited by your imagination relative to what you want the biotechnology industry to put in your corn of tomorrow”

As we look forward to corn availability in the future, we have the scientific and mechanical technologies to meet the demand, and adding the fact that the American farmer wants to plant corn!

I must not leave this paper without the infamous quote:

“The greatest service which can be rendered to any country is, to add a useful plant to its culture”

Thomas Jefferson, A memorandum of Services to My Country, CIRCA, 1800

References


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Weigel, J.C. BASF Plant Science, 1996 – 2010 collection time


WHAT POULTRY NUTRITIONISTS AND MARKETERS NEED TO KNOW ABOUT CONJUGATED LINOLEIC ACID: A POTENT ANTI-INFLAMMATORY OF RUMINANT ORIGIN

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Summary

Conjugated linoleic acid (CLA) has been extensively studied by the scientific community with over 2000 peer-reviewed reports published of its effects on human and animal health. CLA, while a naturally occurring fatty acid in animal fats (mainly fats of ruminating animals), is currently sold as a synthetic formula and approved for select animal and human foods. Dietary consumption or supplementation imparts considerable health benefits to both animals and humans, including disease associated with adiposity, inflammation, cancer and cardiovascular function. While the supplementation of most poultry diets has little economical benefits with regards to animal growth and feed efficiency, there are clearly applications of CLA in the poultry industry. The purpose of this short review to provide some insight into why poultry nutritionists and marketers of poultry products should keep themselves informed with regards to opportunities involving CLA: 1) Dietary CLA has uses for poultry species during select environmental stresses; 2) Limited data suggest it may be useful in the prevention of skeletal abnormalities; 3) Poultry products enriched in CLA may be viewed as a value added product and; 4) CLA present in the muscle may also improve the shelf life of poultry products.

Conjugated linoleic acid (CLA)

During the fermentation of feedstuffs, the microorganisms in the rumen of ruminant animal species, and less so the microbes in the gastrointestinal tract of monogastrics, biohydrogenate unsaturated fatty acids into saturated fatty acids. A number of intermediates of fatty acid biohydrogenation occur during this process, and among them are the CLAs or fatty acid precursors of CLA. Two CLA products made from linolenic (C18:3, cis 9, cis 12, cis 15) and linoleic (cis 9, cis 12) contain positional changes in their double bonds and are known as c9t11- and t10c12-CLA. Other conjugated diene isomers of less known biological activity are also produced (see Bauman et al., 2003). A significant amount of C18:1, t11 (also known as vaccenic acid) is produced during the biohydrogenation process. Vaccenic acid (while considered as a “trans” fatty acid) when absorbed by the animal undergoes hepatic enzyme action (stearoyl-CoA desaturase) to form c9t11-CLA. The feeding practices used by dairy producers can dramatic impact the level of CLA isomers in milk fat and animal tallow. For example, when a ruminant is placed on pasture, the linolenic acids in grasses are biohydrogenated such that the levels of CLA in ruminant fat can increase 5 fold (where c9t11-CLA can increase to 4% of total fatty acids in milk fat). The predominant CLA isomer found in animal fat is the c9t11-CLA isomer (Bauman et al., 2000; 2003). Depending on feeding strategies the t10c12-CLA isomer rarely increases above 0.2% of total fatty acids (Shingfield et al., 2006). The use of animal fats of ruminant origin can serve as a source of CLAs or precursors for CLA for other animals and humans. Hence understanding their biological role in the health of other species is critical.
Most published research on CLA’s biological activities used synthetic forms of the isomers. When oils rich in linoleic acid are subjected to heat and alkali, essentially all the linoleic acid is converted into equal amounts of c9t11- and t10c12-CLA. CLA made from corn oil (50% linoleic acid) yields oil with approximately 25% c9t11 and 25% t10c12. A higher percent of CLA isomers can be achieved using high linoleic acid sunflower oil (60% linoleic acid) or safflower oil (80% linoleic acid). CLA synthesized from vegetables oils is inexpensive and readily available for animal studies. A few animal studies have been conducted using the pure isomers of CLA, simply to determine the isomer responsible for a particular biological activity. Once the active isomer was determined, there was rarely a need to continue the study of CLA in its pure isomer form, unless isomers were found to interact with one another. Hence, follow-up studies often used the more available 50:50 mixture of c9t11- and t10c12-CLA.

CLA current regulatory status

Synthetic CLA was first introduced to the market place as a dietary supplement to prevent body fat accumulation in humans. Numerous human clinical trials were conducted and CLA was found to be “generally recognized as safe.” In 2008, the use of synthetic CLA, in select human foods, was approved by the Food and Drug Administration. Shortly afterward, CLA was approved for use in finishing swine diets. At this time, additional approvals are being sought for other animal applications. The only source of CLA that can be found in animal diets, apart from synthetic CLA in finishing swine diets, is CLA naturally found in animal fats. Chin et al. (1992) analyzed the CLA content of a variety of animal products. They found that turkey meat contained significantly more CLA than meats from other monogastric muscles (2.5mg/g fat vs. 0.9 mg/g fat and 0.6mg/g fat for turkey, chicken and swine, respectively). However, analysis of turkey breast meat for CLA content by another group did not substantiate a significant level of CLA isomers (Yan et al., 2006). It is very possible that the level of CLA in the fat of different monogastric animal species is largely dependent of the diet the animals were fed (Tathong and Worrawattanatam, 2010). If a nutritionist were to use natural sources of CLA (i.e., animal fat), ruminant fat would be the preferred source. The limited natural sources of CLA (with its predominant c9t11-CLA isomer) and the unique fatty acid profile of synthetic CLA (50:50 c9t11- and t10c12-CLA) makes the analysis of CLA a useful biomarker in determining the origin of fats used in animal feeds or type of fat fed to poultry.

Health benefits of dietary CLA

An unidentified growth factor in milk from cattle during summer months was described in the 1940s (Boer et al., 1946), but the nature of this growth factor had never been understood and was somewhat controversial. It was also known that milk contained fatty substances that absorbed at a UV wavelength, and this property was eventually shown to be fatty acids with conjugated dienes (Parodi, 1999). Beginning in the late 1970s (Pariza et al., 1979), studies on the formation of carcinogenic compounds during the grilling of hamburger meat suggested that beef contained a factor that may prevent mutagenesis. Pariza’s group went on to discover that beef contained an anti-carcinogenic factor, and that factor was conjugated linoleic acid (Ha et al., 1987). Synthesis of a mixture of the two primarily conjugated dienes (c9t11-, and t10c12-CLA) permitted a rapid succession of studies that now has grown to include over 2000 peer-reviewed publications.

Discoveries involving CLA focused in three primary fields of study: CLA’s ability to control fat accumulation, CLA’s anti-cancer activity, and CLA’s anti-inflammatory activity (Table 1).

An early study involving the maternal effects of CLA on progeny development showed that dietary CLA promoted growth (Chin et al., 1994). Other observations made were decreased feed intake and improved feed efficiency in mice and chicks fed CLA. Improved growth and reduced feed intake led to studies investigating the effects of CLA on body composition. When these studies were conducted in
mice, the feeding of CLA markedly prevented body fat accumulation (Park et al., 1997). These findings resulted in a rapid launch of human CLA supplements to control body fat in humans as well as human clinical trials to confirm the preclinical studies (Watrus et al., 2006; Whigham et al., 2007). Trials were also conducted in pigs with similar effects (Dugan et al., 1997).

Currently, CLA is largely sold as a human supplement or food additive for the control of body fat accumulation. Early studies also suggested that CLA protected against immune-induced cachexia (Cook et al., 1993; Miller et al. 1994). Studies were conducted to determine if the protection against immune-induced weight loss was a function of immune suppression. Findings strongly supported that dietary CLA promoted select immune reactivity. Enhanced immune reactivity led researchers to investigate if CLA had contraindicated uses in immune disorders. In a model of airway hypersensitivity, dietary CLA was protective (Whigham et al., 2000) and these findings were supported in human studies of type 1-hypersensitivity diseases such as asthma (MacRedmond et al., 2010) and allergies (Turpeinen et al., 2008). Additionally in models of lupus, and arthritis there was a protective effect of CLA against inflammatory diseases (Yang and Cook, 2003; Huebner et al., 2010). More recently an epidemiologic study linked CLA levels in humans to a decreased risk of myocardial infarction (Smit et al., 2010). The anti-cancer effects of CLA continued to largely be based on animal and cell culture models, however some epidemiology data supports an anticancer effect of CLA in humans (Aro et al., 2000). The extensive evidence that CLA provides health benefits in humans and animals suggests that CLA will be increasingly found in human foods either through direct supplementation or enriched animal products.

Poultry and CLA

Questions that poultry nutritionists and marketers of poultry products need answered include: 1) What effects the CLA content of oils and fats fed to poultry? 2) Does supplementation of a poultry diet with CLA provide health or productive benefits? 3) Is there an opportunity to create a CLA enriched food? In the sections that follow, select literature involving CLA and poultry will be presented. Topics presented may be of interest to the poultry industry and if so, the limited references could serve as a starting point for those interested. In some cases, information provided may not come from peer-reviewed scientific literature.

CLA in fats and oils fed to poultry

Nutritionists that feed poultry strict plant based oils and ingredients will not provide a dietary source of CLA. CLA in the tissue of poultry fed strict plant based diets likely came from fermentation in the gastrointestinal tract. Previous work involving germ free rats suggested that there could trace amounts of CLA in the tissues of monogastric animals through gastrointestinal fermentation (Chin et al., 1994b). There may be occasional reports of CLA in plant based poultry diets, but CLA in these diets could be the result of analytical artifact where CLA was synthesized during the preparation of samples for analysis. Birds that are fed potentially fermentative dietary plant material or raised in a manner that they are exposed to fermentation material (including excreta) may have an increased tissue levels of CLA (i.e., pastured poultry), but at this time, experiments are not available to show this method of feeding would increase the CLA content of poultry. A recent report suggests this is an area worth investigating (Tathong and Worrawattanatam, 2010). If poultry are fed animal fats or animal ingredients containing animal fat, particularly those that originate from ruminant species or pigs fed synthetic CLA, there will be CLA found in the tissues and products of the birds. There is the possibility that if the birds consume trans vaccenic acid (C18:1, trans 11) that this fatty acid could be converted to c9t11-CLA via the bird’s hepatic stearoyl-CoA desaturase. Vaccenic acid is primarily a fatty acid of ruminant origin and this fat source would also contain variable amounts of CLA. However, when estimating the total amount of CLA that may be found in poultry meats or products, the vaccenic acid content of a fat should be considered as a significant source through hepatic synthesis.
Table 1 Demonstrated health benefits of animals and humans consuming dietary CLA

<table>
<thead>
<tr>
<th>Animal Tested</th>
<th>Benefit</th>
<th>Key References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, Pig, Human</td>
<td>Prevention of body fat accumulation and increase in lean mass</td>
<td>Park et al 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dugan et al 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Watras et al 2006</td>
</tr>
<tr>
<td>Rat, Mouse, Human</td>
<td>Prevention of prostate and mammary cancer</td>
<td>Ip et al 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cesano et al 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aro et al 2000</td>
</tr>
<tr>
<td>Rabbit, Hamster, Human</td>
<td>Cardiovascular protection, Reduction of atherosclerosis</td>
<td>Lee et al 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nicolosi et al 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smit et al 2010</td>
</tr>
<tr>
<td>Chick, Rat, Mouse</td>
<td>Anti-cachectic and immune-induced weight loss</td>
<td>Cook et al 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Miller et al 1994</td>
</tr>
<tr>
<td>Guinea Pig, Human</td>
<td>Prevents airway disease involved in type I hypersensitivity</td>
<td>Whigham et al 2001, 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MacRedmond et al 2010</td>
</tr>
<tr>
<td>Mouse, Human</td>
<td>Anti-inflammatory in lupus and arthritis</td>
<td>Yang et al 2000, 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Huebner et al 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aryaelan et al 2009</td>
</tr>
</tbody>
</table>

References listed are the first that reported benefits shown. This list is by no means comprehensive. A comprehensive list of references can be found at http://fri.wisc.edu/claph.php

At this time it is unknown if there has been a change in the CLA content of animal fats due to modern feeding methods in cattle (likely a CLA decrease) or if the use of synthetic CLA will influence the CLA content of rendered fats in the future (a likely CLA increase). It is known that when humans switched from the consumption of animal fats to vegetable oils there was an increase in the polyunsaturated fatty acids (mainly linoleic acid) in human foods (and rendered fats) over the last 40 years. Since residual oils from human foods is often rendered into animal feedstocks, there is a potential for CLA to eventually begin to appear in oils used in poultry diets due to its synthetic use or changes that may result from cattle feeding practices. Human decreased consumption of animal fats has probably resulted in a decrease consumption of CLA. An analysis of fats and oils stored in freezers for many years could prove interesting with regards to changes in the CLA content due to human consumption trends and cattle feeding practices. Examination of past published fatty acid profile data will not be useful since often the methods used did not detect conjugated fatty acids. It would be reasonable to hypothesize that the CLA content of both the fats and oils fed to poultry and the level in poultry tissues and products has declined the last 40 years. Increased CLA in animal products (particularly c9t11-CLA) due to feeding practices or the direct addition of CLA to finishing diets may actually return these levels to what was observed 40 years ago. The one exception could be t10c12-CLA. This particular isomer was never, to our knowledge, a significant isomer in animal fats and tissues, and there is no precursor fatty acid that can be found in the diet that the animal will convert to t10c12-CLA. Research suggests that this fatty acid has a rapid turnover, such that when removed from the animal diet, its presence in tissue is greatly reduced (Park et al., 1999). Therefore, its appearance would be a function of how synthetic CLA is fed.
Usefulness of CLA as a dietary supplement in poultry

In an earlier publication (Chin et al., 1994) we described CLA as a “previously unrecognized nutrient.” This was a rather bold statement for a nutritionist, but it is worthwhile to continue to explore this possibility. There are a series of studies that could be done to support this statement and even determine if it is a required nutrient. However, until studies are conducted to prove or disprove its essentiality in the diet for optimal animal health, an open mind must be maintained. Apart from being a potential nutrient, below are some research results that would suggest its usefulness and potential limits (as with all nutrients) with regards to feeding CLA to poultry.

Poultry and skeletal abnormalities

During early studies involving the feeding of broiler chicks dietary CLA in battery cages, an observation was made that broilers fed CLA had less valgus/varus leg deformities (but normal bone mineralization). Data was collected during this experiment and patent applications were made (Cook et al., 1998; Cook and Pariza, 1998). Table 2 presents some of the finding from one of these experiments. Part of the reason that additional studies were not reported on the effects of CLA and valgus/varus leg deformities was because we did not observe a high incidence of the skeletal problem in subsequent trials. Several reports involving the feeding of CLA to broilers in floor pens has been reported. Unfortunately, there have been no additional reports of CLA’s affect on valgus/varus leg deformities. Leg deformities in broilers are common clinical signs of a number of nutrient deficiencies including zinc, manganese, niacin, choline, biotin, pyridoxine, and folic acid. The inability to reproduce clinical signs of disease in broilers suggest that there may be complex environmental factors involved in the defect that are not well understood. The finding of dietary CLA’s correction of this defect, may have been a chance observation. While valgus/varus leg deformities are not the result of poor bone mineralization, Leone et al., 2009 did observe that feeding breeding hens CLA increased the ash content of 25 day-old chicks 16%. These results, as well as other reports on dietary CLA’s effects on bone mineralization, suggest that CLA may play a critical role in skeletal formation.

Table 2. Effects of dietary CLA on valgus/varus leg deformities. 3-week-old broiler chickens were in battery cages. Score of 1=normal and 4=severe*

<table>
<thead>
<tr>
<th>Dietary CLA</th>
<th>Body Weight Gain</th>
<th>Leg Score (± SEM)</th>
<th>% Birds Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>605</td>
<td>2.0 ± 0.2</td>
<td>27 ±7</td>
</tr>
<tr>
<td>0.2</td>
<td>606</td>
<td>1.7 ± 0.2</td>
<td>13 ± 10</td>
</tr>
<tr>
<td>0.4</td>
<td>618</td>
<td>1.6 ± 0.4</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>0.8</td>
<td>601</td>
<td>1.4 ± 0.2</td>
<td>3 ± 3</td>
</tr>
</tbody>
</table>

*Each mean represents 6 pens of 5 chicks

Poultry and cachexia

The first experiments to investigate a role of CLA in poultry involved its use in the prevention of weight loss due to endotoxin injection (Cook et al., 1993). Chicks fed a control diet and injected with endotoxin either stopped growing or lost weight over a 24-hour period. However, if chicks were fed CLA and injected with endotoxin, chicks continued to grow, although at a slightly reduced rate when compared to saline injection. Partial prevention of endotoxin-induced decreases in weight gain was confirmed in both chickens (Takahashi et al., 2002) and mammals (Miller et al., 1994). One area of immune (endotoxin)-induced weight loss that has not been explored involves the laying hen (pullet). The pullet receives multiple vaccines during its growing period, and depending on the vaccine management practices, some of these vaccines can include gram-negative bacteria in oil-based adjuvants. No study has been conducted to determine if the use of CLA in the diet of growing pullets could be cost effective in
helping the pullet reach mature body weight earlier. CLA has no adverse effects on immune responses (and may actually enhance certain immune responses), hence its use in conjunction with vaccination should not be a problem. However, feeding CLA to pullets may have effects on body composition (see later) that could impact their subsequent rate of lay.

**Poultry growth and feed efficiency**

As mentioned, CLA was reported to be a growth factor (Chin et al., 1994) in rats. In the study by Chin et al., c9t11-CLA appeared to be the isomer responsible for improved progeny growth. In mice studies, we also observed that the c9t11-CLA isomer was a growth factor (unpublished). Studies with broilers, where synthetic isomers of CLA were fed in the 50:50 mix, have shown no improved growth response as a result of CLA feeding (our unpublished results agree with these finding). There does appear to be a slight improvement in feed efficiency of 2.5% or four points in some studies (Takahashi et al., 2002; 2003). This level of improved feed efficiency is below that needed to make CLA a cost effective additive when fed at the levels used. Surprisingly, most studies involving the feeding of CLA in poultry have involved high dietary levels (1-3%), particularly when one considers the biochemistry of CLA. Rodent studies often used lower levels (0.5%) and even these levels were fed to achieve rapid tissue saturation (less than 2 weeks of feeding). High levels of dietary CLA could result in decreased weight gain. While it is not likely that lower doses of CLA will improve growth and feed efficiency (Du and Ahn 2002), there may be value in studies feeding lower levels of CLA particularly if other valuable responses were desirable. For example, it has been shown that the feeding of saturated fats to broilers may improve valuable attributes such as meat shelf life (see below). Since dietary CLA increases the saturated fatty acids content of the bird (see below), it may alter its response to heat. Limited reports support a positive effect of dietary CLA on heat stressed broilers (Celik, 2006). Also, if CLA reduces certain types of leg deformities in poultry, low doses may prove to be adequate to improve bone health.

**Body fat and fatty acid content of poultry**

Dietary CLA is well known to reduce fat accumulation in a number of animal species including humans. Reduced body fat in broilers fed CLA required levels of 1% or greater and the reduction of body fat was not as great as observed in some species (Du and Ahn, 2002). Dietary CLA (t10c12-CLA) inhibits hepatic stearoyl- CoA desaturase in animal including poultry. Birds fed CLA have increased tissue saturated fatty acids. Increased tissue saturated fatty acids affects the storage stability of poultry meats (Yan et al., 2006). There may be circumstances where reduced oxidation of poultry meat during storage (e.g., turkey) could be important enough that the additional cost of CLA supplementation was justified. Also, the content of CLA in poultry products is directly related to the length of time CLA was fed and the dietary dose (Du and Ahn, 2002; Aydin and Cook, 2004), hence if a marketer of poultry products could establish value in human food products based on CLA content, ample literature is available to teach nutritionists how to achieve these goals.
dietary CLA (specifically the synthetic formula) deserves an entire review and is complex, some general comments can be made. The decrease in hatchability induced by maternal dietary CLA can be completely prevented by altering the fatty acid composition of the hen’s diet (Aydin et al., 2001). The use of rendered fats from animals fed CLA, even if the animal fat is naturally saturated, such as beef tallow, will not alter egg hatchability even though CLA is present (Aydin and Cook, 2005), and maternal feeding of CLA can result in normal progeny development post hatch (Leone et al., 2009). These findings suggest that nutritionists that use synthetic CLA in a feeding program involving breeding hens, may find value but should be aware that there are specific formulation needs when CLA is fed to breeding hens. CLA is not toxic to the embryo (Aydin and Cook, 2009), but depending on the diet fed to the hen, CLA can influence lipid transport across the yolk sac membrane (Leone et al., 2010). Also, if CLA enriched eggs are produced, nutritionists will need to change the fatty acid content of the diet to assure that yolk hardening does not occur. Dietary changes to achieve normal hatch and acceptable egg quality when hens are fed CLA are not overly cumbersome. Also, since CLA content in the rendered fat source could have potential value in the progeny of breeders fed CLA (Leone et al., 2009), there could be advantages in having an analysis of the CLA content of the fats used in poultry diets.

General Conclusions

Conjugated linoleic acid (CLA) is a natural occurring isomer of linoleic acid that is only found in significant quantities in animal fat (predominately of ruminant origin). Health benefits in the control of adiposity and diseases of inflammation are well recognized and scientifically supported. At this time, the use of synthetic CLA in poultry diets cannot be economically justified based on its effects on growth and feed efficiency, however, there may be reason for considering the use of CLA in poultry diets. Limited data suggest that dietary CLA may have value in skeletal development. Dietary CLA may offer resistance to environmental stress. The shelf life of poultry meats can be extended through the feeding of CLA. Poultry products containing CLA may one day be viewed as being healthy as a human food. Feeding breeder hens CLA, while requiring special dietary consideration, may have benefit to progeny development. Both poultry nutritionists and marketers of poultry products should continually look for opportunities to consider the role of CLA in their business strategies.

Additional Reading:


BUTYRATE REGULATES UREA METABOLISM AND NITROGEN USE IN SHEEP

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ABSTRACT: Butyrate has been implicated in regulation of rumen urea transporter expression and thus urea recycling, yet direct evidence is absent. This study aimed to determine the effect of butyrate on urea recycling by infusion of butyrate into the rumen. Wether sheep (42 kg BW) fitted with rumen cannula were fed to 1.8× energy maintenance a standard diet (130 g CP/kg, 9.3 MJ ME/kg) and infused into the rumen with isoenergetic (1.2 MJ/d) and isonatremic solutions of either Na-Acetate (control) or Na-Butyrate for 10-d periods in a crossover design. $[^{15}N_2]$Urea was continuously infused IV for the last 5 d, and all urine and feces were collected. Compared to no infusion, Butyrate increased (P < 0.05) rumen acetate and butyrate, whereas Acetate increased (P < 0.05) rumen acetate. Compared to Acetate, Butyrate decreased urea entry (17.2 vs 14.3 g urea-N/d, P < 0.05) and urinary urea excretion (9.3 vs 7.7 g urea-N/d, P = 0.11). Although transfer of urea to the gut was not different, Butyrate increased (0.6 vs 2.2 g urea-N/d, P < 0.05) microbial capture of the urea-N that was transferred to the gut. The results suggest that butyrate does not increase urea recycling to the gut compared to acetate. However, the reduction in urea synthesis coupled with increased capture of recycled urea-N by gut microbes suggests that butyrate enhanced overall capture of feed and urea derived ammonia by microbes.
TURKEY LITTER AND HEN MANURE ASH PHOSPHATES AS A DIETARY SUPPLEMENT FOR BROILER CHICKENS

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ABSTRACT: Turkey litter (TL) ash (13.7 metric tons) was generated on a commercial turkey farm from a 586kW boiler made by Blue Flame Stoker and an experimental belted hen manure (HM) ash was generated using a Coaltec Energy USA, Inc. gasification system. TL and HM ash contained 11.6 and 3.8% P and 19.4 and 25.1% Ca, respectively. Three corn and soybean meal diets with 1% Celite (3100 kcal/kg, 22% CP) were formulated with 1% Ca and graded levels of available P (avP) (0.20, 0.25 and 0.35%). TL and HM ash diets were formulated to have 0.35 and 0.27% avP, respectively, with 1% Ca. In a 4 wk study, 280 Ross x Heritage male broiler chickens were fed these diets and provided water ad libitum, with 7 pens per diet and 8 chicks per pen. Weekly feed intake (FI), body wt (BW), and mobility was monitored. At 28 d, ileal digesta was collected from carcasses to determine P and Ca digestibility. Left legs were removed for tibial bone ash determination. Statistical analysis was done with SAS version 9.1 using a one-way ANOVA and Tukey’s mean comparisons; P≤0.05 was deemed significant. BW was significantly reduced by the low P (0.20 and 0.25% avP), TL ash, and HM ash diets compared to the control (0.35% avP) diet in wks 2 and 3. However, in wks 1 and 4, BW did not differ significantly between the TL ash and control diets. Weekly BW gain for the control and TL ash diets averaged 83, 178, 296 and 391 g/bird at wks 1, 2, 3, and 4, respectively, and FI averaged 104, 270, 464 and 646 g/bird in wks 1, 2, 3, and 4, respectively. Both gain and FI were reduced for the low P and HM ash diets. Bird mobility was excellent for the control and TL ash diets in wk 4 (100 and 98.2%, respectively), but was significantly reduced for the low P (0.20 and 0.25 avP) and HM ash diets (60.0, 76.0 and 60.7%, respectively). Overall, mortality was high for the low P (0.20 and 0.25 avP) and HM ash diets (85.7, 39.3, and 55.4%, respectively), particularly in wk 4 of this study; however mortality was low for both the control and TL ash diets (1.8 and 3.6%, respectively). Percent bone ash was significantly greater for the control and TL ash diets (34.5 and 35.3%, respectively) compared to the low P (0.20 and 0.25% avP) and HM ash diets (28.4, 28.6, and 27.1%, respectively). Based on BW and FI, the P in HM ash was utilized at 83.7 and 68.1% efficiency, respectively, compared to monocalcium phosphate used in the 0.25% avP diet. Based on BW, gain, and FI, the P in TL ash was utilized at 88.3, 84.0, and 89.4% efficiency, respectively, compared to monocalcium phosphate used in the 0.35% avP (control) diet. The results of this study indicate that both ash products from the incineration of turkey litter and gasification of hen manure may have recycling potential as a dietary phosphate.
ENERGY BALANCE REGULATION AND CARBOHYDRATE UTILIZATION IN DEVELOPING CHICKEN EMBRYOS

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ABSTRACT: Broiler chicks hatched from small (<55 g) compared to typical size (65-70 g) eggs have greater embryonic and post-hatch mortality, and this has been linked to low glucose status and tissue glycogen stores. We hypothesized that with even more limited resources, carbohydrate utilization and regulation of energy metabolism would differ between the two sizes of eggs. Small (53.2 ± 1.0 g, n=60) and typical size (69.0 ± 1.9 g, n=60) broiler breeder eggs from 26 wk and 42 wk-old hens, respectively, were acquired from Perdue Farms Inc., and measurements were made on embryonic (e) days 11, e14, e17, e20 and on post-hatch day 1. The initial dry yolk:albumen ratio was higher (2.6 vs 1.6) in typical vs small size eggs. At set, glucose and mannose contents in albumen were 500-fold higher than fucose, but by e11 glucose content decreased to negligible levels whereas mannose and fucose remained constant. 5’-AMP-activated protein kinase (AMPK) activity, determined by enzyme linked-immuno-sorbet assay, was higher in livers of embryos from typical vs small size eggs from e11 to day 1 post-hatch. In conclusion, preformed glucose is used preferentially by embryos until e11 whereas mannose use predominates after e11. Liver AMPK activity is higher in embryos from typical size eggs, and this may reflect increased metabolism of the larger lipid stores (yolk) available to these embryos to support their faster growth rate in ovo.
PLASMA AMINO ACID CONCENTRATIONS IN YOUNG QUARTER HORSES FED GRADED DOSES OF DIETARY LEUCINE

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ABSTRACT: Our hypothesis was that an increasing dose of dietary leucine would result in dose associated changes in plasma concentration of metabolically related amino acids. The objective was to measure plasma amino acid concentrations in horses fed graded doses of dietary leucine. There is data to indicate that leucine supplementation may affect regulation of plasma glucose and/or insulin, and additionally glycogen storage in human and equine athletes. Leucine supplementation will also affect the absorption and metabolism of other amino acids. Eight yearling Quarter Horses were used in a replicated balanced latin square design to study the effects of four leucine doses (0, 0.05, 0.1, 0.2 g/kg body weight) on four study days. Leucine was only supplemented on study days (1/week), and the control sweet feed was fed to all horses on off study days. At the beginning of the study, the horses weighed an average of 364 ± 22 kg and their average weight at the completion of the study was 388 ± 21 kg. Horses were fasted overnight, prior to the start of sampling. On study days, baseline blood samples were taken before the meal of sweet feed with leucine dose was fed at 8:00 AM, 0 minutes. Plasma amino acids were measured in blood samples taken at 0, 15, 45, 60, 120, 180, 420 minutes post-meal. High performance liquid chromatography was used to determine the concentration of 24 amino acids. The data was analyzed using ANOVA with repeated measures. Main effects evaluated included time, dose, and time by dose, with differences considered significant at P<0.05. Time effects were seen in 19 amino acids. This was expected, and may be interpreted as a reflection of the absorption and metabolic changes associated with the meal. In addition, clear dose effects were measured for plasma concentrations of branch-ed chain amino acids leucine, isoleucine, and valine. One interpretation of this is that a higher dose of dietary leucine decreases the absorption of isoleucine and valine into the bloodstream. This may be a result of transporters being overloaded with leucine which competitively inhibited isoleucine and valine absorption. It is important to note that the doses of leucine provided in this study were between 150-550% of that found in the control diet. In addition to our transporter interpretation there are numerous other mechanisms that may explain the changes in the plasma amino acids measured in this study. Further research needs to be conducted to define these specific mechanisms. The results of this study indicate that care should be taken to understand the broad impact of dietary fortification with individual amino acids on different metabolic pathways.
BODY CONDITION IMPROVEMENTS IN UNDERWEIGHT HORSES FED SENIOR HORSE FEED

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ABSTRACT: Many horses are below ideal body weight due to the effects of malnutrition, starvation or a serious condition that led to weight loss. Recovering from the underweight state requires a very carefully balanced nutritional program designed to facilitate healthy weight gain. The objective of this study was to document the effects of a horse feed designed to promote weight gain in underweight horses. In this field study eight mature, healthy, but underweight horses of varying age, gender and breed were transitioned to Life Design® Senior Horse Feed (Cargill Animal Nutrition), for six weeks at a feeding rate of 1.5% of target body weight. Feeding rate was based on feed formulation and feeding directions provided by manufacturer then adjusted as needed based on forage component of diet and changes in body condition. All transitions to the new feed were gradual and daily concentrate ration was divided up into two or more meals. Horses also had ad libitum access to hay and water. A total of four evaluations were done on each horse, including a baseline evaluation and follow-up evaluations every two weeks. During each evaluation body condition scores, gaskin circumference, heart girth and body length (for body weight estimation), rump fat thickness (for body fat estimation) and the depth of the left longissimus dorsi muscle were measured by a licensed veterinarian. B-mode ultrasonography was used to obtain body fat and muscle depth data. Data was statistically analyzed using a liner ANOVA in SAS. There was an increase (P = 0.03) in body condition score over the course of the study (baseline, 3.1 ± 0.3 vs. evaluation 4, 4.1 ± 0.3) as well as an increase (P = 0.05) in depth of the left longissimus dorsi (baseline, 3.6 ± 0.2 cm vs. evaluation 4, 4.7 ± 0.3 cm). Data from body weight and body fat estimations, and gaskin circumference were not different between assessments. Results from this study suggest that the test feed did increase body condition and muscle depth; however, did not increase body weight or body fat during the 6 wk time course. The amino acid profile of this feed was a proprietary blend, which could have supported the muscle depth increases. Preference for lean muscle deposition over fat deposition may be beneficial for underweight horses having undergone muscle wasting due to energy and amino acid deficiencies they may have been previously subjected to. Further investigation with horses across regions in the US is being done to determine the extent of the effect the test feed has over a larger population of horses.
CHANGES IN FECAL MICROBIAL POPULATIONS AND ASSOCIATED BLOOD VARIABLES IN MORGAN HORSES MAINTAINED ON PASTURE OR DRY LOT

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ABSTRACT: Our hypotheses were that 1) Equine Hindgut Streptococcal Species (EHSS) would increase in horses maintained on pasture and decrease in horses maintained in stalls fed hay and 2) that glucose and insulin dynamics would be different between horses maintained in either pastures or stalls. The objective was to measure the fecal microbial population and blood parameters in horses kept in the two environments. Insulin insensitivity is associated with obesity and is one of the common features that link various metabolic disorders and laminitis. There is evidence that the Morgan breed is predisposed to obesity and laminitis. Therefore, 8 Morgan horses were used in a crossover design over 4, 2-week periods (June 23 – August 17), with period 1 serving as a baseline period and period 3 serving as a washout period. Horses (n=8) were blocked by age, gender, bodyweight, and body condition score and assigned to either 14’X 12’ box stalls or turned out in a 1.5 acre pasture during periods 2 and 4. Stalled horses were fed hay three times a day while pastured horses were allowed to graze continuously. During periods 1 and 3, all horses were housed in box stalls and fed a hay diet. Body weight was measured on a calibrated livestock scale and body condition score was independently assessed by at least two research technicians on days 7 and 14 of each period. Blood samples were taken on days 3, 7, 10, and 14 of each period. Fecal samples were taken from all horses on days 0, 3, 7, 10, and 14 of each period. Plasma glucose concentrations were measured with a glucose oxidase based colorimetric assay, while plasma insulin was measured with a previously validated radioimmunoassay. The EHSS bacterial population in fecal samples is expressed as a percentage of the total bacterial population detected by a control probe using bacterial ribosomal DNA fragments. Differences were considered significant at $P < 0.05$. The nutrient composition on a percentage basis of hay was 87 DM, 10 CP, 44 ADF, 68 NDF, 11 WSC, and < 1 starch, while pasture was 21 DM, 17 CP, 31 ADF, 54 NDF, 11 WSC, and 1.6 starch. The average plasma glucose and insulin concentrations were 105 ± 4.0 mg/dl and 9.7 ± 8.0 mIU/L, respectively. Glucose was not influenced by treatment, but was higher on day 14. Plasma insulin was higher in horses maintained on pasture versus stalls (13.4 ± 9.2 vs. 6.1 ± 4.1 mIU/L), and there was no effect of study day. The pasture maintained horses had lower concentrations of EHSS than those consuming hay only (0.05 ± 0.04 vs. 0.15 ± 0.15%, respectively). The EHSS numbers also increased from day 0 to 14. The results of this study lead to new questions in regard to the influence of nutritional environment on metabolic and microbial parameters related to laminitis.
DIETARY PROTEIN LEVELS AFFECT NITROGEN EXCRETION IN HORSES

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ABSTRACT: The modern equine diet often includes supplementation with high-protein feed sources. As many horse owners have been shown to overfeed protein, there is a potential for widespread environmental impact should elevated dietary protein lead to higher levels of nitrogen compounds excreted in feces or urine. Blood urea levels after feeding can also have an impact on animal health. The objective of this study was to evaluate nitrogen levels in the urine, feces, and blood, as well as volatized atmospheric nitrogen, when horses were presented with dietary protein in excess. Six mature, healthy, unfit Standardbred mares were fed 700 g/d of soybean meal (SBM) on top of a maintenance ration (1041 g protein/d), while six received only the maintenance ration (700 g protein/d) as a control (CON). The study was run as a randomized crossover design with 14 days of adaptation to each diet, 5 days of collection and a 14 day washout period in between trials. Trial 1 took place between September 27 and October 15, 2010, while Trial 2 took place between November 1 and November 19, 2010. Mares were housed for 16 hours overnight in stalls without bedding during the 5 day collection; the manure was collected at 0800 daily, to be analyzed for nitrogen (N), ammonia (NH3) and organic nitrogen (OrN). Volatized ammonia (air NH3) was tested using Drager Tubes both over an 8 hour period of stall rest and by pump before the morning feeding. Urine was collected every 2 hours overnight during a 16 hour period post feeding by Foley urinary catheter and analyzed for urinary protein. Blood samples were collected at 8 hour and 16 hour post feeding and the serum was analyzed for blood urea nitrogen (BUN). Data was statistically analyzed using a mixed model ANOVA. Significance was determined by P < 0.05. In manure, SBM was shown to be significantly higher than CON for N (P = 0.038), and NH3 (P = 0.015), while OrN had no effect of treatment. In urine, protein varied between Trial 1 and 2 (P = 0.019) and by sample (P = 0.034). Serum BUN varied by treatment, trial, and treatment by sample interaction (P < 0.0001), as well as in a trial by sample interaction (P = 0.004). For air NH3, there was a significantly higher level in the SBM group vs. the CON group (P = 0.029) for the 8 hour collection; however, the morning pump test did not differ between groups. These results show that elevating protein levels in the horse diet past recommended levels increases the ammonia and nitrogen levels excreted in manure, the ammonia in the atmosphere, and the urea nitrogen in the animal’s blood. As animal waste disposal is a known concern in watershed and atmospheric pollution, and normal blood urea and airborne ammonia levels are preferable for health, these results should be pursued as possible signs to adjust the common equine ration.
THE EFFECTS OF THE DIFFERENT LEVELS OF ALOE VERA GEL ON OOCYSTS SHEDDING IN BROILERS WITH COCCODIOSIS

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ABSTRACT: Coccidiosis is the most important parasitic disease in poultry. The disease may result in losses, indigestion, and increased food conversion ratio in chickens. Resistance against anti-coccidiosis drugs is among the major problems resulting from chemical therapy. Therefore, it seems necessary to replace chemical substances with herbs. Aloe vera is a well-known herb shown by recent studies to have useful properties such as anti-bacterial, anti-viral, and anti-parasitic properties. Thus, the present study aims to identify the effects of different levels of Aloe vera gel on performance and oocysts shedding in broilers with coccidiosis. The study was carried out on 200 one-day-old male broilers from Ross 308 strain on a completely randomize design with four treatments each with five replicates each composing of ten chickens. The groups included control group (basal diet), three group with basal diet mixed different level of Aloe vera gel (1.5, 2, and 2.5 % mixed with feed). On the day 28, all chickens with oocysts were challenged by Eimeria maxima. One week later, for a period of five days, three feces samples were taken on daily basis from each group and oocysts per gram of feces were measured. Food conversion ratios were calculated for the whole farming period (42 days). The findings suggested that groups treated by Aloe vera had improved food conversion ratio compared to the control group. The group treated by 2.5 % Aloe vera showed significant difference from the control group. In addition, significant reduction was observed in oocysts per gram of feces in the Aloe vera groups in comparison with the control group. 2.5 % Aloe vera Group showed the lowest level of oocysts per gram of feces. In general, the results of this study indicate that Aloe vera gel can improve food conversion ratio in broilers with coccidiosis and reduces oocysts shedding. In addition, the group treated by 2.5 % Aloe vera showed the best level of such improvements.