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Proceedings

50th Maryland Nutrition Conference
For Feed Manufacturers

and

1st Mid-Atlantic Nutrition Conference

March 27-28, 2003

University of Maryland
Penn State University
University of Delaware
West Virginia University
Rutgers, The State University of New Jersey
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MARYLAND AND MID-ATLANTIC NUTRITION CONFERENCE
Thursday March 27, 2003

6:30 am Registration – Lobby (Coffee Service)

GENERAL SESSION

8:00 am Welcome Address ................................................................. Richard Erdman
8:15 am Update on Issues in the Animal Feed Industry .................... Richard Sellers
8:45 am Effects of the Homeland Security Act on Animal Agriculture ........ Maurice Clarke
9:15 am Issues in the Future of Maryland Agriculture ...................... Bruce Gardner
9:45 am BREAK

10:30 am Emerging Animal Feeding Issues ........................................... Mamduh Sifri
11:00 am Contributions of Animal Nutrition to Human Health: The Selenium Story. ................................................................. Gerald Combs, Jr.
11:30 am Reflections on Fifty Years of the Maryland Nutrition Conference for Feed Manufacturers .......................................................... Gerald Combs, Sr.

12:00 - 1:30 pm LUNCH

CARGILL FEED QUALITY AND MYCOTOXIN SYMPOSIUM

1:30 pm Welcome .............................................................................. Craig L. Wyatt

1:40 pm Considerations in Grain Procurement .................................... Dennis Inman
2:20 pm Nutritional Impact of Mycotoxigenic Fungi ............................. John Doerr
3:00 pm New Developments in the Utilization/application of a Mold Inhibitor in Grain and Feed. ................................................................. Yin-Chieh Li and Craig Wyatt

3:35 pm BREAK

3:45 pm Impact of Sub-clinical Levels of Mycotoxins on Poultry Performance and Physiological Parameters. .................................................. David Ledoux

4:20 pm The Effects, Prevention and Treatment of Mycotoxins in Dairy Cattle .......... Lon Whitlow

5:00 pm Closing Remarks and General Discussion .............................. Craig Wyatt

5:30 - 7:00 pm RECEPTION
Friday March 28, 2003 – Concurrent Sessions

6:30 am  Registration – Lobby  (Coffee Service)

POULTRY NUTRITION: SESSION I

8:00 am  Agristats – Where Is Poultry Going as an Industry and What Economic Challenges Does It Face? ................................................................. Mike Donahue

8:30 am  Effects of Feed Processing and Texture on Bird Performance .................... Scott Beyer

9:00 am  The Effects of Dietary Protein Level and TSAA: Lysine Ratio on Egg Production Parameters, Egg Yield and Molecular Components in Tissues .. Curtis Novak

9:30 am  Corn Quality – What Are We Including in Our Poultry Diets? ............ Tom D. D'Alfonso

10:00 am  BREAK

POULTRY NUTRITION: SESSION II

10:30 am  Broiler Breeder Nutrition – What’s New and What Challenges Do We Face? ................................................................. Steve Leeson

11:00 am  Alternative Feed Ingredients for Poultry: Broiler and Layers ................. Nick Dale

11:30 am  Alternative Ingredients for Poultry: Turkeys ........................................ Sally Noll

12:00  ADJOURN

12:05 pm  Maryland Feed Industry Council and the Mid-Atlantic Nutrition Planning Committee Luncheon Meeting
Friday March 28, 2003 – Concurrent Sessions

6:30 am  Registration – Lobby   (Coffee Service)

DAIRY CATTLE NUTRITION:  SESSION I

8:30 am  Ammonia Emissions from Dairy and Beef Production Systems  ..........  James Ferguson
9:00 am  Nutritional Factors Influencing Reproductive Success in Dairy Cattle ......................................................... William Thatcher
9:30 am  Heifer Nutrition:  Prepubertal Growth and Development ..................... Tony Capuco
10:00 am  BREAK

DAIRY CATTLE NUTRITION:  SESSION II

10:30 am  Natural Products for Manipulation of Fermentation in Ruminants .......... John Wallace
11:00 am  A Model to Describe Ruminal Metabolism and Intestinal Digestion of Fatty Acids ........................................... William Chalupa
11:30 am  Forage Breeding to Improve Nutrient Content for Ruminants ................ Mike Peterson
12:00  ADJOURN

12:05 pm  Maryland Feed Industry Council and the Mid-Atlantic Nutrition Planning Committee Luncheon Meeting
Friday March 28, 2003 – Concurrent Sessions

6:30 am Registration – Lobby (Coffee Service)

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8:15 am  Geriatric Horse Nutrition, An Update on the Care of the Older Horse: Diet and Health ......................................................... Mary Beth Gordon

8:45 am  Feeding of the Future: Nutrition of the Growing Horse …………………………… Erin Petersen

9:15 am  Dietary Considerations for Athletic Horses: Sources and Effects of Dietary Energy ………………………………………………… Ray Geor

10:00 am BREAK

EQUINE NUTRITION: SESSION II

10:30 am  Fat Supplementation in the Equine Diet …………………………………… David Kronfeld

11:00 am  Vitamin and Mineral Requirements in the Horse …………………………… Kathleen Crandell

11:30 am  Neutraceuticals in the Horse Industry …………………………………………… Marty Adams

12:00  ADJOURN

12:05 pm  Maryland Feed Industry Council and the Mid-Atlantic Nutrition Planning Committee Luncheon Meeting
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ISSUES IN THE FUTURE OF MARYLAND AGRICULTURE

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Introduction

The future of agriculture in Maryland is clouded by a number of factors. Some of these, and perhaps the most important ones, are shared with the whole of U.S. agriculture. Low market prices for the main crops are now in their fifth year, and milk prices are at ruinous levels for many producers. I will however focus on issues specific to Maryland (although largely shared with other Mid-Atlantic states), which will continue to require attention even when commodity markets strengthen. Many of those factors are associated with the location of much of the state in a zone of rapid suburban development and with the emergence of environmental issues involving water quality in the Chesapeake Bay watershed. The acreage of farmland in Maryland is continuing to decrease, albeit at a slower rate in recent years than in previous decades. However, the high average age of Maryland farmers and the bleak view of many in the industry indicate that Maryland agriculture faces an uncertain future. In the most suburbanized counties, commercial agriculture is particularly at risk. Even in the more rural parts of the state, the prospect of continuing conversion of farmland to nonagricultural uses raises concerns about the future.

Yet much of Maryland agriculture continues to be competitive with other parts of the country, and farming is an attractive and viable way of life for thousands of people. The farming sector and its related industries (e.g., agricultural inputs, services, and food processing) account for about $5 billion (three percent) of the Maryland gross state product and in 1999 employed 62,700 people (12,400 farm operators, 5,900 farm laborers, and 44,300 in farm input, service, supply, and agricultural processing). Those contributions are not declining over time, even though agriculture’s share of the state’s economic activity is declining because non-agricultural sectors are growing faster.

Maryland must face the question of whether agriculture’s large and varied contributions to the state’s economy and environment can be sustained. What could reasonably be done to foster the future economic health of the sector? Those issues were addressed in a two-year study recently completed at the University of Maryland’s Department of Agricultural and Resource Economics. The study was commissioned and funded by the Maryland Department of Agriculture. This paper reviews our main findings.

Current Situation Summary:

Negatives:

- Many Maryland farms have gone out of business in recent years, especially in hog and dairy production. Acreage of some commodities, notably vegetables for processing, has declined substantially, and tobacco is on the verge of disappearance.

- The age of farm operators has been rising, and the average Maryland farm operator is now over 54 years old; indicating the importance of a flow of new replacement farmers.
• Small-scale and part-time farms on average have expenses greater than receipts, and these farms are increasing as a fraction of the state’s farms. That suggests an eroding base for commercially viable agriculture.

• Farmland continues to be lost to suburban development at a rate that may threaten the continuation of agricultural activity in some areas of the state. Maryland already ranks fifth among all states in percentage of land area that is developed.

• Public perception of farming among the general public appears to have shifted toward seeing agriculture as a threat to water quality and other environmental values.

**Positives:**

• Since 1990, the rates of loss of farms and farmland have moderated from the losses of earlier decades.

• The incomes of farm operator households in Maryland are, on average, favorable as compared to other states. In 2000, Maryland’s average net income per farm was well above Pennsylvania and Virginia, and exceeded the national average substantially. While the majority of the almost 80 percent of Maryland farms with sales of less than $100,000 have negative net cash income (expenses greater than receipts), the larger farms do much better.

• The relatively high value of farmland in Maryland is a source of farm wealth, despite the barriers posed for those who wish to enter farming or add to their land ownership. Maryland farms have lower debt/asset ratios than are typical in other states, and the net worth of the average farm is higher than the nation as a whole, despite the smaller average size of Maryland’s farms.

• At both state and federal levels, policies have recently been enacted, and amplified in the 2002 farm bill, that are aimed at preserving land in farming, assisting farmers in environmental stewardship, and providing support for commodity producers to offset currently low prices.

**Future Prospects**

Many farmers and others closely connected with agriculture have expressed a lack of confidence that current national, state, and local policies are adequate to address agriculture’s problems. Meetings with Maryland farm groups and individuals indicate two basic sources of such worries. The first is that the already fragile economic viability of many farm operations will be subject to further economic stress from low returns and rising costs. The second is that agriculture is underappreciated by the nonfarm population, including policymakers in local and state government, which makes it unlikely that necessary steps will be taken to keep people involved with and investing in agriculture.

One example that Maryland farmers regularly point to as an example of underappreciation is state environmental regulations, either in place or on the horizon, that raise costs and reduce the competitiveness of Maryland farms. Local, state, and federal policies have embodied the view that agriculture’s large land base and intensive, high-yield crop production, as well as regional concentration of animal production, pose risks of significant negative effects on water and air quality. The nutrient management requirements created by the Maryland Water Quality Improvement Act of 1998 (WQIA) are expected to affect both animal operations and crop growers. However, neither data nor reports of
stakeholder groups provided evidence of significant effects so far from the WQIA that would hasten the 
decline of Maryland agriculture.

Further risks to agriculture arise from the possibility that the declines in farms and farm acreage may, over the next 20 years, go so far as to seriously impair the economic health of nonmetropolitan areas of the state. For example, if the grain-broiler economy of the Eastern Shore begins to decline, might that generate an accelerating downward economic cycle as the land or production base falls below some critical level needed to sustain the industry at an efficient scale?

And, even if the nonfarm population’s view of agriculture is more positive than what farmers suppose, that is not sufficient to guarantee policies that will translate to an improved economic situation for traditional, commercially based agriculture. The nonfarm public may be equally happy to see 300 acres devoted to several small recreational horse farms as to a working dairy farm; but many in agriculture would see the conversion from the latter to the former as a substantial social and economic loss. Similarly, the public may desire increased uses of land for environmental protection purposes, but in many instances the land is thereby removed from traditional commercial crop use and commercial agriculture is squeezed further. Particular problems arise for crop producers who must rent land beyond the acreage they own in order to attain an economically viable scale of operation.

In order to project the likely future evolution of Maryland agriculture, in terms of farm numbers, land in farms, and value added to the state’s economy, it is important to understand the reasons underlying recent trends. The reasons are economic. That is, land disappears from farming, and farm operators leave agriculture and are not replaced by a new generation because the economic rewards from farming are less than the rewards from alternative nonagricultural uses of people’s land and labor. The question is then, what forces lay behind the decreased economic opportunities in farming as compared to nonagricultural pursuits? Explanatory factors include:

- weak markets for traditional commodities, causing declining prices,
- development pressures causing land conversion to nonfarm uses,
- environmental regulations and programs,
- labor constraints,
- other costs hindering Maryland’s competitive advantage.

Overall Outlook

In view of the success with which Maryland’s farmers have handled the many economic threats that have appeared over the last two decades, and the evidence that producers are already adapting to the changing market and policy-driven demands placed upon them, our baseline projection for the next decade is for continued decline, but only at a relatively slow and manageable rate. We expect a further loss of about 40,000 acres of farmland by 2010 (2.5 percent of current land in farms), but we do not expect an economic crunch that would cause general economic hardship. With respect to farm numbers, we expect that while the size of dairy operations and some other farm enterprises will increase, the percentage of farms that have relatively small acreage will increase also, and that the number of farms will decline at the same rate as farmland, which would imply a loss of 200 to 400 farms by 2010. The rates or loss of both farms and farmland are lower than historical rates in the post- World War II period, but are similar to those of the 1990s.

Even the most urbanized counties, Baltimore, Montgomery, and Prince George’s, have so far maintained substantial cropland bases. That finding is especially notable in view of the evident continued expansion of housing and commercial development on former farmland. The decline of farming in suburban areas is sometimes seen as inexorable, with farm activity eventually falling below a “critical mass,” after which essentially all the farmland is converted to nonagricultural uses. At the edges of
Maryland’s urban areas, that has indeed occurred so that farming is now absent in large parts of our metropolitan counties. But each of those counties has, at the same time, managed to maintain large areas of farm acreage. Our forecast is that this record will be maintained, at least for the next decade.

What about the longer-term outlook? By 2020, Maryland will have grown by approximately 600,000 additional residents over the population of 5.4 million as of July 2001, to a total of 6.0 million according to Census of Population estimates. The added population plus desire for suburban space for more of the existing population will cause problems, but they appear manageable. The risks are greater and potential problems more intractable if we project those trends further into the future, for example to 2050. The state’s population could easily grow by another million by then. Over the longer time span, the population will gain further in affluence and the average household will acquire more space. If an additional million people have an average of two persons per household and one-half acre of land, they will occupy 250,000 acres. If half of that acreage is converted from farms and half from forest lands (roughly the proportions of the past), the state would still have 1.9 million acres of farmland in 2050 (compared to 2.1 million now).

The preceding projections are a baseline scenario for the immediate future of Maryland agriculture, with commodity market conditions and regulatory policies that essentially continue what is in place as of 2002. The future could easily be substantially worse or better. In part, events will depend upon climatic and market forces that no one can predict or control. But most importantly, what happens will also depend on local, state, and national policies that impact agriculture.

Policy Considerations

We presume that the disappearance of farms and farmland in Maryland is a problem to which a public policy response is appropriate. It might be argued, however, that such changes are the results of farmers’ and others’ well-considered decisions in response to market conditions, and the presumption should be a policy of non-interference with market forces. Our reasons for working from the former rather than the latter presumption are: first, that current farming and land-use decisions are not taking place in an unrestrained market situation but are already influenced by governmental interventions such as zoning, public investment in infrastructure, and a variety of regulations and tax policies; and second, that opinions of individuals and groups and other evidence indicate that farming in Maryland generates external benefits and costs beyond those accrued by the actors involved. The first point militates against the presumption that no further policy is the best policy, and the second supports the presumption that the direction of further policy most likely to be beneficial is in the direction of preserving farms and farmland. Nonetheless, any particular policies chosen should pass appropriate benefit-cost tests.

The report discusses a wide range of federal, state, and local policies that affect the economic health of Maryland’s farm sector. It highlights a general division of opinion that prevails among those interviewed. One general view is that the best focal point for state-level and perhaps even national policy is a set of land preservation and conservation programs. Policies in these areas offer the most promise for maintaining land in farms while gaining support of the nonfarm population by promoting environmental goals and maintaining the scenic vistas that make rural Maryland attractive. An opposing general view is that preservation and conservation programs will accomplish little or nothing in the way of fostering agriculture as a commercial activity supporting traditional family farms. Adherents of this view argue that increased profitability of farming is the only way to attract new entrants to farming, induce new investment, and encourage established farmers not to abandon their existing operations.

The existence of these opposing views reflects the fact that urbanization is a two-edged sword for farmers. On the one hand, urbanization impinges upon farmers, making the farming enterprise more costly and difficult. Development pressures raise the price of land, reducing the economic return to farming and increasing the potential gains by switching land to nonfarm uses. On the other hand, higher
land values can provide security for loans or funds for retirement. Residential expansion has also created conflict between farm operations and residential amenities in many communities. At the same time, urbanization provides opportunities for agricultural enterprises to take advantage of nearby urban markets by altering their marketing and/or changing product mixes. Prospects for off-farm employment also increase with urbanization.

An important issue in this context is the role of landowners who are not farm operators. Maryland has an estimated 11,200 owners of agricultural land who are not farm operators. More than half of Maryland’s farmland is owned by nonfarm operators (57 percent of Maryland’s farmland compared to, for example, 45 percent in Virginia, 36 percent in Pennsylvania, and 42 percent for the United States as a whole). The heavy reliance of farm operators on rented land creates management problems and, at times, a divergence of interest between landlord and tenant. Tensions have arisen, for example, when landlords enroll formerly rented cropland in conservation programs, and under the increasingly complicated provisions of farm commodity program regulations that tie benefits to land but make payments primarily to operators. And non-operator landlords are likely to be particularly susceptible to pressures to convert farmland to development. Increases in cash rental rates, even while commodity prices are at record lows, make these issues even more sensitive.

Environmental Regulations

An issue that affects every region of the state is agriculture’s effect on the environment, and environmental regulations that may raise costs and reduce the competitiveness of Maryland farms. Local, state, and federal policies have embodied the view that agriculture’s large land base and intensive, high-yield crop production, as well as regional concentration of animal production, pose risks of significant negative effects on water and air quality. The nutrient management requirements created by the Maryland Water Quality Improvement Act of 1998 (WQIA) are expected to affect both animal operations and crop growers. However, neither data nor reports of stakeholder groups provided evidence of significant effects that would hasten the decline of Maryland agriculture.

The state regulatory environment, including environmental restrictions, labor management regulations (such as provision of housing and other facilities needed to meet state and federal standards), and permits needed to undertake many improvements such as irrigation or drainage projects, creates a perception that the state is decreasingly friendly to agriculture and farmers. This encourages retirements and other exits from farming, and discourages new entrants. It creates a climate that furthers the current tendency to depreciate the capital stock in agriculture and to avoid new investment. Such investment is essential to make the commodity and market-niche adjustments necessary to stay on the frontier of new production technology and marketing opportunities.

Federal Farm Programs

A policy issue that arises with respect to improving the economic viability of farming is the extent to which profitability can be attained through nationwide commodity programs. Currently, Maryland farmers receive commodity program payments that amount to about 20 percent of net farm income, focused on about half of Maryland’s producers. In order to appreciably improve the economic viability of Maryland producers significantly enough to keep their land in farming, it would take a huge increase over current outlays, and even that would not be enough to make agricultural use of land in the central metro counties competitive with development alternatives. Some in the 2002 farm bill debate argued that a shift of emphasis to spending several billion dollars on conservation/environmental programs would serve Maryland and other Eastern farmers better than current commodity programs. A problem however is that farmers’ receipts of such funds would be tied to costly new undertakings by farmers, while current programs pay them for doing just what they are already doing anyway. On the
other hand, the nonfarm population sees more of a benefit from the conservation/environment approach and is therefore more likely to support the necessary government spending over the long term.

Nonetheless, it remains the case that the net gain to farmers per dollar spent on farm programs is substantially larger for current commodity programs than would be the case for conservation/environmental programs. Maryland farmers have shared as little in conservation program dollars as in commodity program dollars. In 2000, for example, Maryland accounted for 0.8 percent of the nation’s agricultural output but received only 0.3 percent of FAIR Act (production flexibility contract and loan deficiency) payments, and received only 0.2 percent of Conservation Reserve Program payments (see Table 4). The relatively large role of non-program commodities in Maryland means that our state is relatively disadvantaged in the whole range of federal programs (Table 5 provides a list of Maryland’s leading cash commodities).

Budget studies as well as recent trends indicate that our most promising future lies with non-program crops, including niche activities that embody substantial services beyond those of just growing the crops. However, it is important to recognize that all specialty crops, vegetables, orchards, and nursery/greenhouse crops together utilize only about 75,000 acres, while grains and soybeans occupy about 1.2 million acres. Thus, no conceivable expansion of the former set of commodities can serve to keep Maryland’s current cropland in agriculture. The traditionally grown grain and soybean crops will remain crucial. This basic agriculture, centered on the Eastern Shore, has grown symbiotically with the broiler industry -- each is necessary to the other. Maryland’s grain growers are arguably placed in a better long-term economic position by the substantial premiums over Corn Belt grain prices that the demand for chicken feed creates than by any conceivable price support program. So state-level policies that can promote the continued viability of broiler production in Maryland are arguably the most important agricultural policies the state can implement.

State Programs

What else can the state government reasonably do? The general thrust that appears most promising is to undertake public investments and foster private investments that will advance the state’s comparative advantages and create new ones. Every state, including Maryland, across the country supports value-added agriculture in some fashion. The programs offered relate to the types of agriculture in each state, with state-grown product promotion and labeling programs being the most popular.

Agricultural marketing assistance could be used to more effectively exploit alternative marketing channels. Export promotion has been utilized by many state agricultural departments, but this approach is relatively dubious for Maryland, apart from broilers, because Maryland is typically a grain importing area. Maryland has been effective in facilitating the development of farmers’ markets. But further issues could be explored specifically related to the barriers of increased participation in direct marketing and value-added agricultural activities. For instance, small-scale farmers and food processors need assistance in complying with the panoply of food safety, labor, and environmental regulations at the federal, state, and local levels.

Farm labor supply needs are persistent to farm employers and complicated by the unpredictable nature of agricultural production. Currently, foreign workers can be employed temporarily in agriculture under the H-2A provisions of the Immigration and Nationality Act. However, there are a number of limiting factors such as cumbersome lead time for employers, lack of certified housing, and administrative pressures that could be corrected by increased funding and Federal legislative changes. A state program to assist with development of worker housing may facilitate the use of this program. The state could also usefully provide broader services to farmers in assisting them through the labyrinth of employer requirements and regulations.

7
**Farmland Preservation Programs**

Maryland has been a national leader in enacting farmland preservation programs including conservation easements, purchase of agricultural easement programs, right-to-farm laws, and differential assessments. At the local level, Maryland jurisdictions have enacted programs centered on comprehensive planning, right-to-farm ordinances, and transfer of development rights programs. Given the overarching goal of ensuring the survival of the agricultural economy by preserving productive farmland, specific goals for these programs have included: maximizing the number of preserved acres; preserving productive farms; preserving farms most threatened by development; and preserving large blocks of land. While our research indicates that these programs have had some significant effects, much could still be done to improve participation in state and local agricultural land preservation programs and to provide a more effective use of existing resources available to purchase agricultural land easements.

Another issue in farmland preservation is creating a stronger linkage among the various farmland protection, natural resource, and agricultural economic development programs in areas where farmland is threatened. If a farmer has made a commitment to keep the farm’s land in agriculture, it is arguable that the public should provide some assistance in helping to retain a working and profitable farm. Some counties – in particular, those with established offices of agricultural economic development – are well on their way towards fostering such a linkage.

**Conclusions**

In summary, there are many areas in which state as well as federal policy could assist in promoting a prosperous agriculture that contributes to Maryland’s future economic vigor and quality of life. It is noteworthy that the most promising policies are not huge departures from current directions, but rather intensification of what is working and pulling back from what is not. If Maryland’s agricultural economy and policies were to continue on their current path, our projections suggest that the rapid rates of loss of farm and forest resources of past decades will not return over the next ten to twenty years, although some segments of agriculture are at risk. Further losses of farmland will occur, as is inevitable as population continues to grow and affluence expands with its attendant demands for more living space for the average household. But these losses will continue to be manageable, at least for the immediate future.
EMERGING ANIMAL FEEDING ISSUES

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Classifying the Issues

The emerging issues in animal feeding are diverse and complex and often related. In order to have some assemblance and common sense, all issues are reviewed and arbitrarily divided into business issues or technical issues. The reader and the listener have the privilege to classify them in a manner of their choice that should assume a balanced evaluation.

The Business Issues

The market dynamics are in continuous motion providing challenges and opportunities. The animal feed business, whether independent or integrated, is responding to these effectors to survive and stay viable with respect to its umbrella food enterprise. Consequently, companies will continue to focus on consolidation, differentiation, fine-tuning, capital management, and new products in order to succeed.

It is logical to believe the expansion of animal agriculture in the USA will be limited; consequently, the pressure for delivering new products at increasingly lower prices will continue to mount. Conventional food-chain integration has provided some industry relief; however, the basic questions center on the longevity of this process and viability of the outcome. The market continues to be significantly segmented in the global sense for all supply-chain elements that influence feed and food production. In the current environment, it seems natural that this kind of segmentation will not last for long and that new arrangements will emerge gradually. Those businesses that succeed will include comprehensive partnerships (alliances, joint ventures, or other arrangements) that will circumvent current market structure for unique advantages.

For lack of official names, these partnerships will be called “virtual integrations.” These partnerships might include some or all the segments in the animal agriculture food chain. Such opportunities will not be limited to major companies. Intermediate and small companies can take advantage of this process to serve viable niche markets. Consequently, there may be industry dictated guidelines or governmental regulations to address the pertinent and new issues.

The Technical Issues

It is intriguing to note that an internet website search about “emerging animal feeding issues” will invariably leads to titles similar to the following technical issues:

1. Waste and Nutrient Management
   a. Environment
   b. Animal population and density
c. Plant population and density
d. Human population and density
e. Combinations/Interactions
f. Regulations: local, state, federal, international and others

2. Food

a. Composition
b. Shelf life/Preservation (freshness)
c. Convenience
d. Health
   (1) Contaminations/residues
   (2) Irradiation
   (3) Disease transmissions
   (4) Inherent toxicants
e. Designer/Precision Food
   (1) Specific nutrient/medicine delivery
   (2) Organic
   (3) Natural
   (4) Functional
f. Nutritional supplements (nutraceuticals, specific peptides, herb or conventional plant extracts)
g. Combinations

3. Partnership Between Producer and Consumer

a. Effective Communication
b. Effective Education
c. Effective Implementation

4. Potential Opportunities to Address the Technical Issues

In spite of the fact that the emerging animal feeding issues are challenging and complex, they can be addressed successfully. The answer is equally complex, however, it has to be based on logic, science and a tremendous energy of emotional balance. The following is a tabulation of some of the pertinent technical issues that should be addressed.

a. Environmental and waste management challenges such as phosphorous, nitrogen, odor, toxicants and others.
b. Health of the animals and the humans involved in the enterprise
c. Precision use of medicines (drugs), such as antibiotics, growth promotants and coccidiostats.
d. Alternatives to medicines, such as enzymes, prebiotics, probiotics, herbs, modulators, antibiotics and other natural solution strategies, and their respective delivery systems.
e. Animal welfare and human welfare as it relates to housing and production systems.
f. Utilization of alternative ingredients, plant co-products from processing plants and dealing with new compositional challenges.
g. Rendered by-products of animal origin.
h. Assessing nutrient requirements in view of genetic changes, new nutrient sources, energy cost and economics.
i. Renewability of animals and nutrient sources.
CONTRIBUTIONS OF ANIMAL NUTRITION TO HUMAN HEALTH:
THE SELENIUM STORY

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Summary

The last nutrient to be recognized as a dietary essential is selenium (Se). First recognized by animal nutritionists some five decades ago, the need for Se has been characterized for animal, its metabolic roles have been revealed, and its potential in cancer prevention has been demonstrated. This work originated in the arena of animal nutrition because it yielded practical benefits for livestock feeding, and animal-based research proved key to the development of the fundamental understanding that now serves as the base and rationale for clinical trials with humans.

This body of research has revealed that an element once thought to “spare” vitamin E is actually an essential component of a family of enzymes that play essential roles in various aspects of fundamental metabolic functions including antioxidant protection, energy metabolism, redox regulation and some aspects of gene expression. While it has long been clear that the biological activities of Se was a function of the various Se-compounds it which it may occur, and not to the element per se, it is now clear that the metabolic functions upon which its nutritional role is based owe to the activities of these various selenoenzymes.

It is also clear that Se-compounds can be reduce or delay tumor yields in animal models, and limited clinical trials suggest that Se-compounds may be useful in reducing cancer risk in humans. While important questions remain about the efficacy, specificity and safety of Se-compounds, it is likely that Se products will become increasingly important to consumers and, hopefully, increasingly important in improving public health. In such an environment, it will be appropriate for animal producers not to default to the other interests for the implementation of this knowledge, as there are clear opportunities to develop animal-based functional foods containing Se.

Introduction

One of the most important nutritional discoveries of the last 50 yrs has been the recognition of the role of selenium (Se) as a risk modifier of cancer risk. That discovery, which carries the potential for enormous human and economic impacts by reducing a major cause of preventable death, emerged from research that originated in the field of animal nutrition.

It is not unusual that efforts to answer practical questions concerning the health and productivity of livestock should yield information of import to human health. In fact, the a glimpse of Nutrition history shows this progression to be a natural one:

- the chick anti-polyneuritic factor proved to be thiamine which cures beri-beri in humans;
- the preventative of canine “black tongue”, niacin, proved to cure human pellagra;
- the anti-rachitic factor, vitamin D, which was identified in studies with puppies and rats, cures rickets in children;
- the anti-anemic factor for the chick, vitamin B\textsubscript{c} and later vitamin B\textsubscript{12}, proved to prevent pernicious anemia in people.

Some of these cases were driven by practical need, e.g., the need to replace animal by-products in non-ruminant diets resulted in the discovery of vitamin B\textsubscript{12}. Other cases were driven only by fundamental scientific curiosity, e.g., the discovery of the lipid necessary to prevent fetal death in rodents resulted in the discovery of vitamin E as an essential antioxidant component of all biological membranes.

The Se story has features of both the practical and the fundamental. It started with the recognition that Se could be toxic even carcinogenic, then with the finding that it could “spare” vitamin E, then with the discovery of a family of unique selenoenzymes, then with the finding that Se-compounds can be anti-carcinogenic in tumor models and, most recently, that Se-supplements can be effective in reducing cancer risks in people.

**The Selenium Story**

*A Collaborative Discovery*

Since the mid-1930’s, Se had been recognized to be the toxic principle responsible for “alkali disease” and “blind staggers” in livestock grazing on the selenium-rich Dakota prairies. So, imagine the surprise of the late Klaus Schwarz and his colleagues at the National Institute of Health some 20 years later when this little-known mineral proved to be the active component of brewers’ yeast responsible for protecting vitamin E-deficient rats from necrotic liver degeneration. Excited, Schwarz called Milton Scott at Cornell University and told him, “Milt, try selenium!”.

Scott had recently found the brewers’ yeast contained a factor that reduced leg weakness in turkey pouls and prevented exudative diathesis in the vitamin E-deficient chick. He had become acquainted with Schwarz through their mutual interest in that feedstuff. “Try selenium!” Schwarz said, and try selenium Scott did. That year, 1957, the two scientists announced their mutual discovery of the nutritional essentiality of Se (Schwarz and Foltz, 1957; Schwarz, *et al.*, 1957).

**Selenium in Animal Nutrition**

The discovery that exudative diathesis in the chick is a clinical sign of combined Se and vitamin E deficiency, actually preceded the diagnosis of the syndrome in commercial poultry flocks. By the time that this condition, and the related gizzard myopathy in turkeys, was seen in the field, the “basic” research of Scott and his colleagues at Cornell had generated information useful in solving those problems. Others including Van Vleet at Purdue University, Hogue at Cornell University, Oldfield and Whanger at Oregon State University, Levander at the USDA-Beltsville, Poston at the Department of the Interior, Mahan at Ohio State University, and others extended these findings to generate a field of understanding of the role of Se in the nutrition of dogs, rodents, pigs, fish, sheep and cattle (Combs and Combs, 1986a). In most cases, Se appeared to function in concert with vitamin E, reducing the amount of the vitamin needed to prevent pathologies of the vascular system, brain or muscles.

Such findings were taken as evidence that Se must have an analogous function to that of vitamin E, as an antioxidant. But how could this be? Selenium is a mineral element with a chemistry very similar to that of sulfur, whereas vitamin E is a lipid-soluble, alkyl derivative of chromanol. Thus, by 1970 two questions remained: How can Se have antioxidant action? Is Se needed by the vitamin E-adequate animal?
These questions were settled in the early 1970’s with two findings. Scott’s group demonstrated a specific deficiency syndrome resulting from uncomplicated Se deficiency (nutritional pancreatic atrophy\(^1\)) in the vitamin E-fed chick (Thompson and Scott, 1986) which showed that Se was required even in the presence of vitamin E. Then, Hoekstra’s group at the University of Wisconsin found a specific biochemical function of Se: as an essential constituent of the enzyme glutathione peroxidase (Rotruck \textit{et al.}, 1957). Because that enzyme was known to participate in the antioxidant protection of cells by reducing hydroperoxides, this finding was taken to explain the nutritional “sparing” by Se of vitamin E. While the latter finding provided the first plausible hypothesis for the metabolic basis of the nutritional actions of Se. Still, for most of the next two decades it was not clear whether Se had metabolic roles other than as part of GPX, or whether Se status was physiologically relevant except under conditions of oxidative stress.

\textit{Understanding the Mechanisms of Action of Selenium}

Discoveries of multiple Se-enzymes and other Se-proteins over the last 15 years have changed that view. At present, several Se-enzymes are recognized (Gladyshev, 2001):

- five GPX isoforms
- three iodothyronine 5’-deiodinases (TDIs),
- three thioredoxin reductases (TRs)
- and selenophosphate synthetase

In addition, at least four other proteins are recognized as specifically incorporating Se, although their metabolic functions remain unclear:

- plasma selenoprotein P
- muscle selenoprotein W
- two selenoproteins in prostate and placenta

It is now clear that each of these proteins contains Se in a form, selenocysteine (SeCys), that is present only in proteins that depend on the presence of Se for their expression. Selenium is incorporated into these Se-proteins after metabolic conversion to hydrogen selenide (H\(_2\)Se) in which form the element is phosphorylated to selenophosphate, which participates in the co-translational modification of tRNA-bound serinyl residues. The resulting tRNA-SeCys residues are incorporated at certain loci encoded by specific UGA codons containing SeCys-insertion sequences (SECIS) in the 3’-untranslated regions of their respective mRNAs (Gladyshev, 2001, Martin and Berry, 2001). This unique structure enables the decoding of UGA as SeCys (via the conversion of tRNA-serine to tRNA-SeCys) rather than as the stop signal normal for that codon. It is probable that more SeCys-proteins remain to be discovered, as several other Se-containing proteins have been identified.

The nutritional essentiality of Se is, therefore, considered to be due to the functional activities of a relatively small number of SeCys-proteins. Collectively, these have broad physiologic relevance, including:

- antioxidant protection effected by the GPXs
- energy metabolism effected by the TDIs
- redox regulation of transcriptional factors effected by the TRs
- gene expression affected by the TRs

According to this view, Se, by way of these SeCys-enzymes, contributes to normal function of all cells in the body.

\(^1\) i.e., acinar degeneration and periacinar fibrosis
**Selenium as a Cancer-Protective Agent**

The first suggestion that Se may be anti-carcinogenic was based on empirical observation of an inverse relationship of cancer mortality rates and forage crop Se contents in the US (Shamberger and Frost, 1969). Most, but not all subsequent epidemiology has shown Se status to be inversely associated with cancer risk (Combs and Grey, 1998). Prospective cohort studies in several countries have all shown cancer cases to have significantly lower mean pre-diagnostic serum Se levels than controls, and negative associations have been found for various parameters of Se status and risks to cancers or pre-cancerous lesions of the bladder, brain, esophagus, lung, head and neck, ovary, pancreas, thyroid, stomach, melanoma, prostate and colon (Combs and Grey, 1998).

Studies with animal tumor models have shown that Se treatment can reduce tumor yields. Some years ago, I estimated that, of what was then more than 100 studies in which tumor production and/or pre-neoplastic endpoints had been measured, two-thirds showed that supranutritional Se doses reduced the incidences of such outcomes, with half showing reductions of 50% or more (Combs and Combs, 1986b). Further studies have demonstrated similar reductions in tumor yields or experimental metastases. Only four studies have found Se treatment to enhance tumorigenesis; the interpretation of these is not straightforward, as these reports include increased tumors at one site accompanied by reductions at another site, and enhancement only when the carcinogen was administered in a certain way.

The strongest evidence of anti-cancer efficacy of Se in humans comes from a limited number of clinical trials, the most informative of which has been the Nutritional Prevention of Cancer (NPC) Trial (Clark et al., 1996, Duffield-Lillico et al., 2002). That randomized, placebo-controlled trial showed that Se-supplementation (200 mcg Se/day as Se-yeast) of non-deficient skin cancer patients did not reduce their risks to recurrent basal/squamous cell carcinomas. At the same time, the results suggested that Se-supplementation did reduce risks to other cancers: 37% fewer total non-skin cancers, 45% fewer total carcinomas, 50% fewer total cancers, 63% fewer cancers of the prostate, 58% fewer cancers of the colon-rectum, and 46% fewer cancers of the lung.

![Fig. 1. Cancer Risk Reduction by Supplemental Se, Nutritional Prevention of Cancer Trial (Clark et al., 1996)](image)

That Se deficiency could increase cancer risk might be expected if it overcame a condition of limited expression of the selenoenzymes involved in antioxidant protection (GPXs), and redox regulation (TRs), as such conditions compromise metabolic defense against carcinogenic free radicals. Alternatively, that anti-carcinogenic effects of Se have been observed under conditions of maximal selenoprotein
expression would suggest other anti-carcinogenic mechanisms most likely involving Se-metabolites. On this point, a large body of evidence makes it abundantly clear that Se intake in excess of the nutritional requirement can inhibit and/or retard tumorigenesis in experimental animals (Combs and Grey, 1998, Combs and Lü, 2001). Anti-tumorigenically effective Se-exposures in animal models (at least 1 mg/kg diet) have typically been an order of magnitude greater than those required to prevent clinical signs of Se deficiency or to support the maximal expression of known selenoproteins (less than 0.2 mg/kg diet). Accordingly, it is significant that Se-supplements were effective in reducing cancer risks in the NPC Trial (Clark et al., 1996, Duffield-Lillico et al., 2002) few, if any, subjects of which had nutritionally limiting Se intakes as judged by their baseline plasma Se levels.²

While it is plausible that Se-deprivation may enhance tumorigenesis, there is little empirical evidence on that point. In contrast, it is well documented that at least some forms of Se can, in supranutritional doses, reduce cancer risk, thus, suggesting that that one or more Se-compounds/metabolites can function in directly anti-carcinogenic ways. Correction of nutritional Se-deficiency might, therefore, be expected to increase cellular antioxidant protection through the expression of the antioxidant GPXs, the redox-regulatory TRs and, perhaps, the hormone-regulating DI s. The efficacy of Se-supplementation in reducing cancer risk in non-deficient individuals (i.e., at supranutritional intakes) suggests the involvement of other anti-carcinogenic mechanisms (e.g., altered carcinogen metabolism, gene expression, immune surveillance, cell cycle/death regulation and neo-angiogenesis [Combs and Lü, 2001]) that function in addition to or in lieu of mechanisms involving selenoproteins.

There is evidence of anti-carcinogenic activities for several intermediary metabolites of Se. These include selenodiglutathione (GSSeSG), the reductive metabolite of the oxidized inorganic salts (selenite, selenate); hydrogen selenide (H₂Se), the common intermediate of that reductive pathway and the catabolism of selenoamino acids; and the methylated metabolites of selenide ([CH₃]₂Se) that have hitherto been thought of only as excretory forms of the element. The anti-carcinogenic activities attributable to each of these metabolites are summarized in Fig. 2.

²The cohort level was 114±23 ng/ml, suggesting an average daily intake of at least 85 mcg Se/day, or at least 155% of the RDA]
The product of the thiol-dependent reduction of selenite, GSSeSG, would appear to be relevant only when selenite and/or selenate is fed. In contrast, H$_2$Se is the common metabolite of both the oxidized inorganic and the selenoamino acids selenocysteine (Se Cys) and selenomethionine (SeMet). Selenide can be methylated to produce a sequence of excretory metabolites including methylselenol (CH$_3$SeH) that has been shown to be anti-carcinogenic in the murine mammary cancer model (Ip and Ganther, 1990; Ip, 1998). Accordingly, Se-compounds that are readily metabolized to CH$_3$SeH are anti-carcinogenically active; these include the food/feed forms selenobetaine (CH$_3$SeO$_2$H) and methyl-selenocysteine (CH$_3$SeCys).

The common forms of Se in foods and feedstuffs, SeMet and SeCys, are not directly anti-carcinogenic; however, each can be metabolized first to H$_2$Se and, then, to CH$_3$SeH (Figure 2). That conversion occurs directly for SeCys, which cannot enter the general protein pool and, thus, is catabolized to yield H$_2$Se. The process is indirect for SeMet, which can be directed into the general protein pool as a mimic of methionine (Met). The ultimate conversion of SeMet from either dietary or protein-turnover sources necessarily involves its first being converted to SeCys by the Met-transsulfuration pathway. For this reason, most short-term studies have found SeMet to be generally less anti-carcinogenically efficacious than SeCys or selenite, particularly in the absence of luxus amounts of Met. However, under steady-state conditions effected by long-term use, and particularly with high-Met diets, one would expect the anti-tumorogenic efficacy of SeMet to approach those of SeCys and selenite.

A number of synthetic Se-compounds have also been found to be anti-carcinogenic in animal model systems (Combs and Grey, 1998; Combs and Lü, 2001; Ip, 1998). These include a number of alkylselenocyanates, allyl-selenocysteine, and several aryl selenocyanates (e.g., benzylselenocyanate, p-methoxybenzyl-selenocysteine, p-phenylseleno-cyanate). These compounds are thought to undergo initial metabolism through arylselenol, which induces apoptosis without DNA single strand breaks. When compared to selenite, these forms offer comparable anti-tumorogenic efficacy; yet, the are less effective in supporting GPX expression, indicating that they are less effective as metabolic precursors to H$_2$Se.

The anti-carcinogenic activities of various Se-compounds/metabolites may involve their reactions with critical proteins as well as their redox cycling, both of which effects may selectively impact the transformed phenotype. Selenium-compounds may affect cellular proteins through the formation of selenotrisulfide bonds (-S-Se-S-), selenylsulfide bonds (-S-Se-) or diselenide bonds (-Se-Se-), or through the catalysis of disulfide bond formation/dissolution. These reactions would affect the activities of enzymes with critical sulfhydryl groups and of selenoproteins which have SeCys residues at their active centers. One such thiol-sensitive enzyme is protein kinase C (PKC); its inhibition by CH$_3$SeH would be expected to trigger a number of downstream effects including cell cycle arrest, apoptosis and angiogenic switch regulation. Evidence indicates that Se-doses large enough to support high, steady-state concentrations of CH$_3$SeH can effect anti-carcinogenesis by inhibiting critical redox-sensitive factors including PKC and, probably, NF-kB and AP-1, thus, impairing tumor cell metabolism and transformation (Combs and Lü, 2001).

Effects of Se-compounds on cell proliferation may also involve their abilities to form catalytically active, redox-cycling intermediates. Selenite, diselenides and the oxidation product of H$_2$Se, selenium dioxide, for example, can each react with GSH to produce the selenolate ion (RSe$^-$). In the presence of GSH and molecular oxygen, RSe$^-$ can cycle continuously to generate O$_2^-$ and H$_2$O$_2$. This redox cycling, which is thought to be the basis of Se-toxicity, may also contribute to anti-carcinogenesis.
Implications of Selenium Research

**Guidance for Human Health**

In 1980, Se was recognized by the Committee on Dietary Allowances with the establishment of Estimated Safe and Adequate Daily Dietary Intake values (50-200 µg) (NRC, 1980). In 1989, those values were replaced with Recommended Dietary Allowances (RDAs) (e.g., women: 55 µg; men: 70 µg) (NRC, 1989), which were modified (e.g., RDAs for both women and men: 70 µg) in the Dietary Reference Values (NRC, 2000).

It is highly relevant to public health considerations to confirm that Se can, indeed, reduce cancer risk in healthy people. This is being done through the commitment of nearly $200 M of public research funding mostly through the National Institutes of Health. Much of that funding supports the 10-yr, 32,000 subject SELECT trial\(^3\) that was started last year to evaluate the effects of Se and/or vitamin E supplements in reducing prostate cancer risks among American men. Anticipating the policy actions that will be called for upon the publication of positive results of that trial, it is now time to generate other necessary information. Specifically, it is necessary to determine the effects of Se-deprivation on cancer risk, and the minimum Se intake that confers cancer risk reduction. Both of these questions require a better understanding of the mechanisms of anti-carcinogenic and nutritional actions is this element.

**Opportunities in Animal Nutrition**

The dietary requirements of animals for supporting the expression of Se Cys-enzymes are generally satisfied by dietary concentrations of 0.1-0.2 mg/kg, although levels up to 0.3 mg/kg are permitted under current US feed regulations. These were established based on the common practice of the US livestock industry of using inorganic selenium salts (particularly, sodium selenite) in feed supplementation. These inorganic forms, because they yield only H\(_2\)Se and the methylated excretory products metabolically, support only the SeCys-proteins and, therefore, have minimal effects on tissue Se levels at intakes above these required levels. They are unsatisfactory for the purposes of producing animal-based functional foods that deliver anti-carcinogenic forms and amounts of Se in normal servings. For that purpose, there are new problems and opportunities for the animal nutritionist: to determine the amounts and forms of dietary Se (particularly SeMet, CH\(_3\)SeMet and CH\(_3\)SeCys) most effective in supporting the safe enhancement of Se in milk, meats and eggs so as to render those products useful in reducing cancer risk (Combs, 2001).

**Conclusion**

In just five decades a nutrient was discovered to be a dietary essential, characterized for use in animal feeds, elucidated in terms of its fundamental metabolic roles, and found to be effective in reducing carcinogenesis. This work originated in the arena of animal nutrition because it yielded practical benefits. Those needs, in livestock feeding, could be addressed with fairly descriptive information, but it was the opportunity to gain deep and fundamental understanding through experimentation with animal models and, more recently, cultured cells that provided the base and rationale for clinical trials with humans. To date, all of these results point to Se, in various compounds, being useful in reducing cancer risk. While many important questions remain about the efficacy, specificity and safety of Se-compounds, it will be appropriate for animal producers not to default to the pharmaceutical and supplement industries for the implementation of this knowledge. There are clear opportunities to develop animal-based functional foods

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\(^3\) The SELECT trial is a multi-center, national study directed by scientists at the Fred Hutchinson Cancer Center, Seattle, WA.
containing Se. That will call for the continued attention of the animal nutrition community that, after all, gave birth to this field.

References


REFLECTIONS ON FIFTY YEARS OF THE MARYLAND NUTRITION CONFERENCE FOR FEED MANUFACTURERS

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Summary

This presentation celebrates the Golden Anniversary of the Maryland Nutrition Conference for Feed Manufacturers, and the 1st Mid-Atlantic Nutrition Conference, which will be cosponsored by Pennsylvania State University, Virginia Polytechnic Institute, and University of Delaware, as well as the University of Maryland and the American Feed Industry Association. This series of fifty Conferences has been a uniquely successful example of voluntary cooperation between University, Government and Industry in serving as a regular forum for the evaluation of emerging scientific developments. Thus, the Conferences have provided a highly tailored series of useful information for improving the production of safe, wholesome food products for consumers. Few cooperative activities have rendered such valuable service at so little cost. Some of the conditions in the Maryland area, which led to the first Conference, are mentioned. An effort is made to describe the broad scope of topics covered over the past 50 years, with brief mention of major developments. This period in our history has seen a vast amount of scientific progress and continued improvement in the efficiency of animal and poultry production. Finally, speculation is made as to how research challenges may change in the future.

Introduction

This is a time of celebration. This Conference is the 50th Anniversary of the Maryland Nutrition Conference for Feed Manufacturers, and the 1st Mid-Atlantic Nutrition Conference! For half a century the Maryland Nutrition Conference for Feed Manufacturers has provided interpretations of relevant scientific research on nutrition related topics. The annual meetings have served as an exceptionally effective forum for accelerating the evaluation and implementation of new findings in the production of food products for consumers. We also welcome and applaud Pennsylvania State University, the University of Delaware and Virginia Polytechnic Institute for joining the University of Maryland and the American Feed Industry Association in cosponsoring this and future Mid-Atlantic Nutrition Conferences.

This series of fifty Conferences has been uniquely successful, and is an outstanding example of continuing cooperative efforts between University, Government and Industry, as stressed by Dr. G. Lynn Romoser in his 25th Anniversary article in the 1978 Conference Proceedings. Beginning with the first Conference, an advisory committee, composed of representatives from feed manufacturing companies, has helped plan each program. This body was formalized as the "Maryland Feed Industry Council, Inc.," with rotating Industry members. The Council members have been extremely helpful in identifying developing areas of potential interest to the feed industry. The American Feed Industry Association, originally the American Feed Manufacturing Association, has cosponsored the Conferences since 1967. Because the information provided has been valuable, many participating companies have contributed support. In recent years, breakfast meetings and related symposia also have been sponsored.

Each Conference program included approximately 20 scientific presentations on a wide range of research topics, including various aspects of animal or poultry nutrition, feed ingredient quality, disease prevention and control, environmental protection, food safety, economic issues, consumer concerns, changing markets and many others. In the last 50 years, at least 950 scientists or other specialists, many
from other Universities, Government agencies, and Industry laboratories, have presented current research developments and discussed their possible application to the feed and food industries. In this way the Conferences have provided a highly tailored series of useful information for improving the production of safe, wholesome food products for consumers. Few cooperative activities have rendered such valuable service to consumers, as well as to the feed industry, at so little cost.

What led to the first Maryland Conference?

Anniversaries always permit one to review the past and prognosticate the future. Before we consider the rapid progress that has been made in the technology and economic efficiency of producing animal and poultry food products, let us review the conditions in the Maryland area prior to the first Conference. Please forgive my use of the broiler industry to exemplify this progress, but this industry was born on the Eastern Shore, and has become a primary part of Maryland agricultural.

According to the late Professor Wade Rice, Maryland Poultry Extension Specialist, Maryland and Delaware gave birth to the "broiler industry" in 1929. The first significant production of broilers occurred at Preston and Federalsburg, MD and Ocean View and Little Creek, DE, where 5,000 to 6,000 capacity broiler houses had been constructed, with about 10 coal-burning stoves. The feed was a mash concentrate, fed with cracked corn and grit. Skim or clabber milk was provided when available. Greens of all sorts (as grass clippings, lettuce, cabbage and kale) were cut for broilers to eat. He reminisced about his many visits to these locations, where losses commonly approached 45-50% due to faulty diet and disease. Coccidiosis and pullorum disease were real killers. Vitamin D deficiency rickets was a serious problem in confined birds. Although McCollum, at Johns Hopkins, had discovered in 1922 that cod liver oil contained both vitamins A & D, the addition of cod liver oil to broiler feeds was not a regular practice until about 1930.

After vitamins A & D were provided, other nutritional problems appeared in broiler flocks. These included "curled toe paralysis", "slipped tendon", and "crazy chick disease". Riboflavin, required for normal growth and prevention of curled toe paralysis, was not isolated until 1933. Vitamin E, which prevents crazy chick disease, was isolated in 1936. In 1937, manganese was found by the Cornell group to prevent slipped tendon, or perosis. In 1937, it was discovered that soybean protein required heat treatment for satisfactory use in poultry feeds. Then, in 1941, it was shown that added methionine improved the protein value of heated soybean meal. These findings led to heat treatment of soybean meal and the chemical synthesis of methionine for use in corn-soybean type rations for poultry and swine. Perhaps as important as the research leading to identification of essential nutrients of practical importance, was the rapid development of economical supplements by industry for use in practical feeds.

It should be remembered that in 1948 nutrition was still a young, emerging science. Actually, the term "vitamine" was not coined until 1912. Most of the presently known vitamins were isolated in the 1930s. The exceptions were vitamin C and thiamin, both isolated in 1926, and folic acid and vitamin B12, isolated in 1946 and 1948, respectively. (This author fractionated the "animal protein factor" as a Ph D thesis problem at Cornell University; it turned out to be vitamin B12).

The Delmarva Peninsula clearly led in broiler production in 1948, when this author joined the Poultry Husbandry Department staff at College Park. At that time, small and medium-size feed mills and growers already were working together, and the integration of the broiler industry was underway. The first available records reveal that in 1934, approximately 34 million broilers were reared. This number grew rapidly to 1.8 billion in 1959, and is expected to exceed 8 billion this year.

Many questions still remained in 1948. Even after tailored vitamin mixes were commercially available for use by feed manufacturers, the minimum level of specific vitamins, required under varying conditions of growth and reproduction, were not clearly established. More specific quantitative
information also was needed for other nutrients, including energy, protein, certain amino acids, and several minerals. Unidentified or unexplained growth responses were still being obtained from certain ingredients. Information was needed about nutrient composition and bioavailability of many feed ingredients. Nutrient balance received too little attention, especially after stabilized fats became available for use. Consequently, many practical formulas, at least for broilers, contained costly sources of “unidentified factors” and more than adequate levels of other supplemental nutrients, in an effort to achieve adequacy. In retrospect, the rocket-like increase in the broiler industry could hardly have occurred without the simultaneous advances in nutrition. One must hasten to stress that successful application of advances in nutrition depends also upon progress in breeding, disease control, marketing and other factors.

The University of Maryland was most fortunate to have Dr. Morley A. Jull as Head of the Department of Poultry Husbandry in 1948. Dr. Jull (at left) was a prominent internationally known authority on poultry. He authored several widely used textbooks on poultry husbandry and genetics. Jull’s vision and influence led to the timely construction of the Maryland Broiler Substation near Salisbury. The first field trial was conducted in 1951. Early in 1953, five broiler trials had been completed and a one-day "Maryland Poultry Nutrition Conference" was held at College Park on March 6 to present the findings. The following year, the Animal Husbandry and Dairy Husbandry Departments joined with the Poultry Husbandry Department in sponsoring a 2-day meeting. Thus, the 1st Maryland Nutrition Conference for Feed Manufacturers was held on March 25-26, 1954, at the University. The next year, the conference location was moved to Washington, D.C., as it was deemed more convenient for many. Since 1973, the annual conferences have been held in Baltimore, MD.

Founders of the 1954 Maryland Nutrition Conference

A large number of scientists and others, too many to list, have participated over the 50 years in the planning and operation of the annual meetings. Despite this, it does seem fitting to identify, as Founders, those individuals from the University and Industry who were involved in the planning and conduct of the first Conference.

University of Maryland Nutrition Conference Committee: Asst. Prof. Richard E. Brown, Nutrition, Dairy Husbandry Department; Dr. Gerald F. Combs, Nutrition, Poultry Husbandry Department; Dr. Emory C. Leffel, Nutrition, Animal Husbandry Department; Dr. Joseph C. Shaw, Nutrition, Dairy Husbandry Department; Asst. Prof. Perry F. Twining, Extension, Poultry Husbandry Department; and Dr. G. Lynn Romoser, Poultry Husbandry Department, Chairman.

University of Maryland Administrators: Dr. Thomas B. Symons, Acting President; Dr. James M. Gwin, Director of Extension; Dr. I.C. Haut, Director of Experiment Station; Dr. G. H. Beck, Head, Dairy Husbandry Department; Dr. John E. Foster, Head, Animal Husbandry Department; and Dr. Morley A. Jull, Head, Poultry Husbandry Department.

Other University of Maryland Participating Staff: Dr. R. N. Doetsch, Bacteriology Department; Mr. William E. Donaldson, Poultry Nutrition Graduate Student; Mr. William L. Ensor, Dairy Nutrition Graduate Student; Prof. Wade H. Rice, Extension Service, Poultry Husbandry Department; Dr. Clyne S. Shaffner, Physiologist, Poultry Husbandry Department; and Mr. George B. Sweet, Poultry Nutrition Graduate Student.

Maryland State Feed Industry Committee: Mr. Sterling Bowman, Bowman Brothers, Gaithersburg, MD; Dr. Leonard M. Dansky, D. A. Stickel & Sons, Inc., Hagerstown, MD; Mr. Robb Dryden, J. McKenny Willis & Son, Easton, MD; Mr. Otis Esham, Otis Feed Company, Parsonsburg, MD;
Mr. Bowen Quillen, Berlin Manufacturing Company, Berlin, MD; Mr. Theodore Reinke, Crisfield Dehydrating Company, Crisfield, MD; Mr. Merrick Wilson, Wilson Grain Company, Centreville, MD; and Dr. Charles D. Caskey, Cooperative Mills, Inc., Baltimore, MD, Chairman.

The Speakers at the first Conference are shown in the photograph above. First row, left to right: Dr. C.D. Caskey, Mr. Lee Boyd (AMFA), Dr. J.L. Krider, Dr. E.I. Robertson, Dr. L.A. Moore; second row, Mr. W.L. Ensor, Dr. L.M. Dansky, Dr. C.S. Shaffner, Dr. H.L. Wilecke, Dr. G. L. Romoser, Dr. J.C. Shaw; third row, Mr. P.W. Chichester, Dr. G.F. Combs, Dr. R.E. Brown, Dr. E. C. Leffel, and Prof. P.F. Twining. Other speakers, not shown, were Dr. R.N. Doetsch, U. of Maryland, Dept. of Bacteriology; and Prof. W.H. Rice, W.E. Donaldson and G. B. Sweet of the Poultry Husbandry Department.

This report would be remiss if the continued efforts of several University staff members over this fifty-year-period were not cited. The chairpersons or conference coordinators of successive Maryland Nutrition Conference Committees have had a critical role in the timely planning and smooth operation of each Conference. Sincere appreciation is due Lynn Romoser, Richard Creek, Charles Chance, Clyne Shaffner, Joe Soares, John H. Vandersall, Emory Leffel, Owen Thomas, Elton Johnson, John Doerr, and Nickolas Zimmermann, who have served in this capacity. Wade Rice, Charles Chance, Clyne Schaffner, Emory Leffel, John H. Vandersall, and Chuck Wabeck served as treasurer of the Maryland Feed Industry Council and deserve our gratitude. It is regrettable that the late Dr. G. Lynn Romoser cannot be with us on this occasion as he did so much to assure the success of these Conferences in those early years.

The photo at the right shows the speakers at the 1955 Maryland Nutrition Conference.

Over this period, approximately 150 different industry representatives have served one or more 3-year terms on the Maryland State Feed Industry Council. Since 1954, the University of Maryland at College Park has had five successive Presidents, as well as changes in other administrators. There can be no question, but that this Conference has enjoyed the continued support of the University and Feed Industry.

Scope of Research Covered

Despite the fact that copies of all Conference Proceedings have been available for my perusal in preparing this manuscript, the scope of topics covered was far too broad to be summarized here. Most of the various topics covered by speakers at previous Maryland Nutrition Conferences related to one or more of the areas listed:

- Developments concerning nutrition requirements and management of poultry (broilers, layers, starting pullets, broiler breeders, turkey poult & breeders); ruminants (dairy cattle, beef cattle, calves, sheep, rumen microflora for protein synthesis), and non-ruminant animals (swine, gestating sows, horses, fish, dogs, cats);
- Human nutrition concerns (dietary guidelines, "trans" fatty acids, organic foods, designer/functional foods, food safety, and nutritional misinformation);
- Nutritional composition of feeds and foods, nutrient bioavailability, effects of feed enzymes, methods of measuring nutrient composition and bioavailability, and biogenic amines;
- Availability & use of chemical feed additives, including amino acids, vitamin & minerals;
- Use of antioxidants and stabilized fats in feeds; and processing of feed ingredients;
- Biotechnology (growth hormone, somatotropins, genetic engineering, trans genetics);
- Metabolic aspects, including acid base balance, role of microflora and digestion;
- Prevention and control of mycotoxins in feeds;
- Prevention and control of animal and avian diseases (antibiotics, coccidiostats, organo-arsenicals, probiotics, effects of nutrients on immunity, and FDA requirements);
- Feed and grain outlook, economics of production, export policies, role of animals and poultry in sustainable agriculture and in meeting future world food needs;
- Consumer shopping, eating behavior, "functional foods", consumer needs market demand; and
- Environmental protection and animal welfare concerns.

Clearly, these conferences have provided a vast amount of timely information on a broad range of valuable topics to the feed industry. This information transfer clearly has benefited consumers through its application in more efficient production of wholesome food products at low cost.

Due to the broad area covered, my comments will be directed primarily to developments in poultry nutrition. The Maryland Broiler Substation at Salisbury provided an excellent facility in which to conduct nutrition and management studies of immediate practical value to feed manufacturers and broiler growers. At that time, few companies or growers had facilities to conduct research on nutritional problems. The results of the Substation studies usually were presented at a field day at the end of each
trial. They also were promptly published in Feedstuffs and summarized at the next Nutrition Conference. Since these trials were designed to answer current practical questions, conference attendance grew rapidly. In about 1958 or 59, over 500 people, from 28 states and two foreign countries, attended.

**Unexplained growth responses and the Antibiotic Era.**

The inclusion of certain animal protein supplements, as fish meal, dried whey, or certain fermentation by-products usually improved growth and feed efficiency of starting broilers, though the responses were variable and not great. These growth responses persisted, even after vitamin B12 feed supplements were fed. Workers at Lederle, Pfizer and Merck laboratories found that spent mycelia residues from antibiotic production caused growth responses when fed to chicks. This response was found to be due to the residual antibiotic present. These antibiotics failed to improve growth of chicks reared in germ-free environments. This led to studies on mechanism of action on intestinal microflora and subsequent effects on health and nutritional requirements. Without question, the antibiotic era, involving low level feeding of selected antibiotics, has exerted a major health and economic benefit in essentially every areas of animal and poultry production.

The unexplained growth responses were due to a combination of several factors. The response from fish meal and other animal products was probably due to a higher energy density, better protein quality, and improved levels of available methionine and lysine. The response from certain fermentation products undoubtedly was due to their antibiotic content. Molasses fermentation byproducts were high in potassium, which the late Dr. W.C. Supplee found at Maryland explained some growth responses in turkey poults. Zinc, copper or selenium levels also may have been involved, as each was found to improve growth in chicks or poults fed otherwise adequate diets. Dr. Donald Blamberg, while at Maryland, found zinc deficiency in breeder hens to result in impaired hatchability and deformed embryonic development.

These unexplained dietary factors ultimately disappeared as research led to diets higher in energy, balanced with respect to protein and essential amino acid, and adequate in all known required vitamins and minerals. This allowed manufacturers to improve their formulas by omitting certain bulky, unnecessary, energy-diluting "unidentified factor sources", usually at a savings. It also set the stage for linear programming of rations, on a least-cost basis primarily.

**Calory Protein Ratio and Stabilized Fats.**

Drs. Singsen and Matterson, at the University of Connecticut, found broiler growth rate and feed efficiency to be improved when rations higher in energy density were fed. After antioxidants were available commercially to prevent rancidity of feed grade fats, stabilized animal and vegetable fats could be used up to about 5% of the ration with good results. Higher levels were considered “toxic” and led to feather picking and impaired growth, as well as increased fat deposition. Dr. W.E. Donaldson, then a Maryland nutrition graduate student, studied the relationship of protein level to fat (energy) level on the performance of growing chicks. His studies, and those of others, clearly showed that the protein level needed to be increased as the level of energy was raised by the addition of fat. It immediately became clear that the "nutritive ratio" concept used in large animal nutrition must be adapted and applied to poultry nutrition. Several broiler field trials, as well as other studies at College Park, were conducted with widely different levels of dietary fat and protein on growth and body composition. Based on the results, an article was published in March, 1955 Feed Age entitled "A New Approach to Poultry Feed Formulation". This article presents the concept that protein level (or limiting amino acids) must be raised in proportion to increases in energy density of the ration. This led to the widely used term, "Calory/Protein" or "C/P" ratio, and demonstrated how rations containing high levels of fat could be successfully used.
Maryland Broiler Trial Results Over 15 Years

<table>
<thead>
<tr>
<th>Trial</th>
<th>Year</th>
<th>Age (wks)</th>
<th>Wt (lbs)</th>
<th>Feed:Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1</td>
<td>1951</td>
<td>10</td>
<td>3.06</td>
<td>2.81</td>
</tr>
<tr>
<td>S-12</td>
<td>1955</td>
<td>9</td>
<td>3.02</td>
<td>2.40</td>
</tr>
<tr>
<td>S-21</td>
<td>1957</td>
<td>8</td>
<td>3.14</td>
<td>2.25</td>
</tr>
<tr>
<td>S-49</td>
<td>1965</td>
<td>8</td>
<td>3.55</td>
<td>1.92</td>
</tr>
</tbody>
</table>

Interestingly enough, the American Feed Manufacturers Association planned a "Feed to Food" program at their 50th anniversary meeting in May 1958. Dr. C.D. Caskey, Cooperative Mills, Baltimore, and the author presented a special exhibit for the event to illustrate progress made in broiler feeding.

Effect of Fat Level in Various Diets on Feed:Gain Ratio in Broilers Reared to Three Pounds

<table>
<thead>
<tr>
<th>Ration</th>
<th>Type</th>
<th>Fat (%)</th>
<th>Age (d)</th>
<th>Wt (lbs)</th>
<th>Feed:Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial.</td>
<td>Standard</td>
<td>8-9</td>
<td>51</td>
<td>3.03</td>
<td>1.86</td>
</tr>
<tr>
<td>Modified</td>
<td>Practical</td>
<td>14-18</td>
<td>48</td>
<td>3.01</td>
<td>1.61</td>
</tr>
<tr>
<td>Modified</td>
<td>Practical</td>
<td>24</td>
<td>48</td>
<td>3.06</td>
<td>1.32</td>
</tr>
<tr>
<td>Modified</td>
<td>Practical</td>
<td>34-35</td>
<td>47</td>
<td>3.02</td>
<td>1.26</td>
</tr>
<tr>
<td>Experimental</td>
<td>Semi-purified</td>
<td>30-33</td>
<td>48</td>
<td>3.08</td>
<td>1.18</td>
</tr>
<tr>
<td>Experimental</td>
<td>Semi-purified</td>
<td>38-41</td>
<td>49</td>
<td>3.06</td>
<td>1.17</td>
</tr>
<tr>
<td>Experimental</td>
<td>Semi-purified</td>
<td>39-51</td>
<td>51</td>
<td>3.02</td>
<td>1.08</td>
</tr>
</tbody>
</table>

For this, three fast growing strains of crossbred male broiler chicks were fed rations varying in nutrient density. The feeds compared involved a commercial feed, high fat feeds composed only of ingredients available for use in commercial feeds, and experimental semi-purified diets, containing up to 50% total fat. These were formulated to maintain adequate levels of protein and amino acids in relation to their energy levels. One of the experimental diets produced broilers (of one strain) averaging 3.02 lbs. in 44 days with a feed conversion of 1.04! This compared to an average of 3 lbs. in 49 days with a conversion of 1.79 for the commercial feed. One high fat feed, composed only of ingredients normally used in practical rations, produced broilers averaging 3 lbs. in 46 days with a 1.19 conversion. These results, far better than most, were published in Feedstuffs, July 1958, and probably helped swell the attendance to our Conference.

Amino acid requirements, bioavailability, and ingredient composition.

Studies on Calory/Protein ratios soon began to focus on amino acid/energy ratios. The sulfur amino acids, especially methionine, were most likely to be deficient in corn-soybean meal type rations. The bioavailability of lysine can vary appreciably in different protein supplements, as lysine reacts with...
reducing sugars on heating and become unavailable to monogastric animals. Bioassays with chicks were conducted by Mr. Earnest Bossard to determine the bioavailability of lysine in a large number of practical feed ingredients. The late Dr. Owen P. Thomas extended the Maryland studies on nutrient composition and amino acid requirements. He studied the requirement of broilers for methionine, total sulfur amino acids, lysine, isoleucine, valine, leucine, threonine, and glycine plus serine. Similar requirements on laying hens were also done for methionine and lysine. Such studies on quantitative amino acid needs in terms of grams per therm of metabolizable energy content and more complete feed composition data have permitted the widespread use of computer formulation.

**Enzyme feeding, phosphorus bioavailability, and environmental protection.**

In recent years, several investigators have studied ways to reduce the nitrogen and phosphorus contamination of the environment. One approach has been to feed microbial phytase in poultry and swine rations, which contain plant ingredients high in phytin phosphorus. Phytase improves the digestibility, and thus the bioavailability, of organically bound phosphorus in plant feedstuffs, including soybean meal and most cereal grains. This improved phosphorus availability permits a reduction of supplemental inorganic phosphorus and calcium to corn-soybean meal-type rations. If adequate amino acid/energy ratios are maintained by adding amino acids as needed, the level of total protein can be minimized. Usually this results in reducing the level of soybean meal. Such changes in rations for broilers and growing pigs lowers the total dry matter excreted in the litter, with reductions up to 30% phosphorus and 5% nitrogen. However, the use of phytase appears to increase the solubility of phosphorus in stored litter.

**Nutrient Partitioning and Biotechnology.**

Of special note is the research with the hormone, bovine somatotropin (BST), for increased milk production in dairy cattle. DNA technology has allowed preparation of porcine somatotropin (pST) in amounts for studies on nutrient partitioning in swine. In adequately nourished pigs, somatotropin appears to regulate metabolism in such a way that more nutrients are deposited into muscle protein instead of into body fat. These studies offer great promise as a means of improving efficiency and lower fat levels in pork. Emerging studies involving genetic engineering of plants and microorganisms, coupled with advances in regulating metabolic processes, offer unparalleled opportunities for striking progress in the future.

**What Will Be The Future Challenges?**

**Many of the present problems will persist, if in a modified form.**

Despite continued progress in nutrition and related disciplines, there will always be a need for improved concepts and methods for understanding more fully the functions and interactions of nutrients with other metabolic components of living organism. The immediate future should yield much progress, as genetic engineering techniques are applied to both plants and animals in solving specific problems. Stable isotope techniques permit detection of metabolic pathways and changes induced by regulatory agents. Questions remain about the variability and bioavailability of important limiting nutrients in many feed ingredients. Studies on nutrient composition will be needed as genetically altered feed ingredients become available. The restrictions on environmental pollution are likely to increase, and acceptable practices must be devised. Education of policy makers may need strengthening to insure that production restraints are considered realistically.

**Consumer concerns about food, health and environment are increasing.**

The high incidence of obesity, heart disease diabetes and cancer constitute major health problems in our society. Since these conditions can be influenced by diet, there is a growing consumer demand for
functional foods, designed to reduce these risks. Diets with lower intakes of total energy and saturated fatty acids may help reduce the incidence of obesity and coronary heart disease. Certain naturally- occurring (phytogenic) compounds in foods have been found to help prevent cancer in animal models. Even consumer demand for “organic foods” is increasing. In short, increasing consumer concern about food and health will offer market opportunities for functional foods designed to better meet their needs. Sound consumer education programs should be part of any marketing effort to insure that the demand generated is genuine.

The long-term solution of meeting global food needs will demand more focused planning if our world is to remain peaceful.

Any successful international nutrition plan should recognize the need for 1) sustainable agricultural food production systems to the extent practicable in every countries and 2) the essential role of food producing animals in order to optimize the use of all available resources.

National policies should permit free trade of food commodities, especially where mean nutrient intakes are sub-optimal. Free trade, especially with developing countries, would allow imports of many needed food commodities, the production of which could be increased readily in this, and perhaps other, countries.

We can be certain that scientists of the future will continue to find solutions to practical problems, even as they uncover new ones. So let us wish them good fortune, and look forward to the 25th Anniversary of the Mid-Atlantic Nutrition Conference in 2027. As Dr. Romoser said 25 years ago about this Golden Anniversary Celebration, “I hope we are there to hear it!”
AGRI STATS - WHERE IS POULTRY GOING AS AN INDUSTRY
AND WHAT ECONOMIC CHALLENGES DOES IT FACE

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The Economic Challenges of the Poultry Industry (February 17, 2003)

As we go through tough times in the U.S. broiler industry, producers try to re-define what factors drive profitability in the industry. It’s a question that is both simple and difficult to answer.

The economic laws of supply and demand certainly control much of what determines profitability but this is more on the macroeconomic side of the business. The ability to improve in profitability also rests on the microeconomic side of the business and this is what we hope to address in the following thoughts.

Profitability in our industry and most industries can be simply defined as the difference between selling price and the production costs of goods sold. In the broiler industry production cost consists of live production cost and plant cost. Broiler production is an ‘integrated’ industry in that producers control all costs of production from the placement of day old parent stock through the sale of the final lb. of broiler meat and by products.

The following are some of the factors that will affect profitability in the industry.

Macroeconomic Factors

Over the last two decades the U.S. broiler industry has rapidly expanded production to meet consumer demand for poultry meat in the U.S. and overseas. Since 1995 total broiler production has expanded by 25% as producers have increased bird weights, improved yields, quickened line speeds and to a lesser extent, built new production facilities.

<table>
<thead>
<tr>
<th>Year</th>
<th>Live Production (000 Lbs.)</th>
<th>% Increase vs. Prev. Year</th>
<th>Evis. Production (000 Lbs.)</th>
<th>Consumption (lbs./per capita)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>34,222</td>
<td></td>
<td>25,021</td>
<td>68.8</td>
</tr>
<tr>
<td>1996</td>
<td>36,486</td>
<td>6.6 %</td>
<td>26,336</td>
<td>70.8</td>
</tr>
<tr>
<td>1997</td>
<td>37,541</td>
<td>2.9</td>
<td>27,271</td>
<td>71.9</td>
</tr>
<tr>
<td>1998</td>
<td>38,554</td>
<td>2.7</td>
<td>27,963</td>
<td>72.6</td>
</tr>
<tr>
<td>1999</td>
<td>40,830</td>
<td>5.9</td>
<td>29,741</td>
<td>77.0</td>
</tr>
<tr>
<td>2000</td>
<td>41,516</td>
<td>1.7</td>
<td>30,495</td>
<td>77.4</td>
</tr>
<tr>
<td>2001</td>
<td>42,445</td>
<td>3.9</td>
<td>31,266</td>
<td>76.5</td>
</tr>
<tr>
<td>2002</td>
<td>32,354</td>
<td></td>
<td></td>
<td>80.3</td>
</tr>
</tbody>
</table>

Source: USDA  * - Projected for 2002
While production increased by close to 25% from 1995 through 2001, per capita consumption by only 11.2%. Exports and changes in how chicken is consumed must be responsible for absorbing the rest of the increase. However we choose to look at the data it is evident that for the last three years U.S. per capita consumption has been essentially flat.

It is very difficult to raise or even maintain product pricing in an industry that is rapidly increasing production in the face of limited increases in domestic production. When additional disruptions to export demand for poultry products come into play, opportunities for maintaining or increasing profitability becomes even more difficult.

**Cold Storage Levels**

Which came first, the chicken or the egg? Which comes first, dropping net sales prices or increases in cold storage levels of chicken meat?

Whatever the answer, there appears to be a direct link between falling profits and levels of chicken cold storage stocks. In 1998, primarily due to the leucosis ‘J’ virus, production increases were restrained. We couldn’t produce as much chicken as we wanted to. As a result with demand stable or even slightly increasing, demand exceeded supply and cold storage stocks were drawn down to below 550,000 tons. Average profitability per lb. in the industry reached $0.10 per lb. for a brief period of time.

Over the next twelve months as producers increased breeder placements in anticipation of continued high pullet and breeder mortality, the incidence of ‘J’ virus was significantly reduced. Broiler meat production increased rapidly. Prices fell or didn’t rise seasonally as they normally would and more product wound up in the freezer as stocks rose, briefly surpassing 900,000 tons in storage…and all of this meat in storage continued to depress prices and profitability.

**Profits vs. Cold Storage Stock**

![Graph showing profits vs. cold storage stock](image)

In early 2001, the industry flushed cold storage stocks, (primarily leg quarters), levels dropped below 650,000 tons. Prices rose and profit returned to the industry. The broiler industry responded by rapidly increasing breeder placements leading to a significant increase in production for 2002. Difficulties...
in exporting leg quarters to Russia (an understatement), starting in early March of this year led immediately to higher cold storage levels, now surpassing 850,000 tons, contributing to lower profits. If cold storage levels are the indicator of supply exceeding demand, until the higher stocks are reduced, opportunities to increase prices and return to profitability are limited.

**Feed Costs Can Influence but Don’t Determine Profitability**

Rapid increases in feed ingredient costs will greatly influence profitability in the short run but over the long run the effect is diminished. For September of 2002, feed ingredient cost was 53.8% of total live production cost and 33.9% of the total cost per eviscerated lb. of poultry meat.

In 1996, reduced U.S. corn and soybean harvests coupled with an increase of demand for corn and soybeans by the developing poultry and pork industries in Asia resulted in a shortfall in corn and soybean supplies and a doubling in feed prices for the U.S. broiler industry. This increase in ingredient prices drove live production cost per lb. from $0.25 per lb. to $0.32 per lb. Broiler meat production in 1996 increased 6.6% relative to year earlier levels and in light of the increase in volume, producers were unable to pass on the higher production costs associated with the higher feed ingredient costs. Profits dropped from $0.05 per lb. to less than $0.02 per lb.

![Live Cost/Lb. vs. Profits](image)

As previously mentioned, 1998 is an anomaly in that the ‘J’ virus reduced supply and improved prices and profitability. The drop in grain prices in 1998 contributed to the improved profitability for that year. However the abundant grain and soybean meal supply of the last three years, which resulted in live production costs at historically low levels, have not resulted in industry profitability probably due to the increase in meat supply.

**Plant Costs and Yields as Indicators of Profitability**

Plant costs will have some effect on profitability and more importantly improvements in yields can help reduce costs if the improvements are made in cuts that have the greatest economic value.
Over the last seven years plant costs have increased by five cents per lb. Much of this increase is due to the change in product mix in most plants as companies have tried to increase the percentage of further processed items in their total product mix to improve the value of their product mix. In 1995, 8.69% of the average plants product mix was sold in deboned breast meat form. In 2001 this number had increased by 50.6 to 13.09% of all eviscerated lbs. sold. Labor costs also have increased significantly as many areas of the poultry industry were seeing local unemployment rates plummet below 4%. All of these areas contributed to annual increases of 4 to 6% in wage rates and consequent increases in labor cost per lb. of meat produced.

**Plant Cost and WOG Yields**

![Graph showing Plant Cost and % WOG Yields over years 1995 to 2001](image)

The increase in plant cost per lb. of meat would have been higher if not for a steady increase in WOG yields and in Boneless Breast Meat Yields. WOG yields are improving by 0.4 to 0.5% per year with the bulk of the improvement showing up as increases in boneless breast meat production. The increase in breast meat in yields and as a percentage of total meat produced should have had a positive effect on the sales value of all lbs. produced, however flat per capita consumption and the ongoing controversies over leg quarter exports to Russia have reduced the net sales value of all lbs. produced rather than raised the value.

**Net Dock Sales Price**

The trend to produce additional volumes of further processed items, (boneless breast meat, boneless thigh meat, cut wings etc.), over the last few years was intended to improve the average selling price of all products produced by the broiler industry. Unfortunately all of the additional volume has decreased sales price and diminished profitability.

Earlier we discussed the effect of the increase in corn and soybean meal costs in 1996 and 1997, which increased feed ingredient cost by $0.06 to $0.08 per lb. of meat produced. Net Dock Sales Price (average price received for all eviscerated lbs. produced minus selling expense) did increase by $0.04 per lb., not enough to cover the additional production costs. In 1998, low grain prices and higher meat prices led to the best industry profitability of the last six years. From 1998 to 2000, average Net Dock Sales Price fell by $0.08 per lb. making profitability more difficult.
Strong wing prices throughout 2001 and relatively strong leg quarter prices in the second half of 2001 helped Net Dock Sales Price rebound by $0.03 per lb. and improved the profitability of the industry particularly from June through November. These gains were short lived and for all of 2002 we will likely see average price fall to the year 2000 level.

Then How Do Companies Try To Reach Profitability?

Net dock sales price is the dominant factor in profitability. Companies that are profitable have efficient live production systems that lead to good live cost and also have high yields and efficient plant operations leading to competitive plant cost. Having both of these areas in line will help profitability but even with both live and plant running well may not drive a company into the top ranks in profitability.

Two things affect net dock sales price; the price received for each lb. of meat produced and the product mix the company is producing. Poultry markets influence both of these areas and as a result companies that are in the top rank of profitability in one year can fall dramatically by the following year depending on the market.

Let’s take a look at the profitability of the top 25% of tray pack companies in September of 2001 and September of 2002. In September of 2001, the Agri Stats average price for all wings sold was $0.9465 per lb. A year later the average price was holding at $0.6706 per lb., a drop of 29.2%. Leg quarter prices per lb. over the same period dropped from $0.3082 to $0.1796 per lb. Companies constantly choose whether to book business on forward pricing at a guaranteed price per lb./cost plus basis or price off the markets. In periods of strong commodity markets, those selling more bulk commodity products have more flexible pricing and can pass on price increases to their customers than those with booked business. 1998 and the second half of 2001 are examples of periods of high commodity prices.

Conversely, in periods of oversupply like 2000 and 2002 those companies with more booked business or fixed price business hold on to prices better than those pricing off commodity markets.
In September of 2001, eight companies/plants made up the top 25% in tray pack profitability. Commodity prices for wings and leg quarters were high and for this group 53.84% of their products were sold in bulk form while 36.27% of their production was sold in tray pack form.

Swing to a year later. Commodity prices have dropped sharply. Only three of the eight in the top 25% in 2001 remain in the top 25%. Those in the top 25% are selling only 39.19% of their production in bulk form. 47.40% of their production is sold in a tray.

**So What Drives Profitability?**

Companies can’t switch from a commodity program to a ‘booked’ program overnight. Plant costs and live costs are also affected by decisions related to the product mix that the sales department wants to sell. In either case the principal driver of profitability is putting as much of the product mix as possible into the ‘right box’.

Companies that sell into commodity markets need to maximize the Yield Value of what they sell. In increasing WOG yields they need to increase the yield of boneless breast meat, wings etc., products with relatively high sales value as opposed to producing more breast skin, bones etc. that depending on the product mix end up being sold to renderers or other byproduct markets. If the company is selling most of their back halves as leg quarters and deboning the front halves they have to maximize the proportion of their back half to their front half yield, to sell more product in leg quarter form and sell fewer lbs. of frames to rendering or for comminuted meat.

Companies selling more cost/plus or booked business need to get as much product into the desired sales channel, in tray pack operations getting more product into a tray at the right weights. In fast food operations the greater the percentage of product sold in eight and nine piece cuts and other fast food options reduce the volume of product that gets dropped into the bulk commodity business.

As a result, profitability in the industry will shift depending on markets and product mix. Live production and plant efficiencies will contribute to improved profits but sales price will eventually be the driver in all operations.
EFFECTS OF FEED PROCESSING AND TEXTURE
ON BIRD PERFORMANCE

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Although the benefits of various feed manufacturing procedures for agricultural animals were recognized in the early 1900's in the US, most improvements in the process have been directed toward the equipment such as improving it's reliability and efficiency. However, many researchers have seemed to lose focus on the minimum quality factors that are needed to realize any return at the production level due to their manufacturing process. Indeed, as mills have become larger and throughput has increased, it seems that feed quality has been less emphasized. This is surprising considering that properly formed feed could result in saving more feed conversion points than many of the other things we work so hard to implement at so great a cost. Combined with the impact that feed manufacturing may have on nutrients, it would seem that more attention is warranted in this area.

There appears to be some seemingly innocuous parameters that should be revisited since so much has changed with bird genetics, husbandry, and nutrition. For example, ingredient particle size, cost of grind vs. benefit to pelleting, and the effect of particle size on the bird’s anatomy and physiology may be more important than we currently understand. Conditioning parameters, or even pre-conditioning factors have been more focused on the ‘process’ than actual return on investment by factoring in the loss or gain in bird growth and feed conversion. Other factors such as the minimum number of pellets required in a feed, pellet size, crumble quality, etc. are all information that is woefully deficient in the current literature. Furthermore, much of the prior data may be confounded since methods of measuring feed quality may have been poorly chosen.

The oil, protein, starch, and fiber content of corn and corn by-products will affect not only the nutritional value, but also the feed milling characteristics. Although expected composition differences due to weather, hybrid, and drought appear small (protein +/- 1.7%, oil +/- 0.8%, starch +/- 2.0%), because corn is usually the largest component of poultry rations, one could expect the biggest impact on feed milling to be due to even small changes in nutrient composition. The grain moisture content must also be considered when grinding and pelleting rations for meat birds. All of these factors will affect pellet quality. If changes in some of these factors result in reduced pellet quality, it is likely to reduce bird performance, especially feed efficiency. It may be possible to determine the effect of the ratio of these nutrients and make adjustments so that pellet quality is not impacted. For example the effect of just .5% added moisture can increase mill throughput while improving pellet quality. With the introduction of new corn varieties with altered oil and starch properties, more information is needed about the impact of changes in the macro nutrient content of corn so that milling may be optimized.

Not many producers consider moisture and it’s affect on milling when formulating feed. However, adding water to feed will decrease the cost of making pellets and could improve feed conversion and growth rates. When given a choice, birds will chose feed with added water because it is more palatable to them. They tend to consume more wet feed than dry, even after the level of moisture is
adjusted. It has been shown in bird growth competitions, that birds fed feed with water grow at a faster growth rate (Beyer et al., 2002).

Feed manufactures work hard to produce pelleted diets of high quality while minimizing production expenses (Mommer and Ballantyne, 1991). Pellet quality (intact pellets) greatly improves broiler growth and feed conversion (Briggs et al., 1999). Fairchild and Greer (1999) have demonstrated that increasing feed mash moisture at the mixer can increase pellet durability and decrease pellet mill energy consumption, consequently improving pellet quality and reducing milling expense. Decreasing pellet mill energy consumption alone provides an incentive for feed manufacturers to consider moisture addition during the manufacturing process. However, potential improvement in pellet durability adds even more enticement for the use of moisture in broiler feeds since past research has illustrated positive relationships between pellet quality and broiler feed efficiency (Moran, 1989). The evidence these past studies provide warrant further research involving the application of pelleting broiler feeds with added water as well as determining the effect of this process on broiler performance. In our own studies, we have found that moisture addition to feed mash generates extensive differences in pellet durability and starch gelatinization between low moisture and high moisture treatments. High moisture pellets for both starter and grower diet formulations produced higher durabilities and gelatinization percentages compared to their respective low moisture equivalents. Broiler performance is most markedly affected in the three- to six-week period. Pelleted treatments produced significantly higher live weight gains and feed efficiencies compared to mash treatments. Surfactant/water additions to high moisture treatments created a dilution of nutrients. Feed efficiencies were adjusted to a 12.5% calculated moisture content in order to place all treatments at a similar nutrient density. Adjusted feed efficiency values illustrated that high moisture pelleted treatments produced significantly higher feed efficiencies compared to any other treatment. A possible explanation for these findings is that broilers fed high moisture pellets were able to better utilize feed energy for growth (productive energy) as opposed to using feed energy for foodprehension (maintenance). Broilers fed intact pellets of high durability would expend less energy in the act of feeding compared to broilers fed pellets of low durability and high percentages of fines. This speculation has been supported in past research (Moran, 1989). Mortality was not affected by moisture additions; however, pelleted treatments produced significantly greater mortality percentages compared to mash treatments.

Other work has been conducted to clarify the relationships between moisture addition, pellet manufacturing and quality, nutrient density and broiler performance. Studies have shown that adjustments in the moisture content of mash feed could overcome the often detrimental effect of added oil on pellet durability (Moritz et al., 2002).

Under most processing conditions using heat and moisture, starches gelatinize and help bind feed particles together. Starch gelatinization is an order-disorder phase transition that includes the diffusion of water into a granule, hydration and swelling, uptake of heat, loss of crystallinity and amylose leaching. Leached amylose immediately forms double helices that may aggregate (hydrogen bond) to each other and create semicrystalline regions. It is thought that as the gelatinized starch cools, the dispersed matrix forms a gel or paste-like mass that may function as an adhesive or binding agent. Past research has associated dietary gelatinized starch both positively and negatively with pellet quality and broiler performance. However, it has been speculated that gelatinized starch \textit{per se} may affect broiler performance aside from its contribution to pellet binding.

Gelatinizing cereal starch has generally been thought to improve enzymatic access to glucosidic linkages and consequent digestibility. Allred et al., reported a significant improvement in weight gain and feed conversion in chicks fed pelleted/re-ground corn that was incorporated into a complete diet over chicks fed similar diets with unprocessed corn. However, later research examining processed/re-ground
corn-based diets concluded there was no nutritional benefit to broilers despite increased diet starch gelatinization (Naber et al., 1969; Sloan et al., 1971). Moreover, Plavnik et al., found that feeding broilers pelleted/re-ground corn-based diets resulted in decreased bird performance compared to broilers fed similar unprocessed diets.

One strategy for producing high quality pellets has been to gelatinize as much ingredient starch as possible. High quality pellets are desirable as they are correlated with improved broiler performance. However, improving pellet quality through increasing starch gelatinization may negatively affect nutrient utilization, thus antagonizing performance enhancements of pelleting.

We have conducted studies to determine the effect of starch gelatinization on broiler feeding (Moritz, et al., 2002a). Fractions of corn were processed using typical feed industry practices and incrementally incorporated into complete diets at the expense of unprocessed corn (UC). The objective was to create diets with different levels of gelatinized starch produced from different commercial processes. Corn was the only ingredient manufactured to avoid confounding processing effects of high fat or high protein ingredients. Corn was either pelleted (PC) or extruded (EC) and subsequently re-ground prior to diet incorporation. Pelleted corn provided dietary starch gelatinization percentages indicative of conventional pelleted feeds, while EC provided extreme levels of gelatinization. Diets were fed to broilers during the 0-to-3-week starter phase to determine effects of processing-derived starch gelatinization on performance. In general, variation in diet particle size confounded effects of gelatinized starch on broiler performance. However, particle size was likely influenced by starch gelatinization. When performance effects could not be explained by particle size, the amount and derivation of gelatinized starch in diets may have influenced feed intake and/or nutrient utilization. Broiler feed intake may have been modified due to the effect of gelatinized starch on appetite, feed passage rate, gut morphology and related factors. Extrusion processing may have reduced nutrient availability of corn. Nevertheless, the data suggest that gelatinizing starch through commercial feed milling processes does not improve nutrient utilization of broilers during the 0-to-3-week starter phase.

We found that broilers fed gelatinized starch during the 0-to-3-week starter phase did not show improved feed utilization. However, in these studies, gelatinizing the starch may have influenced particle size, even after regrinding the samples, which may subsequently affect broiler performance. Further research is necessary to determine what the nutritional value of starch gelatinization in poultry rations.

Particle size is another manufacturing parameter that has received little attention in previous research. This is particularly surprising since feed particle size imparts influence on the anatomy and physiology of the digestive system, it’s function, it’s relationship between certain organs, and even gut microflora and pH. Birds fed feeds which have been finely ground then pelleted with have atrophied gizzards. This loss of gizzard function may affect other parts of the digestive tract including the small intestine where much nutrient breakdown and absorption occurs in birds. Corn quality impacts milling characteristics and grinding parameters which will affect pellet quality and bird performance. It may be necessary to manufacture feeds which allow us to take advantage of the benefits of pelleting while also maintaining gizzard function. Perhaps many researchers discounted the importance of particle size since the feeds were fed in pelleted form. It is true that a smaller particle size will improve pellet quality. However, when the pellet is exposed to moisture in the crop, the feed dissolves into the particles that are sized according to the grind of the grain and thus are in mash form when exposed to the gizzard. The poultry industry needs to conduct further research on particle size.

We are beginning to determine that the gross composition of grain may have an impact the requirement of certain nutrients. Although wet lab analysis can easily determine the protein and amino acid content of a particular load of grain, what may be more difficult to elucidate is how the relationship of starch, moisture and protein, impacts pellet quality which indirectly impacts the requirement of certain nutrients. If feeds with higher pellet quality increase the productive energy value of the rations, does this
then impact the requirement of other nutrients? Research diets often used to determine digestible amino acid needs of poultry are usually semi-purified or corn-soybean meal-based mash diets. While the lack of feed manufacturing equipment or the cost and time associated with processing research diets may necessitate feeding diets in mash form, this is not a common practice in the broiler industry.

The effect of feed form on amino acid requirements has received little attention even though the physical form of feed could be a factor influencing the variability in digestible requirements. Jensen (2000) has suggested that pelleting diets may increase amino acid requirements when expressed per unit of feed. Pelleting increases the density of a diet which reduces the time spent consuming meals, resulting in an increase in productive energy of a ration without changing metabolizable energy (Reddy et al., 1962). Increasing the energy available for protein synthesis may require higher dietary levels of lysine and other essential amino acids to maximize growth performance and efficiency.

A preliminary trial was conducted to determine the effects of feed form on digestible lysine necessary for maximum BWG and FE of broilers from 16-30 days of age. A corn, soybean meal, and corn gluten meal-based diet (1435 kcal ME/lb. and 21% CP) was formulated on a digestible amino acid basis to obtain a lysine deficient diet. Digestible essential amino acids were estimated from a composite of reported data. A 2x5 factorial treatment arrangement was achieved by feeding diets in two forms (mash and steam-conditioned pellets) with five levels of dietary digestible lysine (0.75, 0.85, 0.95, 1.05, and 1.15%). Six pens of 25 chicks were fed each dietary treatment.

Average feed intake and kcal ME consumed/bird were significantly higher for pellet fed birds. Performance of birds fed pelleted diets with digestible lysine levels above 0.95% was generally superior to that of birds fed the mash diets, regardless of lysine level. Maximum BWG and FE responses were observed at 0.95% dietary digestible lysine for the mash fed birds. Pellet fed birds achieved maximum BWG and FE responses at 1.05% and 1.15% digestible lysine, respectively. Our lab is further investigating the effects of feed form on lysine intake and utilization, but the preliminary data suggests that feed form is a factor affecting estimated lysine needs.

In summary, grain quality and manufacturing methods not only affect the nutritive value of feed, but it also has far reaching effects on milling characteristics and feed manufacturing parameters. Small changes in the starch, protein, and moisture content of the corn will have a major impact since corn makes up the largest fraction of poultry rations. Pellet quality and particle size are affected by changes in the composition of the grain and feed rations. These parameters may have an impact on the requirement of certain marginal nutrients if savings in the productive energy of feed can be realized from improvements in pellet quality.

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References

THE EFFECT OF DIETARY PROTEIN LEVEL AND TSAA:LYSINE RATIO ON
EGG PRODUCTION PARAMETERS, EGG YIELD AND
MOLECULAR COMPONENTS IN TISSUES

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Introduction

A large challenge facing today’s poultry industry is nutrient pollution as a result of over
supplementation of nutrients, such as phosphorus and protein. Over supplementation is required because of
the inefficiency of the hen to utilize these nutrients. Today’s hen only utilizes 40% of the dietary
protein supplied leaving 60% excreted to the environment. Formulation of diets with the ideal levels of
amino acids and limited excess or deficiencies and little of the amino acid being used as energy (Ideal
Protein Concept), may help decrease the cost of feed and reduce nitrogen pollution. Such a concept has
been implemented in the broiler and swine industry, and is feasible in the layer industry as well.

As a result of the direct relationship between dietary protein and nitrogen excretion (Lopez and
Leeson, 1995), the logical solution to excessive excreta nitrogen is to reduce protein in the diet. Many
researchers have been successful in reducing nitrogen excretion by reducing dietary crude protein
with/without supplemental amino acids (Schutte et al., 1992; Summers, 1993; Jais et al., 1995; Jamroz et
al., 1996; Blair et al., 1999), but with mixed affects on production variables. Keshavarz and Jackson
(1992) fed a low protein diet supplemented with amino acids, which performed equivalent to a positive
control in some, but not all performance parameters. When feeding a low protein regimen (14, 13, 12%
protein plus methionine (met), lysine (lys), tryptophan (trp) and isoleucine (ill)) or a moderate regimen
(15, 14, 13% protein plus met and lys) to laying hens from 22 to 66 weeks of age, overall, egg production,
feed consumption, egg weight, feed conversion efficiency (grams feed/ g egg) and egg shell breaking
strength (kg per egg) were similar to the positive control (18, 16.5 and 15 % protein). When hens
consumed the low protein diets, egg mass and body weight were significantly reduced compared to
positive control fed hens. Keshavarz and Jackson (1992) also reported amino acid supplemented low
protein diets increased all production parameters tested (egg production, egg weight, body weight, egg
mass, feed consumption and feed conversion efficiency) when compared to the negative control (14, 13
and 12% protein diet without supplemental amino acids).

Penz and Jensen (1991) reported decreased egg weights when feeding low protein diets with or
without supplemental essential or nonessential amino acids. Penz and Jensen fed Dekalb XL from 28 to
34 wks of age low protein diets (13% protein) supplemented with Lys, Met or Trp individually or in
combination at a level 20% above the NRC recommendations or a low protein diet supplemented with amino nitrogen supplying amino acids (glycine and glutamic acid) to equal the 16% protein diet in total nitrogen. Egg production and feed consumption were not significantly different between treatments. Low protein diets, however, performed inferior to control diets with regard to egg weights and body weight gain with or without supplemental amino acids at any level. Feed conversion (g feed/ g egg; g feed/dozen) was increased with low protein feeding compared to control fed hens, but additional Met, Lys and Trp at a level 20% above NRC performed similar to controls. All other amino acids in the low protein diets were at or above recommendations of NRC. The aforementioned research, suggests, supplementing low protein diets with amino acids can maintain egg production and egg weight, but egg mass is inferior compared to hens fed diets high in protein.

In order to utilize low protein diets, it is imperative to understand the relationship between amino acids (amino acid ratios) to maintain production rates. An ideal protein diet containing a balanced amino acid profile is not new to nutritionists and producers. There has been considerable work in turkeys, broilers and pigs to determine the optimal level of each limiting amino acid on a digestibility basis. Optimal amino acid ratios to diminish interactions and excessive feeding of nitrogen have been established in turkeys and broilers, but not in egg-type chickens. There are many ways to define ideal protein, but theoretically, it is the exact balance of amino acids that meet the animal’s needs. There should be no excess or deficiency of amino acids and a minimum of dietary amino acids should be used for energy. Overall, nitrogen excretion may be reduced as a result of a reduction of dietary protein with proper synthetic amino acid supplementation, while hopefully, maintaining overall production.

There is limited research on ideal amino acid ratios for laying hens. Baker (1997) reported a ratio of .72 for 0 to 3 wk old broiler chicks while Knowles and Southern (1998) reported ratios of .66, .71 and .63 to optimize average daily gain, average daily feed intake and gain:feed respectively, also in broilers. Shafer et al. (1996) reported an optimal diet for layers supplied a TSAA:Lysine ratio of around .85, which is similar to previous studies at UNL in Dekalb Deltas from 40 - 60 wks of age (Novak and Scheideler, 1998). Yakout et al., (2001) reported a TSAA:Lysine ratio of .71 for early producing hens for optimal egg production parameters and egg yield. Novak and Scheideler (1998) determined a TSAA:Lysine ratio of .55 is detrimental during early egg production in hens and .91 is optimal.

The objective of the following research was to evaluate low protein diets combined with TSAA:Lysine ratios to reduce nitrogen excretion while maintaining production parameters and egg yield. Carcass composition and molecular components in tissues (liver and magnum) were evaluated to enhance our understanding of the negative effects associated with feeding low protein diets.

Materials and Methods

A total of 432 Hyline W-98 hens were randomly assigned to one of 9 dietary treatment groups varying in protein and TSAA:Lysine ratios. Each of the 9 treatments was assigned to 8 replicate cages with 6 hens per cage (334 cm²/bird). Hens were housed in an environmentally controlled room (72-74°C) with ad libitum access to feed and water. Hens were maintained on a 16:8 hour light: dark cycle throughout the trial. A phase feeding program (Phase I - 20 to 43 weeks of age; Phase II - 44 to 63 weeks of age) was used during the experiment from 20 to 63 weeks of age. Diets (Table 1 - Phase I) were formulated based on feed consumption and age of birds as recommended by the Hyline W-98 breeder guide. All protein-containing feedstuffs were analyzed for protein, ether extract and dry matter prior to formulation. Utilizing regression equations (Degussa’s), amino acid content of feedstuffs was determined prior to

1Hyline W-98 Commercial Management Guide (‘98 - ’99), Des Moines, IA.
2Amino acid prediction model, Degussa Laboratories.
formulation of diets. Diets were subsequently formulated on an available amino acid basis. The experiment consisted of a $3 \times 3$ factorial arrangement of treatments. Three levels of dietary protein (Calculated - 14, 16 and 18%; Actual 15.2, 17.7, 19.5%) and three TSAA:Lysine amino acid ratios (Calculated - .71, .81 or .91; Actual - .82, .85, .97). During phase II, the dietary protein was lowered to 13, 14.5 and 16% (Actual - 14.3, 14.7 and 16.2% protein) while maintaining TSAA:Lysine amino acid ratios (Predicted - .71, .81 or .91; Actual - .72, .82, .92). Methionine was supplied in excess of the requirement to achieve the TSAA:Lysine ratio desired.

Table 1: Phase I Diets (100 g/head/day)

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**Nutrients:**

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<tr>
<td>(Analyzed)</td>
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<td>TSAA/Lysine Ratio</td>
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</table>

$^1$Min. Prems: Mn, 88 mg; Cu, 65 mg; Fe, 8.5 mg; Zn, 88 mg and Se, 0.30 mg.

$^2$Vit. Prems: Vit. A, 6,000 IU; Vit. D3, 2,000 IU; Vit. E, 10 IU; Vit. K, 2.0 mg; niacin, 44 mg; panto. acid, 6.6 mg; saccharin, 24.2 mg; choline, 110 mg; I, 0.08 mg; B1, 2.5 mg; ethoxyquin, 1.1 mg/kg.

Feed samples were collected using a probe for each phase of feeding and subsequently ground using a 1 mm screen Tecator cyclotec grinder. All diets were analyzed for calcium and phosphorus and protein, according to AOAC (1990) by atomic absorption and Kjeldahl methodology, respectively. Amino acids were also determined on complete feeds by Degussa Laboratories.

31093 Sample Mill, Box 70, 3-263 21 Hoganas, Sweden.

4Atomic Absorption Spectrophotometer, Spectra AA30.

5Diets were analyzed for amino acids at Degussa laboratories, Allendale, NJ 07401.
Feed consumption and egg production were recorded daily, while hens were weighed individually on a monthly basis. Average hen weight was calculated for each replicate cage. Weekly, one day’s egg production was used for measuring egg weight. Egg mass was then calculated by multiplying the egg weight times the weeks percent egg production. Biweekly, specific gravities were determined using one-day’s egg production while two eggs per cage were either used for Haugh unit determination or wet and dry egg component determination.

Every 5 weeks beginning at 20 weeks of age until the end of the trial, three eggs per treatment were used to evaluate the protein content in fresh yolk and albumen individually. The individual components were weighed out onto low nitrogen weigh paper and analyzed for protein by Kjeldahl procedures. Protein values were calculated using nitrogen x 6.25.

At 39 and 59 weeks of age, a chromic oxide digestibility trial was conducted to determine percent protein retained using chromic oxide as a marker. Chromic Oxide was added at a rate of 0.3% of the diet, and fed for 5 days. Three days of manure production was randomly sampled from each cage and collected in aluminum pans, frozen (-20°C) and freeze dried in a FTS System (Dura Dry). Feed samples containing chromic oxide and dried feces were ground using a 1 mm screen Tecator cyclotec grinder. Ground feces were sifted (1 mm screen) to remove feathers prior to analysis. Protein analysis was conducted by Kjeldahl methodology. Chromic oxide was analyzed by the procedure of Williams et al. (1962) and Perkin-Elmer (1971). To determine percent protein retention, the following equation was used:

\[
\% \text{ Protein retention} = 100 - ((\text{Diet Cr}_2\text{O}_3/\text{Fecal Cr}_2\text{O}_3 \times \text{Fecal N/Diet N}) \times 100).
\]

N * 6.25 was assumed to calculate Protein.

At 30, 40 and 60 weeks of age, four hens per treatment were euthanized to evaluate carcass composition. Each carcass was individually weighed, scalded and feathers plucked. Viscera was subsequently removed, including all internal organs, excluding the fat pad which remained with the empty carcass. Weight of both empty carcass and viscera was measured and samples were subsequently frozen separately (-20°C) to prepare for grinding. Feathers were air dried, individually bagged and stored at 4°C until ground. After grinding the de-feathered carcass through a Hobart meat grinder with a 1/8” hole plate, the carcass was then sub-sampled (approx. 500 g) and frozen until analyzed for moisture (Freeze dryer - FTS System (Dura Dry)), protein (Kjeldahl), lipid (Goldfisch ether extraction) and ash (LECO). Carcass composition was determined for moisture, protein, lipid and ash and carbohydrate was calculated as 100 - (moisture + protein + lipid + ash). Viscera was analyzed similar to carcass.

At 40 and 60 weeks of age, 20 hens from 5 treatments (low protein - .71, .81 & .91 ratio; .81 ratio - high and medium protein) were euthanized (4 hens per trt) to evaluate total amounts of RNA, DNA and protein in liver and magnum tissue. Prior to euthanization, hens were monitored to determine time of Oviposition. Hens were euthanized two hours later to ensure that an ovum was present in the magnum. At which time, the liver and magnum were quickly removed, weighed and placed in liquid nitrogen. A modified separation procedure of Jones, 1984 (Ph.D. Dissertation) and Shibko et al., 1967 was used to remove the RNA, DNA and protein from liver and magnum tissue. Following separation, total RNA (Jones, 1984 Ph.D. Dissertation; Lin and Schjeide, 1969), DNA (Jones 1984Ph.D. Dissertation; Burton, 1984), and Protein (Burton, 1984) were calculated.
and protein (BCA Protein Assay Kit) were determined to evaluate the affect of protein and TSAA:Lysine ratio on the working components of protein synthesis.

Experimental design for the aforementioned experiment was a Randomized Complete Block Design (RCBD). Analysis of variance was performed by proc mixed procedures (Proc. Mixed: SAS Institute, 1996). Blocks were considered random, while protein and TSAA:Lysine ratios were fixed. A factorial treatment design was implemented with three levels of protein and three TSAA:Lysine ratios. Utilizing SAS, average values for phase I and II and entire trial were generated and subsequently analyzed separately to determine differences between treatment means.

Analysis of data obtained from liver and magnum tissues (RNA, DNA and protein) was also done utilizing proc mixed procedure of SAS. The RCBD experimental design was used, but the factorial treatment design was not. The main effects of protein and ratio were evaluated utilizing contrast statements. A significant difference between treatments was determined by using the lsmeans statement in SAS.

Results and Discussion

Increased concern over the impact of modern poultry production systems on the environment (nitrogen pollution) has led to the manipulation of our current diet formulations to decrease the level of nitrogen being excreted in the feces. Because chickens only utilize about 40% of dietary protein, it seems logical to decrease the level of protein in the diet (Lopez and Leson, 1995). To do so, synthetic amino acids must be used to meet the requirements of limiting amino acids as a result of the dilution of amino acids as dietary protein is reduced. An ideal protein diet is one way to reduce dietary protein in turn decreasing fecal nitrogen while maintaining egg production parameters.

| Table 2: The Effect of Protein and TSAA:Lysine on Egg production parameters |
|---------------------------|----------------|----------------|----------------|
| Diets (Phase I)           | Feed Consumption | Egg Production | Feed Efficiency |
| (g/48h)                   | (grams/day)     | (%)            | (g feed/g egg mass) |
|                          | 1    | 2 Average | 1 | 2 Average | 1 | 2 Average | 1 | 2 Average |
| Protein Ratio             |      |           |   |         |   |           |   |         |
| 18.9                      | 97.9 | 101.0*    | 99.4 | 85.2 | 83.2 | 84.3 | 1.604 | 1.749 | 1.678 |
| 16.3                      | 96.4 | 99.4*     | 97.8 | 86.7 | 81.9 | 84.4 | 1.579 | 1.737 | 1.659 |
| 14.4                      | 92.9 | 93.2*     | 93  | 85.3 | 77.4 | 81.6 | 1.531 | 1.717 | 1.618 |
| 18.9                      | 97.1 | 100.3*    | 98.6 | 83.8 | 84.2 | 84  | 1.607 | 1.751 | 1.681 |
| 16.3                      | 97.4 | 100.3*    | 98.7 | 83.9 | 82.3 | 83.2 | 1.617 | 1.779 | 1.695 |
| 14.4                      | 95.7 | 96.8*     | 97.1 | 85.5 | 79.9 | 82.9 | 1.589 | 1.736 | 1.664 |
| 18.9                      | 96.4 | 101.0*    | 98.6 | 85.9 | 83.1 | 83  | 1.621 | 1.737 | 1.68 |
| 16.3                      | 94.8 | 99.4*     | 96.9 | 84.7 | 85.5 | 83.1 | 1.577 | 1.694 | 1.637 |
| 14.4                      | 95.3 | 98.2*     | 96.7 | 84.6 | 79.3 | 82.2 | 1.58 | 1.724 | 1.654 |
| **SBM**                   | 1.15 | 1.28      | 1.17 | 1.18 | 1.27 | 0.9 | 0.02 | 0.02 | 0.02 |

Protein

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<th>Feed Efficiency</th>
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<tr>
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Ratio

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<th>Egg Production</th>
<th>Feed Efficiency</th>
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Main Effects

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Contrasts

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</tbody>
</table>

**Means with different superscript in the same column and for the same parameter are statistically different.**
In the present study, decreasing protein decreased feed consumption (Table 2) by as much as two g/hen/day, but the medium and high protein diets performed similarly. Feeding low protein diets has been reported to increase feed consumption in the past, which is in contrast to the present study. Bartov (1979) reported an increase in feed intake when feeding low protein diets to chickens, which was explained as the result of an amino acid “appetite” which results from feeding diets marginal in amino acids. Hurwitz et al. (1998) determined this may only be the case for certain amino acids, such as lysine and TSAA, because diets marginal in arginine did not result in an increase in feed intake in growing chickens (male Cobb). In the present study, the first four limiting amino acids were at or above breeder guide recommendations, which may have satisfied the bird’s appetite for amino acids.

Overall, egg production (Table 2) was reduced by as much as two percentage points during the trial by feeding the lowest protein diet, while hens fed the high and medium protein diets performed similarly. The effect of feeding low protein diets was more dramatic during phase II, resulting in a linear decrease in egg production (83.5 to 78.9%). This may indicate a delayed response to reduced protein intake or amino acids. The reduction in protein or amino acids and/or energy, as a result of reduced feed intake, may also have affected egg production. Harms and Russell, 1993 fed a low protein diet (13%) supplemented with essential amino acids (meeting NRC ’84 recommendations) and reported similar egg production when compared to feeding a diet with 17% protein. This was a short trial (6 weeks) and may not hold true for the entire production trial. The reduction in egg production may also be the result of interactions among amino acids as a result of an excessive intake of essential amino acids used in low protein diets. Also, essential amino acids may be becoming limiting because of their conversion to nonessential amino acids that are in low concentration in low protein diets.

Keshavarz and Jackson, (1992) also reported similar egg production (18 to 66 weeks of age) when using low protein diets in a phase feeding program (14, 13 and 12% protein) with supplemental methionine, lysine, tryptophan and isoleucine compared to positive control (18, 16.5 and 15% protein) diets. The same response was reported when utilizing a phase feeding regimen of 15, 14 and 13% protein diets with adequate methionine and lysine. During the present trial, as a result of decreased feed consumption when feeding the low protein diet, hens were consuming less TSAA than recommended by the Hyline W98 breeder guide which may have contributed to the reduction of egg production during phase II. Results of this trial indicate that reducing protein intake from 18.9 to 16.3 g/hen/day and 16.3 to 14.6 g/hen/day during phase I and II, respectively, with supplemental lys, met, trp and thr maintained egg production.

Feed efficiency (Table 2) (g feed/g egg mass) was improved linearly (1.611 to 1.567) as dietary protein decreased. As a
result of decreased feed consumption and only a slight decrease in egg weight with decreased protein, it was expected efficiency would be improved. It is also possible the hens became more efficient in utilizing the protein available. Hen weight gain (Table 2) decreased linearly as dietary protein decreased, but the low and medium protein diets performed similarly during Phase II. Ratio of TSAA:Lysine did not significantly affect hen weight gain. This was surprising as a result of lysine’s role in tissue protein synthesis. It was expected the ratio of .91 would be optimal for maintaining body condition. As the hens ages the requirement for lysine would decrease and the requirement for TSAA would increase as a result of their roles in maintenance.

Egg weight (Table 3) was reduced when feeding the lowest levels of protein (14.4 and 13.8 g/hen/day for phases I and II, respectively) compared to the medium and high protein diets. Even with supplemental amino acids (met, lys, trp and thr), egg weight was reduced significantly in the very low protein diets. It has been reported that even when supplementing certain amino acids, egg weight is reduced as a result of other marginal amino acids (Penz and Jensen, 1991). A reduction of dietary protein reduces intake of non-essential amino acids such as glutamic acid, cystine and glycine, which are important nitrogen sources. These amino acids may become limiting or essential amino acids may be converted for non-essential purposes, which may result in a limitation of protein (egg) synthesis. Penz and Jensen (1991) reported decreased egg weights when feeding a low protein diet (13%) supplemented with lys, met or trp individually or in combination at a level 20% greater than NRC recommendations compared to control fed hens on a 16% protein diet. Egg mass (Table 3) decreased linearly during the second phase of feeding as protein decreased. With a combination of low egg production and decreased egg weight, egg mass was decreased in hens fed the lowest protein diets (14.4 and 13.8 g/hen/day). The negative effect of low protein diets with or without supplemental amino acids on egg mass has also been reported by other researchers (Keshavarz and Jackson, 1992; Penz and Jensen, 1991; Summers et al., 1991 and Ross and Herrick, 1976). In contrast, Harms and Russell (1993) reported similar responses in egg production parameters including egg mass when low protein diets (15 or 13%) with supplemental lys, met, trp, arg, thr, val and ile were compared to high protein (17.6 or 15.5%) diets.

| Table 4: The Effect of Protein and TSAA:Lysine ratio on Allumina Parameters. |
|-------------------------|-----------------|----------------|-------------------|-----------------|-----------------|-----------------|-----------------|
| Diets (Phase I)         | (g/day)         | Phase          | Albumen%         | Dry Albumen%     | Albumen Carbs%  | Albumen Protein%| Feeding Units    |
| Protein %                |                |                |                 |                 |                 |                 |                 |
| 18.9 0.97                | 61.7 60.3 61   | 8.07 7.18 7.62 | 13.65 11.38 12.47| 8.6 10.08       | 85.1 81.5 83.4 |
| 16.3 0.97                | 61.4 60.7 61   | 7.99 7.17 7.58 | 12.94 11.93 12.43| 8.77 10.24       | 93.2 90.8 87.3 |
| 14.4 0.97                | 61.4 59.7 60.6 | 7.82 6.92 7.37 | 12.74 11.59 12.17| 8.62 9.82        | 86.1 82.7 84.2 |
| 18.9 0.85                | 62.0 60.3 61.3 | 8.28 7.32 7.8  | 13.27 12.11 12.69| 7.88 10.6 4       | 85.4 81 83.3  |
| 16.3 0.85                | 61.8 60.1 61   | 8.14 7.08 7.61 | 13.14 11.76 12.45| 8.49 10.1 7       | 87 86.6 83.9   |
| 14.4 0.85                | 61.5 59.8 60.6 | 8.08 6.91 7.49 | 13.09 11.54 12.31| 8.5 9.78         | 88.5 84.3 86.5 |
| 18.9 0.82                | 61.6 60.4 61   | 8.09 7.27 7.68 | 13.11 12.02 12.56| 8.97 10.39       | 87.7 83.5 85.7 |
| 16.3 0.82                | 61.9 60.4 61.1 | 8.33 7.25 7.79 | 13.43 11.99 12.71| 8.76 10.28       | 87.7 82.5 85.2 |
| 14.4 0.82                | 61.8 59.8 60.8 | 7.97 6.92 7.44 | 12.87 11.56 12.21| 8.48 9.42        | 89.7 83.5 86.1 |
| SEM                      | 0.247 0.266 0.223| 0.249 0.073 0.142| 0.374 0.114 0.212| 0.149 0.103 0.392| 3.392 1.03 1.932|
| Protein                  |                |                |                 |                 |                 |                 |                 |
| 18.9                     | 61.9 60.3 61.1 | 8.15 7.26 7.70 | 13.14 12.04 12.57| 7.80 10.37       | 86.1 82.8 84.1 |
| 16.3                     | 61.7 60.2 60.9 | 8.15 7.16 7.66 | 13.17 11.89 12.53| 8.67 10.20       | 89.3 83.1 85.5 |
| 14.4                     | 61.6 59.8 60.8 | 7.95 6.92 7.44 | 12.9 11.5 12.28  | 8.54 9.6 87.1    |
| Ratio                    | 0.97           | 61.5 60.8 61.8 | 7.96 7.09 7.53 | 12.91 11.8 12.36| 8.66 10.04 | 88.1 81.7 85.1 |
| 0.85                     | 61.9 60.6 61   | 8.17 7.1 7.63 | 13.17 11.8 12.49| 8.59 10.17       | 87 83.9 84.6 |
| Main Effects             |                |                |                 |                 |                 |                 |                 |
| Protein                  | NS P<0.03 P<0.06| NS P<0.001 P<0.01| NS P<0.001 NS | P<0.03 P<0.05 P<0.04| NS P<0.02 NS |
| Ratio                    | NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS |
| Protein*Ratio            | NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS |
| Contrasts                |                |                |                 |                 |                 |                 |                 |
| Protein linear           | NS P<0.01 NS P<0.02| NS P<0.001 NS | P<0.001 P<0.001| NS P<0.05 P<0.02| NS P<0.05 NS |
| Ratio Linear             | NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS |

*Means with different superscript in the same column and for the same parameter are statistically different
Egg components (Table 4 & 5) were affected by protein level but not by TSAA:Lysine ratio during the trial. Wet albumen, dry albumen and albumen solids percentages all linearly decreased when protein intake decreased and are probably one of the factors responsible for the reduced egg size. The decrease in albumen percent may have been a result of a decrease in albumen synthesis. The lack of response during phase I may be an indication that the hen is able to cope with the low protein intake and still maintain both growth, development, and egg production. At this time the hen was receiving a higher level of protein when compared to the second phase of production and may have been close to the hens requirement of protein and/or amino acids. Growth of the hens was reduced during this time by feeding low protein diets, but the amino acids present may have been at a concentration high enough to maintain

| Table 5: The Effect of Lysine and TSAA level on Yolk Parameters. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Yolk            | Dry Yolk        | Yolk Solids     | Yolk Protein    |
|                  | (g/100g)        | (g/100g)        | (g/100g)        | (g/100g)        |
| Protein Ratio    | Phase           | 1   | 2   | Avg | 1   | 2   | Avg | 1   | 2   | Avg | 1   | 2   | Avg |
| 18.9  | 0.97 | 25  | 27  | 26  | 13.98b | 14.68 | 14.33 | 55.8 | 54.5 | 55.1 | 15  | 16.71 |
| 16.3  | 0.97 | 25.5 | 27.3 | 26.4 | 14.34a | 14.96 | 14.65 | 56.1 | 54.8 | 55.4 | 14.82 | 16.26 |
| 14.4  | 0.97 | 25.4 | 27.4 | 26.4 | 14.21b | 14.86 | 14.53 | 55.9 | 54.3 | 55.1 | 14.72 | 16.81 |
| 18.9  | 0.85 | 25  | 27.1 | 26  | 13.81b | 14.77 | 14.29 | 55.3 | 54.5 | 54.9 | 15.22 | 16.82 |
| 16.3  | 0.85 | 25.4 | 27.3 | 26.4 | 14.17b | 14.88 | 14.52 | 55.3 | 54.4 | 54.9 | 15.1 | 16.56 |
| 14.4  | 0.85 | 25.4 | 27.4 | 26.4 | 14.23b | 14.98 | 14.63 | 56.1 | 54.7 | 55.4 | 14.71 | 16.43 |
| 18.9  | 0.82 | 25.5 | 27  | 26.2 | 14.25a | 14.62 | 14.43 | 56  | 54.3 | 55.1 | 15.33 | 16.62 |
| 16.3  | 0.82 | 25.2 | 27.2 | 26.1 | 14.11b | 14.6 | 14.36 | 55.9 | 54.2 | 55.2 | 15.09 | 16.63 |
| 14.4  | 0.82 | 25.2 | 27.3 | 26.2 | 14.01b | 14.87 | 14.44 | 55.6 | 54.5 | 55. | 14.92 | 16.57 |
| SEM   | 0.21 | 0.203 | 0.177 | 0.12 | 0.142 | 0.105 | 0.161 | 0.278 | 0.172 | 0.139 | 0.174 |

Protein

| 18.9  | 25.1 | 27.0b | 26.1 | 14.01 | 14.69b | 14.35a | 55.7 | 54.4 | 55 | 15.18b | 16.72 |
| 16.3  | 25.4 | 27.2b | 26.3 | 14.21 | 14.81b | 14.51a | 55.8 | 54.5 | 55.1 | 15.09b | 16.48 |
| 14.4  | 25.3 | 27.4b | 26.3 | 14.17 | 14.90b | 14.51b | 55.9 | 54.5 | 55.2 | 14.79b | 16.6 |

Ratio

| 0.97 | 25.3 | 27.2 | 26.3 | 14.18 | 14.94 | 14.51 | 56  | 54.5 | 55.2 | 14.25b | 16.6 |
| 0.85 | 25.3 | 27.3 | 26.3 | 14.09 | 14.88 | 14.48 | 55.6 | 54.6 | 55.1 | 15.01b | 16.6 |
| 0.82 | 25.3 | 27.1 | 26.2 | 14.12 | 14.69 | 14.41 | 55.8 | 54.3 | 55.1 | 15.11b | 16.61 |

Main Effects

|   | Protein | NS | NS | NS | NS | NS | P<.06 | NS | NS | NS | P=.009 | NS |
|   | Ratio   | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
|   | Protein*Ratio | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

Contrasts

|   | Protein Linear | NS | P<.03 | P<.08 | NS | NS | NS | NS | NS | NS | NS | NS |
|   | Ratio Linear  | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

*Means with different superscript in the same column and for the same parameter are statistically different.
Shell quality (Table 6) was also affected by protein and ratio, which could potentially have consequences for egg laying operations. Reducing dietary protein increased wet shell percentage only during phase II. From this information, hens consuming the low protein diets may be producing an egg that has more adhering albumen on the shell than the other protein treatments or a smaller egg. Specific gravity was linearly decreased by low protein diets, which indicates that shell quality was being reduced. Feeding low protein diets did not significantly reduce shell breaking strength, but there was a trend of reduced shell strength with reduced protein intake. Although the data doesn’t indicate a serious problem related to egg shell quality, there is some type of relationship between low protein diets and shell quality that needs investigation. Increasing the TSAA:Lysine ratio increased shell quality indicating the sulfur amino acid requirement for synthesizing shell protein matrix, which is comprised of 70% protein (Simkiss and Taylor, 1957), needs to be considered to optimize shell quality. Also, increasing the sulfate groups present in the shell matrix significantly increases the calcium binding ability. This in turn may increase both shell percent and specific gravity and overall shell quality. Other researchers have also indicated that decreasing dietary protein will decrease shell quality (Leeson and Caston, 1997; Keshavarz and Nakajima, 1995; Keshavarz and Jackson, 1992).  

### Table 6: The Effect of Lysine and TSAA level on Egg Shell Quality

<table>
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<th>Diets (Phase I)</th>
<th>Wet Shell (%)</th>
<th>Dry Shell (%)</th>
<th>Specific Gravity (%)</th>
<th>Shell Breaking Strength (kg)</th>
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</thead>
<tbody>
<tr>
<td><strong>Protein Ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10%</td>
<td>12.89</td>
<td>12.23</td>
<td>12.56</td>
<td>9.65</td>
</tr>
<tr>
<td>14%</td>
<td>12.91</td>
<td>12.48</td>
<td>12.69</td>
<td>9.57</td>
</tr>
<tr>
<td>16%</td>
<td>12.48</td>
<td>12.05</td>
<td>12.27</td>
<td>9.35</td>
</tr>
<tr>
<td>18%</td>
<td>12.69</td>
<td>12.27</td>
<td>12.48</td>
<td>9.46</td>
</tr>
<tr>
<td>14%</td>
<td>12.68</td>
<td>12.35</td>
<td>12.51</td>
<td>9.43</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>0.118</td>
<td>0.126</td>
<td>0.102</td>
<td>0.078</td>
</tr>
</tbody>
</table>

**Main Effects**

- **Protein**: NS for <0.04, NS for <0.06, NS for <0.01, P<0.01, NS for <0.04, P<0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01
- **Ratio**: NS for <0.04, NS for <0.06, NS for <0.01, P<0.01, NS for <0.04, P<0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01

**Contrasts**

- **Protein**: NS for <0.04, NS for <0.06, NS for <0.01, P<0.01, NS for <0.04, P<0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01
- **Ratio**: NS for <0.04, NS for <0.06, NS for <0.01, P<0.01, NS for <0.04, P<0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01, NS for <0.01

Haugh unit (Table 4) was not affected during phase I or overall, but was increased by decreasing dietary protein during phase II. Deaton and Quisenberry (1965), Aitken et al. (1973) and Leeson and Caston (1997) all reported similar responses as in the present study for haugh units when feeding low protein diets. In contrast, Hamilton (1978) reported no observable change in haugh units when feeding low protein diets to 4 different strains of laying hens.

Protein, RNA and DNA (Table 7) values were in similar range when compared to other researchers. Akinwande et al. (1973) had similar values for total RNA, DNA and protein of 7.0, 7.6 and 237.5 mg/g fresh tissue, respectively. In the present study, protein levels tended to decline in the magnum
during phase two as a result of reduced levels of dietary protein in the diet and lower TSAA/lysine ratio (P < 0.08). As protein levels decreased, there was an increase in total RNA content in the magnum. Since higher protein levels caused increase in protein content in the egg, it could be assumed that RNA levels would also increase to accommodate increases in protein synthesis. However, this was not the case. The decreases in tissue protein may have increased the concentration of RNA and possibly DNA in the tissue, but although not significant, tissue protein decreased as dietary protein intake decreased indicating that the tissue was depleting itself in order to maintain egg weight and albumen synthesis. RNA level only increased when there was a decrease in tissue protein content indicating that change in dilution of other materials within the tissue is increasing the amount of RNA per gram of tissue. Johns and Bergen, (1976) reported reduced total tissue RNA, DNA and protein in the liver when feeding a low protein (7%) diet to lambs (60 to 120 days of age) compared to control (15%). When looking at RNA and DNA in terms of mg/g fresh tissue, RNA and DNA values increased in the liver of lambs consuming the low protein diet whereas protein remained constant.

A similar response was observed when feeding a diet containing .82 TSAA/lysine ratio during phase I. Decreasing the ratio from .97 to .82 resulted in an increase in total RNA while the protein content decreased in the magnum. The effect of decreased ratio and protein intake on magnum tissue was a depletion of the tissue to maintain egg yield. It was also stated by Akinwande et al., (1973) that depending on the physiological state, age and strain of hens, variation might occur. Yu, J. Y-L., et al., (1972) reported RNA and DNA values of 5.14 and 1.67 mg/g-wet tissue in 56-week-old Dekalb hens.

There is very little data on dietary effect on carcass composition in egg-type poultry. Early in production (23 to 30 weeks of age) the hens increase in lean body mass while producing eggs. Increasing protein intake increased the percentage of protein present in the carcass. As the hen ages, amino acid required for growth decreases so there is excess energy to be converted into fat and stored as indicated by the significant increase in carcass lipid percentage during the last collection. There was also an increase in ash percentage with decreased protein intake during the last collection period. The results of feeding low protein diets to laying hens are contradictory to research in broiler chickens. Ajang et al. (1993) reported an inverse relationship between dietary protein content and carcass (carcass without blood and feathers) lipid content, however, carcass protein was proportional to dietary protein. Whitehead et al. (1990) reported increases in abdominal fat pad and total body lipid.

### Table 7: The effect of Protein and TSAA/Lysine ratio on chemical changes in the magnum.

<table>
<thead>
<tr>
<th>Protein</th>
<th>RNA</th>
<th>DNA</th>
<th>Protein/RNA</th>
<th>RNA/DNA</th>
<th>Protein/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>259</td>
<td>253</td>
<td>7.38</td>
<td>6.87</td>
<td>0.262</td>
</tr>
<tr>
<td>Med</td>
<td>286</td>
<td>212</td>
<td>7.13</td>
<td>7.98</td>
<td>0.365</td>
</tr>
<tr>
<td>Low</td>
<td>261</td>
<td>212</td>
<td>7.42</td>
<td>8.81</td>
<td>0.268</td>
</tr>
<tr>
<td>P&lt;.08</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;.01</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Table 8: Protein Retention

<table>
<thead>
<tr>
<th>Diets (Phase I)</th>
<th>Protein Degradability</th>
<th>Protein (%)</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g/kg)</td>
<td>Protein</td>
<td>Ratio</td>
<td>Period</td>
<td></td>
</tr>
<tr>
<td>18.9</td>
<td>0.97</td>
<td>38.92</td>
<td>45.15</td>
<td></td>
</tr>
<tr>
<td>16.3</td>
<td>0.97</td>
<td>44.41</td>
<td>47.85</td>
<td></td>
</tr>
<tr>
<td>14.4</td>
<td>0.97</td>
<td>41.79</td>
<td>41.14</td>
<td></td>
</tr>
<tr>
<td>13.9</td>
<td>0.83</td>
<td>33.83</td>
<td>46.07</td>
<td></td>
</tr>
<tr>
<td>16.3</td>
<td>0.83</td>
<td>45.16</td>
<td>47.37</td>
<td></td>
</tr>
<tr>
<td>13.4</td>
<td>0.83</td>
<td>29.34</td>
<td>46.31</td>
<td></td>
</tr>
<tr>
<td>13.9</td>
<td>0.82</td>
<td>34.29</td>
<td>46.21</td>
<td></td>
</tr>
<tr>
<td>16.3</td>
<td>0.82</td>
<td>26.59</td>
<td>47.92</td>
<td></td>
</tr>
<tr>
<td>14.4</td>
<td>0.82</td>
<td>46.07</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>2.24</td>
<td>1.698</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 8: Protein Retention

<table>
<thead>
<tr>
<th>Protein</th>
<th>18.9</th>
<th>35.67</th>
<th>45.81</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.3</td>
<td>38.72</td>
<td>47.71</td>
<td></td>
</tr>
<tr>
<td>14.4</td>
<td>39.97</td>
<td>44.97</td>
<td></td>
</tr>
</tbody>
</table>

**Ratio**

| 0.97 | 41.72 | 44.41 |
| 0.85 | 36.11 | 46.58 |
| 0.82 | 35.62 | 46.71 |

**Main Effects**

<table>
<thead>
<tr>
<th>Protein * Ratio</th>
<th>352</th>
<th>P&lt;.06</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Protein * Ratio</th>
<th>P&lt;.002</th>
<th>NS</th>
</tr>
</thead>
</table>

**Contrasts**

<table>
<thead>
<tr>
<th>Protein Linear</th>
<th>NS</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio Linear</td>
<td>P&lt;.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Means with different superscript are statistically different.
while total body protein decreased with increased dietary protein. Typically, there is an increase in feed intake when feeding low protein diets, which contributes to increased energy intake. This increased energy intake increases lipid deposition in the hen, but neither was observed in the present trial.

Protein retention (Table 8) was generally improved when feeding low protein diets and by increasing the TSAA:Lysine ratio from .71 to .91. During phase I, there was a 16% improvement in protein retention when increasing the ratio in the diet from .82 to .97. The increased improvement in retention indicates that the ratio of TSAA/lysine is closer to what the hens needed to produce optimally. Although not significant, decreasing protein intake from 18.9 to 14.4 g/hen/day improved protein retention by 9%. A nine percent improvement in protein retention in combination with the lower protein ration resulted in 32% less protein excreted. This decrease could have a significant environmental impact. Summers (1993) reported a 40% reduction in nitrogen excretion when feeding a diet containing 11% protein compared to 17%, which is comparable to what was observed in the present study. During the second phase of feeding, protein retention was not significantly affected by dietary protein intake. Hens consuming 13.8 g protein/day gained significantly less weight (22.3 vs. 90.7 g/hen - consuming 16.3 g protein/day), which may indicate that these hens used less protein per day. It is possible these hens were in a negative nitrogen balance state, which reduced any improvement in retention. An improvement of 4% in retention was noted when dietary protein was decreased from 16.3 to 14.6 g/hen/day which amounts to a 14.6% decrease in protein excretion.

The utilization of low protein diets for laying hens has great potential to reduce dietary costs and protein excretion. Reducing protein intake from 18.9 to 16.3 g/hen/day (20 to 43 wks of age) and 16.3 to 14.4 g/hen/day (43 to 63 wks of age) will decrease protein excretion without changing the production and egg yield of the hens. Further development of an ideal amino acid pattern diet will be needed to reduce the protein intake further. The implementation of such a diet (ideal protein diet) will require a cost reduction of currently available synthetic amino acids and changes in formulation of diets.

References


CORN QUALITY:
WHAT ARE WE INCLUDING IN OUR POULTRY DIETS?

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Summary
Corn quality is variable due to many sources that can be quantified. In particular, the dietary energy of corn can be predicted based on these factors, and the improvement in dietary energy when appropriate enzymes are added to the feed can also be predicted. Ninety-three commercial corn samples were obtained globally, ground, and one mash broiler diet was formulated with 55% corn for each corn sample. Each diet was separated into two portions and one was supplemented with a commercial enzyme blend of xylanase, amylase and protease (Avizyme™1502, Danisco Animal Nutrition). Diets were fed to one pen of 25 male broilers from days 1-28 and body weights and feed intake were measured. Digesta samples were collected at the terminal ileum from six birds per pen and digesta from each bird were analysed for energy content. Corn samples were analysed for starch, protein, oil and gross energy content. Ideal digestible energy (IDE) was computed using the relative proportion of titanium dioxide marker in the feed and digesta. The performance of broilers fed different sources of corn without enzymes was variable. Feed conversion ratio (FCR) ranged between 1.43 and 2.67. Mean = 1.81, s.d. = 0.30 (CV = 16%). One outlier with FCR = 3.36 was removed from the analysis, this being a corn sample from France with 25% moisture. The weight gain ranged between 680 and 1301 g (outlier of 375 g removed). Average = 909, s.d. = 114 (CV = 13%). The addition of enzymes improved performance (P < 0.01 for FCR) and significantly reduced variance of FCR by 30% (P < 0.01).

The amount of energy is relatively constant in corn oil, corn starch and corn protein: 9300, 4150, and 5490 kcal/kg, respectively (Noblet, 2000); however, the amount and the digestibility of these energy sources are variable. The average IDE of corn was 3246 kcal/kg DM with an s.d. of 487 kcal/kg DM. Using least-square estimators, digestibility coefficients for starch, protein, oil and other fractions (fiber and ash) were found to be 86.3%, 81.6%, 90.2% and 11.4%, respectively. With the addition of enzymes, the IDE was raised by an average of 5% to 3405 kcal/kg DM. Digestibility coefficients were found to be raised to 91.3%, 82.4%, 90.7%, and 13% for starch (+5.0%), protein (+0.8%), oil (+0.5%) and other (+1.6%), respectively. Digestibility of starch was significantly related to the rate of starch digestion measured in-vitro (P < 0.01). The addition of the enzymes increased the rate of starch digestion proportionally to the improvement in IDE. It is proposed that xylanase and protease act to increase accessibility to the starch, and amylase increases the rate of starch digestion.

Practical applications of these results include a more precise knowledge of corn energy for feed formulation, leading to reduced feed costs and reduced variability of dietary energy. The optimal formulation of this enzyme blend may be determined. These results form a basis to monitor corn quality by harvest year and geographic region, giving more information on commodity value. In-vitro measures of starch quality at critical points in the feed manufacturing process may be used to make recommendation on temperature, moisture and time of processing related to the effects on corn quality. These measurements may also be used as a tool to monitor the efficiency of feed manufacturing equipment and to predict when maintenance is required.
Introduction

Corn quality is affected by genetics, growing and harvesting conditions, drying process, and feed manufacturing. Variability in composition and quality affect the metabolizable energy content, in particular, and this is why there is such an impact on poultry performance. Energy comes from the protein, oil, and carbohydrate sources in the corn; however, not all of this energy is metabolizable. Corn (see Figure 1) is comprised of a pericarp (P), which is the outer covering, or hull, that protects the kernel from the environment, insects and pathogens, although the tip cap (T) may provide access into the kernel. The kernel is comprised of the endosperm (E) and the germ (G). The endosperm is the source of energy for the seed, and this energy comes mainly from starch, and some protein. The germ is the living part of the kernel, and contains enzymes, vitamins, minerals and the genetic information for the kernel to grow into a corn plant. Depending on the genetic variety, approximately 25% of the germ is corn oil, high in linoleic acid. On average, the dry matter composition of corn is 68% starch, 8% protein, 4% oil, 2.5% cellulose, and trace amounts of vitamins and minerals.

Figure 1. Corn kernel.

Starch is the major component of corn, and other grains, and is thus the largest source of energy in the poultry corn-soy diet, typical in the United States. Regardless of its source in the diet, starch is made up of glucose molecules amylose and amyllopectin. The amyllopectin molecule is nearly identical to amylose, except that amyllopectin has an alpha 1-6 glucose linkage that causes it to form branches. The branching structure (A, B) of a starch granule (C) formed by the alpha 1-6 glucose linkage of amyllopectin (D) is illustrated in Figure 2.

A global corn quality survey (D’Alfonso and McCracken, 2002) provides data on the variability of corn composition. As can be seen in the figures below, as starch level increases, protein and oil content tend to decrease, especially oil. Protein and oil content tend to go up and down together, but this relationship is not as strong. What this implies is that the physiology of the corn limits its range of nutrient composition. If something goes up in concentration, something must go down. Starch, protein and oil are the sources of dietary energy in corn.

The amount of energy is relatively constant in corn oil, corn starch and corn protein: 9300, 4150, and 5490 kcal/kg, respectively (Noblet, 2000). These values could change if the corn is overheated and the starch is retrograded, for example. Figure 2. Starch structure (A-D) and amylose and amyllopectin molecules.

Amylose

Amylopectin
Figure 3 Relationships among starch, protein and oil content of corn.

\[ y = -0.1428x + 17.939 \quad R^2 = 0.1228 \]
\[ y = -0.1715x + 16.299 \quad R^2 = 0.3232 \]

The rate of starch digestion (RSD) was measured in-vitro in a 2 stage process in which the sample was incubated with a solution of pepsin with HCl, followed by pancreatin digestion. This process was designed to simulate digestion in the chicken (D’Alfonso and McCracken, 2002). Sub-samples were taken at 7, 15, 22, 30, 45, 60 and 120 minutes (see Figure 4). Digestibility of starch measured in-vivo was compared to the RSD.

Figure 4 Rate of starch digestion determined in-vitro among 98 corn samples.

The purpose of this research is to quantify the sources of variance in corn quality, and to predict the dietary energy of corn based on these factors. In addition, it is also an objective to predict the improvement in dietary energy when appropriate enzymes are added to the feed. In order to do this, it is first necessary to consider the proportion of corn in the diet. This will determine the impact of corn quality on the performance of birds fed a corn-based diet. It is also necessary to choose a method of measuring dietary energy that is closely related to poultry performance. Ileal digestible energy is the preferred measurement of energy availability to the bird because other systems over-estimate the amount of energy available by counting the energy consumed by the microbes in the lower intestine. Furthermore, microbial populations vary from animal to animal and among production environments, decreasing the accuracy and increasing the variance of metabolizable energy estimation. Finally, it is necessary to measure the IDE of each source: protein, oil and carbohydrates (starch and fiber) and the effect of enzyme supplementation on the IDE.
Methodology

Ninety-three commercial corn samples were obtained globally, ground, and one mash broiler diet was formulated with 55% corn for each corn batch. Diet formulation is shown in Table 1. Each diet was separated into two portions and one was supplemented with a commercial enzyme blend of xylanase, amylase and protease (Avizyme™ 1502, Danisco Animal Nutrition). Diets were fed to 25 male broilers per pen from days 1-28 and body weights and feed intake were measured. Digesta samples were collected at the terminal ileum from six birds per pen and digesta from each bird were analysed for energy content. Corn samples were analysed for starch, protein, oil and gross energy content. The IDE was computed using the relative proportion of titanium dioxide marker in the feed and digesta.

Table 1 Dietary composition and calculated nutrient levels.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>(%)</th>
<th>Calculated Nutrient Levels</th>
<th>Amino Acids</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>corn</td>
<td>54.84</td>
<td>Protein %</td>
<td>23.0</td>
<td>Met %</td>
</tr>
<tr>
<td>soybean meal (49%)</td>
<td>36.34</td>
<td>ME kcal/kg</td>
<td>3090</td>
<td>Cys %</td>
</tr>
<tr>
<td>fishmeal</td>
<td>1.07</td>
<td>Calcium %</td>
<td>1.04</td>
<td>Me+Cys %</td>
</tr>
<tr>
<td>lysine</td>
<td>0.02</td>
<td>Phos %</td>
<td>0.776</td>
<td>Lys %</td>
</tr>
<tr>
<td>dl methionine</td>
<td>0.24</td>
<td>Avail Phos %</td>
<td>0.491</td>
<td>His %</td>
</tr>
<tr>
<td>soy oil</td>
<td>3.60</td>
<td>Fat %</td>
<td>6.2</td>
<td>Tryp %</td>
</tr>
<tr>
<td>dicalcium phosphate</td>
<td>1.82</td>
<td>Fibre %</td>
<td>2.6</td>
<td>Thr %</td>
</tr>
<tr>
<td>limestone</td>
<td>1.22</td>
<td>Na %</td>
<td>0.16</td>
<td>Arg %</td>
</tr>
<tr>
<td>salt</td>
<td>0.32</td>
<td>CI %</td>
<td>0.25</td>
<td>Iso %</td>
</tr>
<tr>
<td>sodium bicarbonate</td>
<td>0.10</td>
<td>K %</td>
<td>0.97</td>
<td>Leu %</td>
</tr>
<tr>
<td>vitamin/mineral mix</td>
<td>0.27</td>
<td>Linoleic acid %</td>
<td>2.53</td>
<td>Phe %</td>
</tr>
<tr>
<td>choline chloride</td>
<td>0.04</td>
<td>Magnesium %</td>
<td>0.21</td>
<td>Tyr %</td>
</tr>
<tr>
<td>titanium dioxide</td>
<td>0.30</td>
<td>Sulphur %</td>
<td>0.19</td>
<td>Val %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Choline ppm</td>
<td>1370</td>
<td>Gly %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ser %</td>
</tr>
</tbody>
</table>

Gross energy of the corn was measured and partitioned into that coming from starch, protein, oil, and other sources. Subtracting the amount of moisture, starch, protein and oil from 100% determined the amount of other sources. Since the amount of gross energy coming from each source was a constant, and the gross energy of the corn was measured, the IDE of each source can be described as a percentage digestibility multiplied by the gross energy values of 9300, 4150, and 5490 for oil, starch, and protein, respectively. Other sources of gross energy were determined by subtraction. This is detailed in the results section of this paper. Finally, the same statistical analysis was carried out on the data where the feed was supplemented with the enzyme blend. Improvements in IDE were partitioned into improvements in the digestibility coefficients of starch, protein, oil and other sources.

Results

The performance of broilers fed different samples of corn without enzymes was variable. Feed conversion ratio (FCR) ranged between 1.43 and 2.67. Mean = 1.81, s.d. = 0.30 (CV = 16%). One outlier with FCR = 3.36 was removed from the analysis, this being a corn sample from France with 25% moisture. The weight gain ranged between 680 and 1301 g (outlier of 375 g removed). Average = 909, s.d. = 114 (CV = 13%). As can be seen in Table 2, the addition of enzymes improved performance (P < 0.01 for FCR) and significantly reduced variance of FCR by 30% (P < 0.01). Variability in starch, protein, oil and gross energy composition among the samples are provided in Table 3. Values are on a dry matter basis.
Table 2 Performance of 28-day broilers fed diets containing 55% of one of 98 different corn batches, and with enzymes (Enz = Avizyme™ 1502, Danisco Animal Nutrition, xylanase, amylase, and protease blend).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain (g)</td>
<td>909</td>
<td>114</td>
<td>13%</td>
</tr>
<tr>
<td>Gain w/ Enz.</td>
<td>915</td>
<td>114</td>
<td>12%</td>
</tr>
<tr>
<td>FCR</td>
<td>1.81</td>
<td>0.30</td>
<td>16%</td>
</tr>
<tr>
<td>FCR w/ Enz</td>
<td>1.73</td>
<td>0.20</td>
<td>12%</td>
</tr>
</tbody>
</table>

Table 3. Dry matter composition of corn, average and variance of 98 samples.

<table>
<thead>
<tr>
<th>Dry Matter</th>
<th>Starch (%)</th>
<th>Protein (%)</th>
<th>Oil (%)</th>
<th>Gross Energy (kcal/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>89.1</td>
<td>68.8</td>
<td>8.1</td>
<td>4.4</td>
</tr>
<tr>
<td>sd</td>
<td>0.87</td>
<td>1.8</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Min</td>
<td>87.4</td>
<td>65.8</td>
<td>7.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Max</td>
<td>90.7</td>
<td>72.0</td>
<td>9.7</td>
<td>5.9</td>
</tr>
<tr>
<td>CV</td>
<td>1%</td>
<td>3%</td>
<td>9%</td>
<td>13%</td>
</tr>
</tbody>
</table>

The ileal digestible energy was measured and partitioned into starch, protein, oil and other sources. The first step of this process was to measure the dry matter percentage of each of these fractions, assign the appropriate energy values of 9300, 4150, 5490, and 4340 kcal/kg for oil, starch, protein and the other sources, and then to compare them with the measured gross energy (GE) of the corn. An example is provided in Table 4. The measured GE for this sample was 4534 kcal/kg. The root mean square error of this method was +/- 25 kcal/kg (+/- 0.5% of the mean) and the model is highly significant (P < 0.0001).

Table 4 Sources of gross energy in an example corn batch.

<table>
<thead>
<tr>
<th>Dry Matter Basis</th>
<th>Percent</th>
<th>GE kcal/kg</th>
<th>kcal / kg of Corn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>71.6</td>
<td>4150</td>
<td>2970</td>
</tr>
<tr>
<td>Protein</td>
<td>7.7</td>
<td>5490</td>
<td>421</td>
</tr>
<tr>
<td>Oil</td>
<td>4.2</td>
<td>9300</td>
<td>389</td>
</tr>
<tr>
<td>Other</td>
<td>16.6</td>
<td>4340</td>
<td>720</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>4500</td>
<td></td>
</tr>
</tbody>
</table>

As can be seen in Figure 5, starch contributes over two-thirds of the gross energy in corn. Protein and oil each contribute approximately the same percentages, although this would be different for high oil corn. Fiber and other sources contribute approximately 20% of the gross energy, but these sources are not very digestible, as will be shown in the IDE values.
The average ileal digestible energy of corn was 3246 kcal/kg DM with a standard deviation of 487 kcal/kg DM. Using least-square estimators, digestibility coefficients for starch, protein, oil and other fractions were found to be 86.3%, 81.6%, 90.2% and 11.4%, respectively. With the addition of enzymes, the IDE was raised by an average of 5% to 3405 kcal/kg DM. Digestibility coefficients were found to be raised to 91.3%, 82.4%, 90.7%, and 13% for starch (+5.0%), protein (+0.8%), oil (+0.5%) and other (+1.6%), respectively. Figure 6 illustrates the percentage of IDE coming from corn components and other ingredients.

There was a marginal residual energy enhancement observed for the entire diet of approximately 4 kcal/kg on average. This is the energy release in ingredients other than corn and is specific to the diet used in this experiment. It is assumed that the IDE of soybean meal may have been improved some, perhaps due to the protease; however, soybean meal composition was not varied in the diets, so IDE of soybean meal could not be estimated.

Digestibility of starch was significantly related to the RSD measured in-vitro (P < 0.01). While RSD ranged from 37% to 53%, with an average of 42%, digestibility of starch ranged from 84% to 90% with an average of 86%. The addition of the enzymes increased the rate of starch digestion proportionally to the improvement in IDE. It is proposed that xylanase and protease act to increase accessibility to the starch, and amylase increases the rate of starch digestion.
Table 5 illustrates an example calculation for corn with an estimated GE of 4500 kcal/kg DM. The IDE of this corn sample was estimated to be 3340 kcal/kg DM, and improved to 3505 kcal/kg DM with the addition of enzymes. In a diet containing 55% of this corn, the increase in IDE due to corn alone is 91 kcal/kg DM. Least square estimators were used to determine average IDE of the other ingredients in the diet.

**Table 5.** Computation of gross energy (GE) and ileal digestible energy (IDE) of corn, a diet containing corn, and the uplift in IDE for a diet containing specific feed enzymes (ENZ).

<table>
<thead>
<tr>
<th>GE of Corn</th>
<th>% in Corn</th>
<th>kcal/kg of Corn</th>
<th>% Digestible</th>
<th>IDE</th>
<th>ENZ Uplift</th>
<th>Digestible ENZ</th>
<th>UPLIFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>4150</td>
<td>71.6%</td>
<td>2970</td>
<td>86.3%</td>
<td>2583</td>
<td>5.0%</td>
<td>91.3%</td>
</tr>
<tr>
<td>Protein</td>
<td>5490</td>
<td>7.7%</td>
<td>421</td>
<td>81.6%</td>
<td>344</td>
<td>0.8%</td>
<td>82.4%</td>
</tr>
<tr>
<td>Oil</td>
<td>9300</td>
<td>4.2%</td>
<td>389</td>
<td>90.2%</td>
<td>351</td>
<td>0.5%</td>
<td>90.7%</td>
</tr>
<tr>
<td>Other</td>
<td>4340</td>
<td>16.6%</td>
<td>720</td>
<td>11.4%</td>
<td>82</td>
<td>1.6%</td>
<td>94</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100.0%</td>
<td>4500</td>
<td>74.2%</td>
<td>3340</td>
<td>77.9%</td>
<td>3505</td>
<td>5.0%</td>
</tr>
</tbody>
</table>

- **w/ ENZ:**

<table>
<thead>
<tr>
<th>GE of Corn</th>
<th>% in Corn</th>
<th>kcal/kg of Corn</th>
<th>% Digestible</th>
<th>IDE</th>
<th>ENZ Uplift</th>
<th>Digestible ENZ</th>
<th>UPLIFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>55%</td>
<td>4500</td>
<td>2475</td>
<td>74%</td>
<td>3340</td>
<td>1387</td>
<td>5.0%</td>
</tr>
<tr>
<td>Other</td>
<td>45%</td>
<td>4920</td>
<td>2214</td>
<td>66%</td>
<td>3235</td>
<td>1456</td>
<td>0.3%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>4689</td>
<td>3393</td>
<td>3387</td>
<td></td>
<td></td>
<td></td>
<td>95</td>
</tr>
</tbody>
</table>

**Conclusions and Directions for Future Research**

IDE and its improvement due to enzyme supplementation is predictable. A spreadsheet was developed to estimate:

1. The IDE in corn based on starch, protein and oil composition;
2. The IDE of a diet containing that corn and based on the inclusion level; and,
3. The improvement in IDE due to supplementation with this particular blend of enzymes.

Practical applications of these results include a more precise knowledge of corn energy for feed formulation, leading to reduced feed costs and reduced variability of dietary energy. The optimal formulation of this enzyme blend (xylanase, amylase, and protease) may be determined. These results form a basis to monitor corn quality by harvest year and geographic region, giving more information on commodity value. In-vitro measures of starch quality at critical points in the feed manufacturing process may be used to make recommendation on temperature, moisture and time of processing related to the effects on corn quality. These measurements may also be used as a tool to monitor the efficiency of feed manufacturing equipment and to predict when maintenance may be required.

**References**


BROILER BREEDER NUTRITION:
WHAT’S NEW AND WHAT CHALLENGES DO WE FACE

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Feeding any breeding stock is always a balance between meeting their needs for growth, maintenance and reproductive output. Growth and reproductive output are usually negatively correlated, and so controlling growth is often a major objective in feeding for reproduction. For many animals, the aim is to stimulate appetite of high producing females since in the case of gilts and heifers for example, a negative energy balance is often a real possibility. In broiler breeders, we have something of a unique situation in that a positive energy balance is never difficult to achieve, and in fact, oversupply of energy and other nutrients is our major worry. As the geneticist strives for ever greater growth potential in broilers, this trait must be passed down through the parental lines and so heavier weight-for-age on a yearly basis is an accepted phenomenon for both grandparent (GP) and parent (PS) lines. The major breeding companies do not yet see broiler growth rate per se as an insurmountable management problem, and so we can expect to see continued increase in growth potential in most parental lines for at least the next 5 years. To a large extent, increased growth rate is an indirect effect of selection for increased appetite. There has been virtually no change in true digestibility of diets over the last 20 years, and so increased growth of broilers is merely a consequence of increased feed intake. However, increasing appetite is obviously a challenge in feeding parental breeder lines.

As the growth potential of broilers continues unabated, there is always a question about changing nutrient requirements of breeders. This situation has been further fueled by the concept that strains developed for breast meat yield have different nutrient needs than do classical breeder strains. In reality, there is little evidence for such assumptions, and in fact, there is little foundation for strain specific diets. Table 1 summarizes the suggested diet specifications for the main breeder strains.

<table>
<thead>
<tr>
<th>Table 1. Adult Breeder Nutrient Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ME (kcal/kg)</strong></td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>CP (%)</td>
</tr>
<tr>
<td>Ca (%)</td>
</tr>
<tr>
<td>AvP (%)</td>
</tr>
<tr>
<td>Amino acids (g/Mcal):</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>M+Cys</td>
</tr>
<tr>
<td>Lys.</td>
</tr>
<tr>
<td>Trypto</td>
</tr>
<tr>
<td>Adapted from Leeson and Summers (2000)</td>
</tr>
</tbody>
</table>

As shown in Table 1 there is general consensus by the breeding companies regarding diet specifications and it is difficult to defend strain-specific diets based on these data. However it is also realized that nutrient intake of breeders is ultimately controlled by the schedule for feed allocation. Table
2 indicates daily nutrient intake of breeder hens at 28 weeks as calculated from information on diet specifications (Table 1) together with data on daily feed intake for each strain.

<table>
<thead>
<tr>
<th></th>
<th>Hubbard</th>
<th>Ross</th>
<th>Cobb</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME (kcal)</td>
<td>458</td>
<td>478</td>
<td>469</td>
</tr>
<tr>
<td>CP (g)</td>
<td>24.8</td>
<td>26.7</td>
<td>25.8</td>
</tr>
<tr>
<td>Ca (g)</td>
<td>5.1</td>
<td>5.0</td>
<td>4.7</td>
</tr>
<tr>
<td>AvP (mg)</td>
<td>640</td>
<td>668</td>
<td>724</td>
</tr>
<tr>
<td>Meth (mg)</td>
<td>560</td>
<td>567</td>
<td>563</td>
</tr>
<tr>
<td>M+C (mg)</td>
<td>928</td>
<td>967</td>
<td>1,030</td>
</tr>
<tr>
<td>Lys (mg)</td>
<td>1,136</td>
<td>1,336</td>
<td>1,256</td>
</tr>
</tbody>
</table>

Adapted from Leeson and Summers (2000)

Again, there is close agreement from the three breeding companies regarding daily nutrient needs of their breeders at this age.

A more difficult question to answer relates to the most appropriate mature body weight of breeders. As growth potential of the broiler increases, so there is upward pressure on body weight of both male and female PS stock. In practice, there has been little change in mature weight of breeders over the last 10 to 15 years. Likewise, the growth curve of PS hens is little changed over the years. Any change that has occurred in ‘mature weight’ has been a consequence of delayed light stimulation, rather than any change in growth rate per se. Modern high-yield strains of breeder are somewhat later in maturing, which is a correlate with the characteristics of meat yield (roaster type strains) that have been introduced at the pure line level. Therefore, it is more common to light stimulate at 22 to 23 weeks rather than 19 to 20 weeks as was standard in the 1980’s. This 3-week ‘delay’ in age at maturity equates to an increase in mature weight of 200 – 250 g. Obviously this change in age at maturity has been made possible because of the general acceptance of light-controlled growing houses, a situation that was not always possible in the 1980’s.

Greater control over juvenile and adult light programs is leading to greater synchronization of sexual maturity. Consequently, we are seeing higher and more sustained peak production, which makes it easier to more accurately allocate feed. In allocating feed to breeders, there is an attempt to estimate needs for growth, maintenance and egg production. Of these parameters, egg production is easily the most variable, especially during the early weeks of production. If birds are not synchronized in onset of maturity, then a flock mean production of 60% often means that a portion of the flock will be at 90% production, while some birds will be immature. Feeding for 60% egg production with non-synchronized maturity means that the earliest and latest maturing birds are under-fed and over-fed respectively. A consequence of this inaccurate feed allocation is lower peak production and less sustained peak. Today we have greater confidence in allocating feed based on production needs, due to synchronized maturity, and hence greater lifetime egg production from these flocks.

The feed needs for maintenance are always open to discussion. When allocating a breeder hen 450 to 480 kcal ME/d, the vast majority will be for maintenance. Only about 100 kcals will be for production, and less than 20 kcals is needed for growth and so at least 300 kcal/d is needed for maintenance. This maintenance allocation is largely a factor of body weight and environmental temperature. There is surprisingly little information available on maintenance energy needs of adult breeders, and many estimates are extrapolated from trials involving Leghorn lines. Spratt et al. (1990) suggest that daily heat production of a 3 kg caged breeder is 280 kcal while egg energy is 86 kcal and growth represents 21 kcal daily. Presumably breeders in a conventional floor managed environment will have slightly higher maintenance needs due to increased activity. One of the major factors affecting this
maintenance allocation is environmental temperature. In fine tuning feed allocation, it is important to account for changes in environmental temperature. For a bird consuming a diet providing 2850 kcal ME/kg then a 3 kg breeder hen needs an extra 1.5 g feed for each 1°C decline in environmental temperature, using 26°C as a thermoneutral standard. A challenge in such calculations is defining environmental temperature. Sometimes this is taken as mean daily temperature, while the average of day and night time temperatures is another option. It is likely that cool night time temperatures are not as deleterious as suggested by thermometer readings, since birds invariably huddle at night, either on litter or on slats, and in so doing conserve heat. By sitting, rather than standing, they are also losing less heat. Our best estimate of Effective Environmental Temperature is:

\[
\frac{(\text{day time high } ^\circ\text{C} \times 2) + (\text{night time low } ^\circ\text{C})}{3}
\]

Using this calculation, Table 3 shows extra feed needed by a 3 kg breeder hen fed a diet with 2,850 kcal ME/kg at various day vs. night time temperatures.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Nighttime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>22 18 14 10 6 2</td>
</tr>
<tr>
<td>22</td>
<td>8 10 12 14 16</td>
</tr>
<tr>
<td>18</td>
<td>14 16 18 20</td>
</tr>
<tr>
<td>14</td>
<td>20 22 24</td>
</tr>
<tr>
<td>10</td>
<td>26 28</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
</tr>
</tbody>
</table>

In addition to influencing feed requirements, environmental temperature will also influence feed clean-up time. At higher temperatures, it takes much longer for flocks to consume their allocation. Samara et al. (1996) indicate that feed and clean-up time can at least double, from 3 to 6+ hours when breeders are maintained at 25 to 35°C vs. 10 to 20°C (Figure 1). Data in Figure 1 also show an interesting trend for breeders kept at cooler temperatures when comparing oviposition vs non-oviposition days. On the day that birds lay an egg, their feed clean-up time is greatly prolonged compared to birds not laying on that day. As breeders get older, their proportion of non-laying days increases, and perhaps this fact should be considered in assessing their clean-up time.

The aim of any breeder nutrition program is ultimately assessed in terms of broiler performance. There is surprisingly little information available on the effects of breeder diet nutrient profile and/or nutrient intake on the performance of broiler offspring. It is assumed that egg size is to some extent influenced by breeder nutrient intake, and that this subsequently affects chick size. There are reports of +5g to +10g increase in weight of 42 to 45d broilers for each 1g increase in chick size. For an integrated company therefore, egg size and chick size assume greater importance than merely assessing breeder performance in terms of number of hatching eggs. The balance of protein:energy seems to have an effect on chick weight (Figure 2) and presumably this will influence broiler growth.
We have recently conducted a study in which breeders were fed a range of peak feed allowances, from 140 g/b/d to 175 g/b/d. Breeders fed the highest allowance received a rapid increase in feed to peak, while the increase was much slower for birds peaked at 140 g/b/d. Following peak feeding, feed was withdrawn at variable rates, with birds fed the highest peak allowance subjected to the greatest subsequent decreases. By 65 weeks of age, birds peaked at 140 g received 135 g/d, while those peaked at 175 g received 155 g/d. All roosters were separately fed a rooster diet at levels corresponding to the breeder.
management guide. Table 4 summarizes breeder and broiler performance for the extreme treatments of breeder feed allowance.

<table>
<thead>
<tr>
<th>Peak feed allowance (g/b/d)</th>
<th>Breeder performance (20-64 wks)</th>
<th>Female broiler performance at 49d&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total eggs</td>
<td>Fertility (%)</td>
</tr>
<tr>
<td>140-147</td>
<td>180</td>
<td>93.0</td>
</tr>
<tr>
<td>169-175</td>
<td>163</td>
<td>85.1</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mean of hatches from 30 and 40 week old breeders.

While breeder performance and especially fertility is greatly reduced by the highest level of feeding, there was a surprisingly large increase in growth characteristics of female broiler offspring. These same effects were seen in male broilers, although differences were not as dramatic as those seen with females as shown in Table 4.

While breeder performance will be optimized with moderate peak feed allowances, it seems as though this will be at the expense of broiler performance. In the future, the basis for breeder feeding programs should be based on the overall goals of the poultry company and not merely related to breeder performance per se.

References

ALTERNATIVE FEED INGREDIENTS FOR POULTRY: BROILERS AND LAYERS

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Introduction

In a nation such as the United States with abundant production of corn and soybeans, research in the area of alternative feed ingredients might seem almost an academic exercise. However, at the present time, a combination of factors has stimulated substantial interest in what might be termed non-traditional feed ingredients for poultry. Economic conditions continue to place increasing pressure on the nutritionist to reduce feed costs, while at the same time several underutilized feed ingredients are attracting increased attention.

At the outset, we should give some consideration to the term “alternative ingredients”. For our purposes, such ingredients will be those which are either: 1) totally or relatively new, 2) becoming available in greater quantities than previously, 2) may find use in a niche position, or 4) may become available in somewhat altered form, with possible new applications.

The purpose of this presentation is not to simply provide analytical data on the alternative ingredients to be discussed. Rather, it is our objective to consider what both vendors and purchasers need to know about these ingredients so they can be properly positioned for effective use by the feed industry. Obviously, a determination of the nutrient values of the ingredients in question will be central to this process. However, as will be noted below, a great deal of practical perspective is called for in deciding which are the critical studies to be first conducted to establish a possible role for an ingredient in commercial feeds. Clearly, not all research can be done immediately, so some order of priority must be established. It will be our objective to review several alternative ingredients which are presently available or becoming available to the feed industry, and explore the thinking behind studies undertaken to assess their potential value in commercial feeds.

Distiller’s Dried Grains with Solubles

This ingredient, commonly called DDGS, is certainly not new, being known to the feed industry for many decades. An extensive literature exists on the use of distiller’s by-products, a valuable source being the Proceedings of the Distiller’s Feed Conference. Reviews by well-known nutritionists such as Milton Scott, 1970 and Leo Jensen, 1981 several decades ago clearly documented the role of this ingredient in animal nutrition. An excellent, current source of information on DDGS is a website initiated by the University of Minnesota (www.ddgs.umn.edu). It should be noted that in the past, a major source of DDGS was the alcoholic beverage industry. By contrast, the staggering quantities (by some estimates 8 million tons per year) of DDGS which are projected to be available in the near future will come from the ethanol industry and are derived almost exclusively from corn. Because of the volumes of DDGS becoming available, there is little doubt this product will be used heavily by certain sectors of the feed industry. Thus, the researcher has little to offer if he or she were to begin studies at the present time, and after several years conclude that the ingredient was in fact suitable for use. The author has taken the position that the best way to serve industry in this particular case is to first quickly conduct those analyses needed for initial formulation (metabolizable energy,
amino acids, mineral scan) and on the basis of this conduct several studies to investigate if the determined values employed in the matrix for feed formulation lead to deleterious effects on animal performance. Thus, we will be looking for skeletons in the closet, be these either gross errors in estimating nutrient content and availability, and/or unexpected toxic or antinutritional factors. In our laboratory, when moving into these initial live bird assays, we prefer to use very conservative values for key nutrients. What must be avoided is to find what is thought to be a growth depressing effect of, in this case DDGS, when the problem really lies in an overestimate of a given nutrient, and subsequent errors in formulation. If the live bird studies (which are presently underway with both broilers and layers) yield no negative effects, then detailed studies can be undertaken to more closely define parameters such as lysine and phosphorus availability. It is fully recognized that this is the reverse of the ideal situation. One would prefer to confirm all the subtle details of an ingredient’s nutritive value, and then move on to pen studies with broilers and large scale laying tests. In this scenario, the large scale studies would almost certainly lag far behind industry needs.

Noll et al., 2001 at the University of Minnesota has recently reported satisfactory results using up to 15% DDGS in turkey rations. This paper is available at the website listed above. Studies initiated at The University of Georgia have found nutrient compositions that generally agree with table values, exceptions being a higher metabolizable energy in some “new generation” DDGS samples and highly variable sodium.

In an initial test of “new generation” DDGS in broiler starter diets, Lumpkins et al. (unpublished) at The University of Georgia included 15% of the ingredient (source, Land O’ Lakes) in sets of diets with two density levels. Results (Table 1) show no detrimental effect of DDGS on either weight gain or feed conversion. In a subsequent floor pen study, broilers were reared to 42 days of age on equinutrient diets containing 0, 6, 12, or 18% DDGS. Partial results, shown in Table 2, indicate no adverse effect of using at least 12% DDGS in broiler feeds. Whether higher levels can be recommended may well depend on the amino acid composition of the formula. Normally, protein from corn constitutes about 25% of the total protein in a broiler starter feed. However, with 18% DDGS, whose protein is largely of corn origin, slightly over 50% of dietary protein is from corn.

<table>
<thead>
<tr>
<th>Table 1. Performance of chicks receiving either 0 (control) or 15% DDGS in equicaloric, isonitrogenous diets.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Higher density diets</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>15% DDGS</td>
</tr>
<tr>
<td>Lower density diets</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>15% DDGS</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>(P<0.05)(Batal, et al., unpublished)

Note: Nutrient values for DDGS used in formulating test diets: 2770 kcal/kg; 1260 kcal/lb; 27.0% protein; 0.70% lysine; 1.00% methionine + cystine; 0.62% available phosphorus; 14% moisture.

65
Table 2. Performance of broilers (1-32 days) receiving DDGS.

<table>
<thead>
<tr>
<th>DDGS (%)</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g/chick)</td>
<td>1467</td>
<td>1473</td>
<td>1449</td>
<td>1426</td>
</tr>
<tr>
<td>Feed conversion</td>
<td>1.57</td>
<td>.156</td>
<td>1.57</td>
<td>1.59</td>
</tr>
</tbody>
</table>

In a study with laying hens, Lumpkins et al. (unpublished) employed a 2 x 2 factorial design to evaluate the use of DDGS. Either 0 or 15% DDGS was incorporated into either control (2871 kcal/kg, 18.5% protein) or lower density (2805 kcal/kg, 17% protein) layer rations. The study was initiated when hens were 21 wk of age and continued until 43 wk. As no differences in egg production (Table 3), egg weight, shell strength, interior albumen quality, nor pigment were noted between treatments, it was concluded that DDGS is a highly satisfactory ingredient for laying hen diets. As was noted above, when high levels of DDGS are incorporated into balanced rations, the contribution of corn protein can exceed that of soy. In the present study it will be noted that in the lower density ration with 15% DDGS there appeared to be a slight reduction in egg production. It is possible that if commercial egg producers elect to use lower density feeds, a lower level of DDGS (perhaps 10%) might be considered the maximum recommended level of inclusion.

Table 3. Effect of DDGS on egg production (%), 22-42 wk.

<table>
<thead>
<tr>
<th>Diet Density</th>
<th>DDGS (%)</th>
<th>0</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher</td>
<td>90.2</td>
<td>89.7</td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>89.2</td>
<td>87.6</td>
<td></td>
</tr>
</tbody>
</table>

Pearl Millet

As was the case with DDGS, pearl millet is hardly a new feed ingredient (Sullivan et al., 1990, Kumar et al., 1991). However, its use by the commercial feed industry has been so limited that, again like DDGS, for many nutritionists it will be a completely new ingredient. Interest in pearl millet is based on two rather unique attributes: it can tolerate much poorer quality soils and can thrive on much less moisture than corn or other standard grains. In many areas of the southeastern United States, and in fact in much of the world, the prospects of a successful harvest are much higher with millet than with virtually any other commodity crop. Initial studies with pearl millet in the state of Georgia were conducted in the mid-1990's with the cooperation of GoldKist (Amato and Forrester, 1995). Unfortunately, varieties of millet best suited to the southeast proved susceptible to rust disease, which dramatically reduced yields. A USDA-University of Georgia group headed by Dr. Wayne Hanna spent several years selecting for rust-resistant cultivars of pearl millet at the Tifton, Georgia experiment station. As of 1999, limited quantities of a new cultivar were available for chick tests, with greater quantities available in subsequent years. Metabolizable energy determined at this laboratory has consistently been close to 3350 kcal/kg (88% DM), but protein has varied according to crop year. Geneticists expect protein will stabilize at about 12%. In tests conducted at The University of Georgia (Davis et al., 2002b), rust-resistant millet was included at various levels in test diets, replacing both corn and a portion of soybean meal. Body weight gains, feed conversions, and carcass yield were not affected by inclusion of millet in the feeds (Table 4).
Table 4. Performance of 42 day broilers receiving either corn/soy or corn/millet/soy diets.

<table>
<thead>
<tr>
<th>% Millet in diet</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0%</td>
<td>33.3%</td>
</tr>
<tr>
<td>Body weight (g/chick)</td>
<td>2126&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2229&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feed/Gain</td>
<td>1.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carcass yield (%)</td>
<td>73.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>(P ≤ .05) (Davis et al., 2002)

Note: Nutrient values for pearl millet used in test diets: 3365 kcal/kg; 1530 kcal/lb; 12.0% protein; 0.36% lysine; 0.53% methionine + cystine; 12% moisture.

The Georgia poultry industry is aware of the clear benefit of producing more grain locally. A major north Georgia integrator has this year contracted 700 acres of pearl millet so as to be able to investigate the potential of this grain on a commercial scale.

As with any alternative feed ingredient, bin space at feed mills becomes a major logistical concern. In the case of millet, while grinding presents no problem, it may prove difficult to store multiple ground grains prior to addition to the mixer. A study currently in progress at the University of Georgia is evaluating the possibility of using unground millet at levels up to 10% of the finished feed. Not only would this facilitate the use of this alternative grain, but may well have an effect on the efficiency of the digestive process by incorporating larger size particles. While initial results appear positive, more complete studies now in progress should indicate whether use of unground millet could be recommended.

**Cottonseed Meal**

Whereas both DDGS and pearl millet are being received with interest by the poultry industry, cottonseed meal (CSM) arouses little enthusiasm. Compared to soybean meal, CSM has less energy, less protein, less lysine, less methionine, lower availabilities of these amino acids, along with gossypol and cyclopropenoid fatty acids. It is certainly not difficult to understand why nutritionists would prefer to simply use soybean meal and avoid these problems. Cottonseed meal has a much higher shadow price in layer feeds than for broilers, reflecting the lower nutrient density of hen feeds. However, the risk of discolored yolks is an understandable impediment to use by the layer industry (Davis et al., 2002a). Studies from Waldroup’s laboratory at the University of Arkansas confirm there is little reason why high protein CSM can not be used in broiler feeds at some level of inclusion (Watkins and Waldroup, 1995). Limitation on bin space is frequently cited as a reason why CSM is generally not used in broiler feeds. What is clearly needed is to find a niche for CSM in which its lower nutrient density might actually prove to be an advantage.

A hypothesis to this effect was developed at The University of Georgia. It is well known that many firms prefer not to use animal protein in breeder pullet diets. Thus, soybean meal represents the major protein source for these feeds. High protein soybean meal contains about 3% lysine, of which approximately 91% is available. This creates a major problem. While low protein feeds are formulated so as to help control pullet growth, the preponderance of lysine in soy protein precludes this amino acid from becoming limiting as a percent of protein. It was hypothesized that by substituting CSM for a portion of soybean meal in breeder pullet diets, growth could be more easily controlled and flock uniformity improved as somewhat greater quantities of feed could be provided. Two initial studies, the second and larger of the two being still in progress, give strong support to the use of CSM in breeder pullet feeds (until 18 weeks of age). In agreement with the hypothesis, greater quantities of feed were provided without compromising the weight restriction program, this leading to improved flock uniformity (Table 5). Total feed cost was approximately the same on...
a per pullet basis, as CSM is less expensive than SBM. In a subsequent study, liver gossypol levels of hens receiving CSM decreased rapidly after the ingredient was removed from the diet (Table 6). This suggests no long-lasting negative effect on fertility.

<table>
<thead>
<tr>
<th>Table 5. Performance of pullets/hens reared on diets with and without 20% cottonseed meal (CSM) until 18 weeks of age.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Corn/Soy</strong></td>
</tr>
<tr>
<td>Body weight, g (18 wk)</td>
</tr>
<tr>
<td>Feed/Pullet (relative)</td>
</tr>
<tr>
<td>Flock uniformity (%)</td>
</tr>
<tr>
<td>(Davis et al., 2002a)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 6. Accumulation and depletion of gossypol from hen liver¹.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>¹Diet contained 20% CSM, which had 0.14% free gossypol.</td>
</tr>
</tbody>
</table>

³abcd(P ≤ .05) (Davis et al., 2002a)

**Peanut Meal**

In past years, the use of peanut meal in poultry feeds has been limited due to three main factors: availability of product, severe deficiencies of essential amino acids such as threonine and lysine, and concerns about possible contamination with aflatoxin. However, due to changes in agricultural policy, the development for rapid tests for aflatoxin, and the availability of synthetic amino acids such as lysine and threonine, all favor a re-evaluation of peanut meal for poultry rations. It might be mentioned that peanut meal contains approximately 30% more arginine than does dehulled soybean meal, an attribute which may prove significant in diets limiting in this amino acid.

In point of fact, peanut meal is a very common feed ingredient in other parts of the world, and so long as quality control concerns can be satisfactorily addressed there is no reason the ingredient should not receive favorable consideration. For the past several decades, satisfactory use of peanut meal has been confirmed in a number of studies, including that of Costa et al., 2001 with broilers and Pesti et al., 2003 using laying hens. Based on very limited testing at this laboratory, the metabolizable energy of peanut meal may be somewhat
higher (approximately 2700 kcal/kg) than that reported by the NRC. This level, being somewhat higher than that of soybean meal, appears reasonable when considering the low digestibility of soy carbohydrates.

**Catfish and Tilapia Meals**

While innumerable species of fish can be employed in the manufacture of fish meals, such ingredients can be divided into two general groups. The first is comprised of those meals produced from a rendering of the whole fish, such as anchovy or menhaden. The second group would include those meals made from the offal remaining after fillets of some species have been removed for human consumption. Tuna, catfish, and tilapia meals can be included in this group.

In evaluating ingredients of this type, the researcher must determine the degree of commitment he/she wishes to dedicate to the specific evaluation. This laboratory has taken the approach that the primary need of industry nutritionists is to have at their disposal a reasonable nutrient profile of such meals so as to determine whether a basic interest exists in possible use. Should the researcher, or industry nutritionist, wish to investigate further, graded levels of the respective meals can be included in practical feeds. The efforts of this laboratory in evaluating the three above-mentioned meals has been limited to defining nutrient levels. Zaviezo and Dale, 1994 reported the nutrient composition of tuna meal. A more recent publication (Dale, 2001) reported results of studies evaluating the energy and nutrient content of catfish meal. Samples of the latter product were obtained from a single rendering facility in Mississippi, which, it is the understanding of the author, was the only facility producing this ingredient at that time. Results of the study are presented in Table 5. An interesting aspect of catfish meal is that it contains high levels of protein in addition to calcium and phosphorus. In the case of tuna, when fillets were also removed for human consumption, a lower protein content was found.

<table>
<thead>
<tr>
<th>Table 7. Nutrient composition of Catfish Meal and Tilapia Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Catfish Meal</strong></td>
</tr>
<tr>
<td>TME_n (kcal/kg)</td>
</tr>
<tr>
<td>TME_n (kcal/lb)</td>
</tr>
<tr>
<td>Protein (%)</td>
</tr>
<tr>
<td>Lysine (%)</td>
</tr>
<tr>
<td>Methionine (%)</td>
</tr>
<tr>
<td>Met + Cystine (%)</td>
</tr>
<tr>
<td>Fat (%)</td>
</tr>
<tr>
<td>Ash (%)</td>
</tr>
<tr>
<td>Calcium (%)</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
</tr>
<tr>
<td>Sodium (%)</td>
</tr>
</tbody>
</table>

1^Dry matter = 94.2%

2^Dry matter = 93.8%

**Conclusion**
A number of alternative ingredients have become or are becoming available to the feed industry. It is understood that a first step in presenting such ingredients to potential buyers is to completely document their nutritive value. In addition, where possible, niches where such ingredients may have a special value should be identified. Finally, consideration must be given to possible problems, such as consistent supply, transportation logistics, and flow characteristics of the materials. Any potential problems that might be encountered either in feed formulation or the logistics of handling the ingredient must be fully appreciated and resolved prior to making a realistic commercial presentation.

1 Laboratories exist to document flow characteristics of materials. Two commercial laboratories providing this service are: Jenike & Johanson, One Technology Park Drive, Westford, MA 01886-3189, Ph: (978) 392-0300, www.jenike.com and JR Johanson, Inc., 712 Fiero Lane, Ste. 37, San Luis Obispo, CA 93401, Ph: (805) 544-3775, www.jrjohanson.com.

References


Alternative ingredients are usually defined as those ingredients other than corn and soybean meal, however, this is also dependent on the availability of those and other competing ingredients in the region. In some areas, what might be considered as alternative are ingredients that are already commonly used i.e., wheat products, meat and bone meal, milo, and so forth. For the purposes of this paper, the focus will be on distiller dried grains with solubles and their potential incorporation into diets with various types of alternative ingredients.

Distiller grains with solubles (DDGS) is not a new or novel feed ingredient. However, increased supplies of DDGS are anticipated throughout the US as a result of ethanol production and this has rekindled the interest in utilization of this corn co-product in animal feeds. DDGS as a feed ingredient has a moderate protein content. In the Midwest US, corn is the primary feedstock although other grains can be processed as well. In the dry mill production of ethanol two products are produced – liquid solubles and grain residue. Each could be dried separately but are mixed together and dried to form DDGS as a dry ingredient. Some of the liquid solubles has been fed experimentally with acceptable results (Hunt et al., 1997) but usually the product is fed after drying. Newer production methods (“new generation plants”) are thought to produce a higher quality ingredient.

An early use of DDGS in poultry diets was primarily as a source of unidentified factors that promoted growth and hatchability. Distillers dried solubles (DDS) or DDGS were used in diets at low levels of inclusion usually less than 10%. Couch et al. (1957) found 5% inclusion of DDS variably improved turkey growth rates with the response ranging from 17 to 32%. Day et al. (1972) reported broiler body weight improvements to DDS and DDGS in broiler diets at 2.5 and 5% in one of 3 trials. Improved performance in reproduction have also been indicated for turkey breeder hens. Couch et al. (1957) found improvements in turkey breeder hatchability during the second half of lay with inclusion of dried alfalfa meal, condensed fish solubles, and DDS. Manley et al. (1978) found 3% DDGS improved egg production in hens late in lay and experiencing a low rate of egg production. In diets low in phosphorus DDGS was particularly valuable in improving egg production. However, in a subsequent report, no benefits were observed without low dietary phosphorus (Grizzle et al., 1982). Use of DDGS has also been examined at high levels of inclusion. When lysine levels were adjusted in turkey diets, similar body weights were obtained with DDGS inclusion up to 20% of the diet to 8 wks of age; but feed conversion worsened (Potter, 1966).

Considerations for the use of this product or any alternative product are fairly similar to that of other ingredients. Information would be needed regarding its nutrient composition and variability, amino acid digestibility, amino acid balance, energy, mineral availability, maximum inclusion levels and cost relative to other ingredients. Unfortunately there is limited recent research for this ingredient with modern strains of poultry.
Nutrient Composition and Variability

To assess composition of material from “new generation” processing plants, DDGS samples were collected from four ethanol processors in Minnesota over a period of time during spring, 2002.

Four representative samples were obtained from each ethanol processor. Each sample was analyzed chemically for proximate components (protein, fiber, fat, ash, moisture), amino acids, and minerals. In addition, the samples were submitted to Dr. Parsons’ laboratory at the University of Illinois for in vivo determination of amino acid digestibility using cecatomized roosters. Samples are also being assessed for energy in turkeys using the True Metabolizable Energy (TME) assay developed by Sibbald.

Preliminary results indicate that nutrient content of the DDGS varies among sources but is relatively consistent within processing source. Sources were found to vary in proximate composition especially protein and fat content. Mineral content also varied among sources. Magnesium, sodium, potassium and phosphorus accounted for most of the inorganic component of the feed ingredient.

### Nutrient Profile and Range in Analytical Values Among DDGS as Compared to NRC.

<table>
<thead>
<tr>
<th>Content</th>
<th>UMN</th>
<th>NRC, 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>25.5-30.7</td>
<td>27.4</td>
</tr>
<tr>
<td>Fat</td>
<td>8.9-11.4</td>
<td>9.0</td>
</tr>
<tr>
<td>Fiber</td>
<td>5.4-6.5</td>
<td>9.1</td>
</tr>
<tr>
<td>Calcium</td>
<td>.017-.450</td>
<td>.170</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>.62-.78</td>
<td>.72</td>
</tr>
<tr>
<td>Sodium</td>
<td>.05-.17</td>
<td>.48</td>
</tr>
<tr>
<td>Chloride</td>
<td>.13-.19</td>
<td>.17</td>
</tr>
<tr>
<td>Potassium</td>
<td>.79-1.05</td>
<td>.65</td>
</tr>
<tr>
<td>Amino acids (selected EAA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>.44-.56</td>
<td>.60</td>
</tr>
<tr>
<td>Cystine</td>
<td>.45-.60</td>
<td>.40</td>
</tr>
<tr>
<td>Lysine</td>
<td>.64-.83</td>
<td>.75</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.02-1.23</td>
<td>.98</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>.19-.23</td>
<td>.19</td>
</tr>
<tr>
<td>Threonine</td>
<td>.94-1.05</td>
<td>.92</td>
</tr>
</tbody>
</table>

In the report presented by Cromwell and coworkers (1993), nine different samples of DDGS were analyzed and tested in chick diets. A large range of lysine contents were noted (.43 to .89%). Chick responses to inclusion of these same samples (20%) in isonitrogenous and isocaloric diets ranged from 63 to 84% of the corn-soy-starch control. Samples higher in lysine tended to perform better but some samples did not follow this pattern.

As distiller grains undergo heating to produce the dried product, concern exists over amino acid digestibility especially for heating of lysine in the presence of sugars. Indeed the limited previously literature citations indicates poorer availability of lysine. Combs and Bossard (1969) found lysine availability to range from 71 to 93% by chick growth assay. Parsons et al. (1983) found slightly lower availability of 66% by chick growth assay. Lysine digestibility with roosters was found to 82%. Other sources also assign a low digestibility to DDGS.
In the survey conducted, digestibility of several essential amino acids was affected, in particular that of lysine, threonine and cystine. Digestible lysine was in general much improved over previously published values in three of the four sources. The data indicate that while there are product differences among sources the product is relatively consistent for each source.

### Digestible Amino Acid Content and Range Among DDGS.

<table>
<thead>
<tr>
<th>Amino acids (selected EAA)</th>
<th>% Digestible amino acid</th>
<th>Digestibility Coefficient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine</td>
<td>.35-.53</td>
<td>85.6-90.0</td>
</tr>
<tr>
<td>Cystine</td>
<td>.28-.57</td>
<td>66.3-85.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>.37-.74</td>
<td>59.1-83.0</td>
</tr>
<tr>
<td>Arginine</td>
<td>.73-1.18</td>
<td>80.5-90.0</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>.14-.21</td>
<td>76.4-87.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>.61-.92</td>
<td>66.8-80.7</td>
</tr>
</tbody>
</table>

The higher digestibility of some sources of DDGS for lysine can definitely add value to the DDGS. An economic analysis of turkey grower diets containing DDGS with either lower or higher lysine digestibility (60% vs. 78%) resulted in an opportunity cost of 50 cents greater for the DDGS with higher digestibility. Different scenarios of corn and soybean meal (SBM) prices were used.

### Influence of Digestible Lysine Content on Value of DDGS ($/cwt)

<table>
<thead>
<tr>
<th>Ingredient and price ($/cwt)</th>
<th>DDGS – Low digestible lysine</th>
<th>DDGS – High digestible lysine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, 3.10</td>
<td>4.28</td>
<td>4.78</td>
</tr>
<tr>
<td>Corn, 3.50</td>
<td>4.54</td>
<td>5.00</td>
</tr>
<tr>
<td>Corn, 5.30</td>
<td>5.70</td>
<td>6.02</td>
</tr>
<tr>
<td>SBM, 8.25</td>
<td>4.54</td>
<td>5.00</td>
</tr>
<tr>
<td>SBM, 8.70</td>
<td>4.72</td>
<td>5.21</td>
</tr>
</tbody>
</table>

### Amino Acid Content and Balance

As a sole source of protein in diet, Parsons and coworkers (1983) found DDGS to be limiting in tryptophan and arginine after lysine. Parsons et al. (1983) found that DDGS could replace up to 40% of soybean meal protein when lysine content was adjusted without an effect on body weight. A comparison of amino acid content as percent of protein also indicates that tryptophan and arginine could become limiting as DDGS replaces SBM in the diet. Isoleucine also could be potentially limiting especially considering other ingredients such as blood meal and meat and bone meal.

In Midwestern diets, canola meal is often used in combination with meat and bone meal. Besides soybean meal, meat and bone meal and canola meal is often available. Along with corn and SBM, these ingredients are often used in market poultry diets. Meat and bone meal is a good source of protein and offers other nutrients such as calcium and phosphorus and contributes energy (fat) to the diet. Canola meal has benefits for pellet quality and mill throughput. Utilization of other ingredients such as DDGS needs to be evaluated in such diets with an emphasis on protein quality or amino acid balance as performance and breast meat yield is greatly impacted by intake of specific amino acids.
Thus a study was designed to examine if significant levels of canola meal and DDGS can be used in market turkey diets and to determine which amino acids (tryptophan, isoleucine, arginine) may limit performance with diets containing canola and DDGS.

Nicholas male poults were placed in starting pens at one day of age and reared to 5 weeks of age. At 5 weeks of age the birds were randomly distributed into 98 pens with 10 birds per pen. Room temperature at 5 wks was targeted at 70 F. In the other room temperature was gradually decreased to 60 F at 14 wks of age and a minimum of 55 F held for the remaining experimental period. Starting at 5 wks of age, the toms in each environment (cool and warm temperature environments) were fed one of seven dietary treatments with 7 replicates per treatment.

Treatments

1. Control - Corn/soy/animal protein
2. As 1 plus corn DDGS
3. As 1 plus Canola meal
4. As 1 plus DDGS and Canola meal
5. As 4 plus Tryptophan to Trt 1
6. As 4 plus Tryptophan and Isoleucine to Trt 1
7. As 4 plus Tryptophan, Arginine, and Isoleucine to Trt 1

All major diet ingredients were analyzed for nutrient content and digestible amino acids. Ingredients were chemically analyzed for protein, minerals and amino acids. Samples of each ingredient were submitted to Dr. Parsons at the University of Illinois for determination of digestible amino acids using cecatomized chickens.

Sample diets are shown in the tables below for the respective 5 to 8 and 17 to 19 wk periods for Treatments 1 through 4. The control diet (Treatment 1) includes animal protein because of its obvious economic advantage and widespread use. Valine content (as a percent of protein) is similar across ingredients; therefore diet protein in these sample diets was fixed by setting a valine specification. Supplemental lysine, methionine, and threonine were used so that all diets contained adequate amounts of these amino acids. For Treatments 5, 6, and 7 supplements of tryptophan, arginine and isoleucine were used to achieve amino acid levels similar to that of Treatment 1. All diets contained 60 gm Coban and 20gm Stafac from 5-8 wks and 20 gm Stafac per ton alone from 8 to 19 wks of age.

Weights and feed consumption were determined at 8, 11, 14, 17 and 19 wks of age. At 19 weeks, toms were processed and carcass and breast meat yield determined. At this time samples of breast meat representing each treatment and environment were measured for meat quality by obtaining color, pH, and purge loss.
## Selected Diet Composition 5 to 8 Wks of Age (Exp. TG002)\(^1\)

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>Control (C-S-MBM)</th>
<th>DDGS</th>
<th>Canola</th>
<th>Canola &amp; DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trt 1</td>
<td>Trt 2</td>
<td>Trt 3</td>
<td>Trt 4</td>
</tr>
<tr>
<td>Corn</td>
<td>59.95</td>
<td>54.09</td>
<td>54.81</td>
<td>48.95</td>
</tr>
<tr>
<td>SBM 47%</td>
<td>26.78</td>
<td>20.49</td>
<td>18.68</td>
<td>12.39</td>
</tr>
<tr>
<td>Poultry blend</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>DDGS</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Canola meal</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.943</td>
<td>1.055</td>
<td>0.954</td>
<td>0.865</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.6833</td>
<td>0.748</td>
<td>0.567</td>
<td>0.632</td>
</tr>
<tr>
<td>Scarb</td>
<td>0.3817</td>
<td>0.366</td>
<td>0.338</td>
<td>0.324</td>
</tr>
<tr>
<td>Salt</td>
<td>0.0401</td>
<td>0.004</td>
<td>0.044</td>
<td>0.008</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>0.0044</td>
<td>0.036</td>
<td>0.060</td>
<td>0.093</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.1844</td>
<td>0.179</td>
<td>0.131</td>
<td>0.125</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.2751</td>
<td>0.405</td>
<td>0.301</td>
<td>0.432</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.0771</td>
<td>0.091</td>
<td>0.069</td>
<td>0.082</td>
</tr>
<tr>
<td>Vitamin/mineral mix</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Choice white grease</td>
<td>2.0600</td>
<td>2.120</td>
<td>3.570</td>
<td>3.630</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.0</strong></td>
<td><strong>100.0</strong></td>
<td><strong>100.0</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

### Calculated Nutrient Content

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control (C-S-MBM)</th>
<th>DDGS</th>
<th>Canola</th>
<th>Canola &amp; DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein (%)</td>
<td>22.7</td>
<td>22.5</td>
<td>22.9</td>
<td>22.7</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>6.4</td>
<td>7.4</td>
<td>7.9</td>
<td>8.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient (%)</th>
<th>Digest. Total</th>
<th>Digest. Total</th>
<th>Digest. Total</th>
<th>Digest. Total</th>
<th>Digest. Total</th>
<th>Digest. Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met plus cyst (%)</td>
<td>0.8190</td>
<td>0.9050</td>
<td>0.8190</td>
<td>0.9120</td>
<td>0.8190</td>
<td>0.9220</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.2877</td>
<td>1.4184</td>
<td>1.2877</td>
<td>1.4187</td>
<td>1.2877</td>
<td>1.4332</td>
</tr>
<tr>
<td>Arginine (%)</td>
<td>1.3361</td>
<td>1.4433</td>
<td>1.3421</td>
<td>1.3421</td>
<td>1.3421</td>
<td>1.4171</td>
</tr>
<tr>
<td>Tryptophan (%)</td>
<td>0.2254</td>
<td>0.2543</td>
<td>0.2084</td>
<td>0.2238</td>
<td>0.2299</td>
<td>0.2584</td>
</tr>
<tr>
<td>Valine (%)</td>
<td>0.9000</td>
<td>1.0031</td>
<td>0.9000</td>
<td>1.0061</td>
<td>0.9000</td>
<td>1.0191</td>
</tr>
<tr>
<td>Threonine (%)</td>
<td>0.7858</td>
<td>0.8867</td>
<td>0.7858</td>
<td>0.8910</td>
<td>0.7850</td>
<td>0.9010</td>
</tr>
<tr>
<td>Isoleucine (%)</td>
<td>0.7888</td>
<td>0.8610</td>
<td>0.7590</td>
<td>0.8320</td>
<td>0.7920</td>
<td>0.8430</td>
</tr>
</tbody>
</table>

\(^1\) For all diets, ME was set at 3070 kcal/kg, calcium at 1.18%, inorganic phosphorus at 0.6%, potassium at 0.8%, sodium at 0.19%, and chloride at 0.22%.
Selected Diets for 17 to 19 Wks of Age (Exp. TG002)  

<table>
<thead>
<tr>
<th>Nutrient (%)</th>
<th>Control (C-S-MBM)</th>
<th>DDGS</th>
<th>Canola</th>
<th>Canola &amp; DDGS</th>
<th>Trt 1</th>
<th>Trt 2</th>
<th>Trt 3</th>
<th>Trt 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>74.46</td>
<td>70.55</td>
<td>71.04</td>
<td>67.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBM 47%</td>
<td>12.67</td>
<td>8.48</td>
<td>7.28</td>
<td>3.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry blend</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDGS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canola meal</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.768</td>
<td>0.709</td>
<td>0.674</td>
<td>0.615</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.563</td>
<td>0.606</td>
<td>0.485</td>
<td>0.529</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scarb</td>
<td>0.333</td>
<td>0.322</td>
<td>0.304</td>
<td>0.294</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>0.110</td>
<td>0.085</td>
<td>0.112</td>
<td>0.088</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>0.011</td>
<td>0.033</td>
<td>0.049</td>
<td>0.070</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.042</td>
<td>0.039</td>
<td>0.006</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.154</td>
<td>0.241</td>
<td>0.171</td>
<td>0.258</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>0.022</td>
<td>0.031</td>
<td>0.017</td>
<td>0.026</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin/mineral mix</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choice White Grease</td>
<td>5.51</td>
<td>5.55</td>
<td>6.52</td>
<td>6.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calculated Nutrient Content

| Crude Protein (%) | 14.6 | 14.6 | 14.8 | 14.7 |
| Crude fat (%)     | 9.9  | 10.5 | 10.8 | 11.5 |

<table>
<thead>
<tr>
<th>Met + Cys (%)</th>
<th>Digest.</th>
<th>Total</th>
<th>Digest.</th>
<th>Total</th>
<th>Digest.</th>
<th>Total</th>
<th>Digest.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine (%)</td>
<td>0.756</td>
<td>0.845</td>
<td>0.756</td>
<td>0.845</td>
<td>0.756</td>
<td>0.855</td>
<td>0.756</td>
<td>0.855</td>
</tr>
<tr>
<td>Arginine (%)</td>
<td>0.829</td>
<td>0.901</td>
<td>0.766</td>
<td>0.834</td>
<td>0.812</td>
<td>0.884</td>
<td>0.749</td>
<td>0.816</td>
</tr>
<tr>
<td>Tryptophan (%)</td>
<td>0.134</td>
<td>0.154</td>
<td>0.123</td>
<td>0.143</td>
<td>0.137</td>
<td>0.157</td>
<td>0.126</td>
<td>0.146</td>
</tr>
<tr>
<td>Valine (%)</td>
<td>0.600</td>
<td>0.675</td>
<td>0.600</td>
<td>0.677</td>
<td>0.600</td>
<td>0.685</td>
<td>0.600</td>
<td>0.688</td>
</tr>
<tr>
<td>Threonine (%)</td>
<td>0.490</td>
<td>0.564</td>
<td>0.490</td>
<td>0.567</td>
<td>0.490</td>
<td>0.573</td>
<td>0.490</td>
<td>0.576</td>
</tr>
<tr>
<td>Leucine (%)</td>
<td>1.303</td>
<td>1.396</td>
<td>1.352</td>
<td>1.446</td>
<td>1.274</td>
<td>1.37</td>
<td>1.323</td>
<td>1.419</td>
</tr>
<tr>
<td>Isoleucine (%)</td>
<td>0.505</td>
<td>0.555</td>
<td>0.485</td>
<td>0.535</td>
<td>0.487</td>
<td>0.543</td>
<td>0.467</td>
<td>0.523</td>
</tr>
</tbody>
</table>

For all diets, ME was set at 3390 kcal/kg, calcium at .8%, inorganic phosphorus at .4%, potassium at 0.5%, sodium at 0.18%, and chloride at 0.22%

The experimental design was factorial with diet and environment as the main effects. Analyses of variance were conducted to determine the effects of diet, environment and their interaction on gain, feed conversion, and breast meat yield.

Body weight and feed efficiency (feed/gain) were affected primarily by environment temperature. Turkeys grown in the warm temperature environment had less body weight especially at 19 wks of age with somewhat better feed efficiency. Inclusion of moderate levels of canola meal and DDGS had no adverse effects on performance in comparison to the control diet in either environment. Both environment and diet affected breast meat yield (amount and percentage). Warm temperatures depressed yield by 1.2 lbs. or 2% of the carcass. Inclusion of either DDGS or canola meal alone had little effect on breast meat yield. However, the inclusion of both into the diet depressed percentage meat yield significantly.
Supplementation of the diet with tryptophan restored some of the lost yield in comparison. Isoleucine was without effect, while supplementation with arginine (in combination with tryptophan and isoleucine) restored breast meat yield completely.

**Performance of Male Market Turkeys (Exp. TG002). Main Effect of Diet.**

<table>
<thead>
<tr>
<th>Diet # Description</th>
<th>Body Weight</th>
<th>Feed Efficiency</th>
<th>Carcass Weight</th>
<th>Breast Weight</th>
<th>Breast Meat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11 wks</td>
<td>19 wks</td>
<td>5-19 wks</td>
<td>lbs</td>
<td>lbs</td>
</tr>
<tr>
<td>Control (Corn-Soy-Animal Protein)</td>
<td>18.6</td>
<td>41.6</td>
<td>2.437</td>
<td>32.39</td>
<td>9.99</td>
</tr>
<tr>
<td>As 1 + DDGS</td>
<td>18.7</td>
<td>41.9</td>
<td>2.477</td>
<td>33.21</td>
<td>10.13</td>
</tr>
<tr>
<td>As 1 + Canola Meal</td>
<td>18.9</td>
<td>42.2</td>
<td>2.470</td>
<td>33.30</td>
<td>10.30</td>
</tr>
<tr>
<td>As 1 + DDGS &amp; Canola</td>
<td>18.8</td>
<td>41.8</td>
<td>2.470</td>
<td>32.71</td>
<td>9.81</td>
</tr>
<tr>
<td>As 4 + Tryptophan to Trt #1</td>
<td>19.0</td>
<td>41.9</td>
<td>2.494</td>
<td>32.84</td>
<td>10.01</td>
</tr>
<tr>
<td>As 5 + Isoleucine to Trt #1</td>
<td>18.7</td>
<td>41.7</td>
<td>2.449</td>
<td>32.85</td>
<td>9.88</td>
</tr>
<tr>
<td>As 6 + Arginine to Trt #1</td>
<td>18.8</td>
<td>42.2</td>
<td>2.466</td>
<td>33.25</td>
<td>10.25</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>18.8</strong></td>
<td><strong>41.9</strong></td>
<td><strong>2.466</strong></td>
<td><strong>32.93</strong></td>
<td><strong>10.05</strong></td>
</tr>
</tbody>
</table>

P Value

<table>
<thead>
<tr>
<th>Diet</th>
<th>Environment</th>
<th>Diet x Env</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>NS</td>
<td>0.0001</td>
</tr>
<tr>
<td>0.0001</td>
<td>0.0001</td>
<td>0.1415</td>
</tr>
</tbody>
</table>

Least Significant Difference (P<.05)

| Diet | 0.3 | 0.7 | 0.046 | 0.86 | 0.39 | 0.65 |

Digestible amino acid content of the DDGS used in this project was much better than reported elsewhere. Warm environmental temperatures depressed body weights by 1.8 lbs. at 19 wks of age and breast meat amount by 1.2 lbs. Inclusion of significant levels of either canola and/or DDGS had no effect on growth performance. Breast meat yield (as a proportion of carcass weight) was sensitive to amino acid quality as reflected by the depression in yield when the combined diet of canola and distiller grains were fed. The amino acids tryptophan and arginine appeared to play a role in restoring yield.

A second trial (Exp. TG013) was conducted to compare the performance of DDGS as protein level of the diet decreased. The experimental design was factorial (2x3) with 2 diet series comparisons (corn-soybean meal-poultry byproduct meal vs. inclusion of DDGS); and 3 protein levels (100,95 and 90% NRC dig thr. Diet protein level was established by using intact protein to provide the desired digestible thr target without supplemental thr. All diets were supplemented with lysine and methionine plus cystine to meet the estimated digestible amino acid requirement.

Diets were formulated using digestible amino acids as determined prior to the start of the trial. Thus corn, soybean meal, DDGS, and poultry byproduct meal would be assayed as described above. Diets were isocaloric and fed in mash form. Each diet was fed to 8 replicate pens of turkeys. The trial started at 8 weeks of age. At 7 weeks of age the birds were randomly distributed into 96 pens with 10 birds per pen. All diets contained 60 gm Coban and 50 gm (BMD) bacitracin from 0-8 wks and 50 gm BMD per ton alone from 8-17 wks of age. The trial was conducted during the approximate time period of February to April.
Weights and feed consumption were determined at 8, 11, 14, 17, and 19 wks of age. At 19 weeks of age, toms were processed, carcass and breast meat yield determined. Analyses of variance were conducted to determine the main effects of diet series, protein, and thr addition, and their interaction on gain, feed conversion, and breast meat yield for the main factorial design.

Diet protein as digestible thr (90, 95, 100% NRC) had the most consistent effect on body weight at the different ages and on breast meat yield at 19 wks of age. The response to diet protein was similar for both diet series. Body weights and breast meat yield per bird were reduced when diet protein was reduced to 90% NRC thr.

**Effect of protein (as dig NRC thr), and diet series on market tom turkey performance (Exp. TG013).**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Diet % NRC thr</th>
<th>Corn/soy/meat</th>
<th>Corn/soy/meat/DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>27.1</td>
<td>27.5</td>
</tr>
<tr>
<td>14 wk BW, lbs</td>
<td>95</td>
<td>27.4</td>
<td>26.9</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>26.8</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>42.4</td>
<td>42.5</td>
</tr>
<tr>
<td>19 wk BW, lbs</td>
<td>95</td>
<td>42.4</td>
<td>42.3</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>41.6</td>
<td>41.9</td>
</tr>
<tr>
<td>Breast meat, lb/bird</td>
<td>100</td>
<td>10.7</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>10.0</td>
<td>10.2</td>
</tr>
</tbody>
</table>

Information from the feeding trial indicates that when DDGS are incorporated into market turkey diets on a digestible amino acid basis, performance was equivalent to that of the control at all protein levels. Greater responses to threonine were observed in the diet series without DDGS at a lower protein level.

In summary, DDGS was found to be an acceptable source of protein in diets for heavy toms at moderate levels of inclusion. Diets should be formulated taking into consideration specifications for tryptophan and arginine. While isoleucine was not found to be limiting, combined use of DDGS with alternative ingredients may lead to shortages of isoleucine and/or arginine. More information regarding DDGS can be found at the following University of Minnesota website: www.ddgs.umn.edu.

**Acknowledgments**

Thanks to the Minnesota Turkey Growers Association, Nutrition subcommittee members for their suggestions and technical expertise and to the staff at the Rosemount Agricultural Experiment Station for their assistance and care of the flock. The project was partially funded by the Minnesota Turkey Research and Promotion Council, Heartland Lysine, MN Ethanol Distillers, and Minnesota Corn Growers Association. Central Bi-Products, FMC, Pfizer and Elanco also donated supplies to the project.
References


AMMONIA EMISSIONS FROM DAIRY AND BEEF PRODUCTION SYSTEMS

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Summary

Environmental increases in the reactive forms of N have occurred over the 20th century. Increases have occurred as a result of anthropomorphic activities associated with combustion of fossil fuels, urbanization, and intensification of agriculture. Effects of increases in reactive forms of N in aquatic systems has resulted in eutrophication and acidification, in ground water has resulted in higher nitrate concentrations, in the atmosphere has resulted in increases in 2.5 µm particulates which increase haze, human health risks, and green house gasses, and in land systems has increased acidification, plant and tree growth and reduced biodiversity. Inventories accounting for sources of atmospheric N indicate that animal production systems are the primary source of atmospheric reactive N in the form of ammonia. Environmental reactive N is a major concern of the U.S. Environmental Protection Agency (EPA) with three offices with major efforts underway to understand and control N: Water, Air and Research and Development. Dairy and beef production systems primarily contribute to ammonia emissions through excretion of waste N as urea in urine. Urease activity in feces is high and rapidly converts urea to ammonia after excretion. Conversion of urea to ammonia is temperature and pH dependent and volatilization further dependent on depth of fluid column. With open lots and housing systems developed for improved ventilation for animal health, ammonia from urea is rapidly volatilized into the atmosphere from many housing systems. Furthermore, degradation of organic nitrogen in animal waste to ammonia can occur during storage. Ammonia may be also lost from storage structures and land application of slurry. Even during grazing, at least 8% of urinary nitrogen is volatilized. A typical dairy cow producing 27 kg of milk may consume 417 g of N and excrete 127 g of N in urine, 30% of intake. Urea may comprise 86% to 90% of urinary nitrogen. Thus 109 g may volatilize from the housing facility from this cow. In dry cows and heifers, 39% to 50% of intake nitrogen may be lost each day in urine and be subject to volatilization. Erickson has estimated that 50 to 63% of intake N may be volatilized from feed lots. To control ammonia emissions, rations must be formulated to optimize rumen microbial capture of N in bacterial protein and reduce over feeding of protein. In addition, rapid collection of urine and separation from feces can minimize contact time and reduce ammonia losses from barns. Capping lagoons and pits and injection of slurry at time of land application at time of high crop uptake can reduce losses from field application of animal wastes. Acidification of slurries or alkalizing alleyways may also reduce ammonia volatilization. Ammonia losses from dairy and beef facilities cannot be reduced to zero, but they can be minimized and will need to be to meet EPA air quality standards.
Introduction

Nitrogen use in agriculture as fertilizer in agronomic systems and as protein supplements in animal systems has increased productivity and benefited mankind over the last 150 years. However, forms of reactive N (ammonia – ammonium ions, nitric oxides, nitrates and nitrites, nitrous oxides) have increased in air, land and water systems with negative effects (Anon, 2002; Cowling et al., 2001). Increased loading of N into aquatic systems has increased eutrophication and acidification, with negative effects on aquatic plants and fish. Atmospheric deposition of reactive N on land has stimulated tree and plant growth, but has also decreased biodiversity and acidified soils. Atmospheric loading of reactive N increases haze, 2.5 micrometer particulate, greenhouse gases, and acid precipitation. Ammonia emissions are a major concern and there is international momentum to set policy measures to reduce these emissions (Cowling et al., 2001). According to US EPA national air pollutants emissions trends report, ammonia emissions come predominantly from agricultural sources, primarily from livestock.

Ammonia emissions primarily arise from animal waste. Nitrogen in excreta can be converted to ammonia through bacterial degradation, primarily the conversion of urea to ammonia (Muck, 1982). N content of dairy cattle excreta is directly influenced by N intake (Wilkerson et al., 1997). Historically, NRCS was the governmental agency with the primary responsibility to oversee the collection and land disposal of animal waste, and the USDA through the National Research Council was the agency responsible for suggesting feeding standards for dairy and beef cattle of all age classes. However, due to the intense concern over N pollution, the EPA is becoming the major agency with oversight on N in animal waste, and ultimately may have indirect control over N in animal feeding. The EPA is the coordinating agency establishing the national standards for nutrient management programs in concentrated animal feeding operations with cooperation from the NRCS and USDA. Therefore, dairy and beef operators need to be aware of factors within their operations that can contribute to ammonia emissions.

Protein Feeding Systems

Both the Beef and Dairy NRC publications (NRC, 1996; 2001) utilize metabolizable protein systems to define requirements for growing, and lactating cattle. Metabolizable protein is calculated as the sum of absorbed true protein from the small intestine from feed protein and rumen bacterial cells. Small intestinal feed protein is protein which escaped degradation in the rumen. Bacterial cells are produced from fermentation of organic matter in the rumen and digestion of these cells that flow to the small intestine provides bacterial protein to the cow.

The core of metabolizable protein systems in ruminants is to predict the extent of rumen degradation of feed proteins and the amount of microbial protein produced. NRC Dairy (NRC, 2001) assumes the yield of bacterial crude protein (BCP) is 130 g/kg of TDN (discounted) intake. Dietary rumen degraded protein (RDP) is 1.18 x MCP yield. When RDP is less than 1.18 x RDP then BCP yield is calculated as .85 x RDP supply. The Beef NRC uses a value of 13 g/100 g of TDN to predict BCP synthesis when forage comprises over 40% of the diet. When forage is less than 40% of the diet, then a 2.2% reduction in BCP synthesis for every 1 percent decrease in forage effective neutral detergent fiber (eNDF) less than 20%. The dairy NRC considers BCP to contain 80% true protein and 20% nucleic acid N. True protein is considered to be 80% digestible, thus 64% of BCP is absorbed by the cow.

Rumen degraded feed protein supplies N necessary to support BCP synthesis. Some N may be provided from urea recycling in saliva, thus it may be possible to slightly under feed RDP and still maintain BCP yields. However, RDP above that needed for BCP is absorbed as ammonia from the rumen and ultimately is converted to urea by the liver and excreted from the body in urine and milk.
Requirements for MP are the sum of protein necessary to support maintenance, lactation, growth and gestation. A factorial approach is used to determine requirement. Each physiologic process is associated with an efficiency of utilization. The conversion of MP to net protein in product releases N from tissue metabolism, which eventually is lost as urea in urine and milk. Provision of optimal blends of amino acids may enhance production efficiency. In dairy cows, the requirement for MP for production is milk true protein yield divided by 67%.

When supplies of RDP and rumen undegradable protein (RUP) are balanced for milk production, plasma urea will fall within a specific range for a group of cows (Roseler et al., 1993). In addition milk urea was as useful as blood urea to monitor protein status (Baker et al., 1992; Roseler et al., 1993). Urea moves into milk via passive diffusion from blood and rapidly equilibrates with blood values (Baker et al., 1992). Based on work by Baker and Roseler, we have previously proposed that the mean optimal urea values for a group of cows would fall between 10 to 14 mg/dl and 95% of the individual cows in the group would range +/- six units from the mean milk urea nitrogen (MUN) value (DePeters and Ferguson, 1992). The minimum value of 10 mg/dl agrees with Hof et al. (1997) observation for the minimum MUN value below which rumen available N may impair performance. Higher values of MUN would be associated with reduced efficiency of protein utilization and would be wasteful. Jonker et al. (1998) proposed a model to assess N intake from MUN and provided guidelines for expected MUN values based on milk production and cattle grouping. Therefore, MUN serves as a useful tool to assess protein feeding efficiency (Hof et al., 1997; Jonker et al., 1998). In nonlactating cattle, particularly beef animals, blood samples would need to be used to assess urea-N concentrations, but the same principals would apply.

**Urinary Nitrogen**

Forms of N in urine include urea, creatinine, allantoin, uric acid, ammonia, alpha-amino nitrogen, peptide N and hippuric acid. The major N sources are urea, creatinine and purine derivatives, allantoin and uric acid. Of the purine derivatives, allantoin is the major constituent found in cattle urine. By far the major N component in urine is urea. In cattle consuming a similar ration the urinary urea-N concentration ranged from 185 mg/dl to 1250 mg/dl with a mean of 697 mg/dl (sd 252 mg/dl). Creatinine ranged from 11.0 to 78.2 mg/dl with a mean (standard deviation) of 77.7 mg/dl (sd 35.2). Creatinine contains 33.6% N on a weight basis, therefore this represented 26 mg/dl N from creatinine. Nitrogen from urea in urine is about 25 times the amount of N from creatinine. Urine contains a small amount of ammonia, which is excreted as a urinary buffer. These cows had .10 mg/dl to 9.43 mg/dl ammonia in urine with a mean of 1.97 mg/dl (sd 1.95). Urea-N may be 92% of N in urine; creatinine 2% and other nitrogenous compounds including ammonia, 6% of urinary N. Diet will influence the urea excretion to a major extent. Creatinine excretion is relatively constant and is a function of body weight (Ganong, 1999).

We sampled blood and urine a total of 275 times from 91 cows. Samples were collected monthly across one year. Cows were on different diets throughout the year. Mean values for plasma urea –N and creatinine were 12.6 mg/dl (sd 4.2) and 1.05 mg/dl (sd .2), respectively. Urinary urea-N and creatinine were 705 mg/dl (sd 234) and 92.0 (sd 34.7), respectively. Urinary creatinine and urinary urea were inversely related (r=-.276, p<.0001). High concentrations of creatinine tended to occur later in lactation, when cows had lower urinary urea. Urinary creatinine was independent of dietary CP content but was associated with plasma creatinine (r=.299, p<.0001). Urinary urea was correlated with plasma urea (r=.151, p<.01) and dietary CP content (r=.136, p<.05). Our main interest in this trial was to monitor urea and assess its loss from the dairy barn through out the day.

Baker (1992) observed that as urea-N was infused intravenously, 95% of the infused dose was collected in urine and 5% in milk. Thus, MUN could serve as an indicator of urea-N excreted in urine. Jonker et al. (1998) observed a direct relationship between MUN and urinary output. Smits et al. (1995) found that urinary nitrogen excretion was dependent on protein in the diet. Urinary urea is rapidly broken.
down to ammonia nitrogen when in contact with bovine feces due to urease enzymes present in bacteria in feces (Muck, 1985). The rate of ammonia production was temperature and pH dependent. With temperatures above 15°C most urea-N was converted to ammonia. At temperatures above 20°C, additional organic N was converted to ammonia in manure. Therefore, urinary urea-N as a minimum represents what may lost from a dairy or beef facility as volatile ammonia. MUN would serve as a predicator of potential ammonia losses.

**Ammonia Volatilization**

Bovine feces is rich in urease activity (Muck, 1985). Activity was influenced by pH and temperature. Optimum pH was between 6.8 to 7.6 (Muck, 1985), which is typical pH for cattle feces. Warm weather would increase the rate of ammonia production from urea, particularly with temperatures above 15°C. This means substantial losses of ammonia may occur from a dairy facility and open beef feed lots.

Assuming all urinary nitrogen may volatilize, we (Ferguson et al., 2001) estimated potential losses from a typical Pennsylvania dairy farm using data from Dou et al. (2001). For a farm with 69 lactating cows, 4.62 tonne/year of ammonia would be volatilized. This would represent 30.9 kg/animal unit/year. This is 32% of N intake in feed. Voorburg and Kroodsman (1992) reported a value of 1 kg of N per month per cow lost from Dutch housing facilities, which would represent 12 kg/cow/year. This is lower than our estimate, possibly due to different protein feeding or manure handling in the Dutch situation.

To more closely assess these losses, we have monitored N fates at the Marshak Dairy over 2000 and 2001. Monthly, weekly samples of feed, milk, feces, urine, flush water, and blood were collected from cows at the Marshak Dairy, University of Pennsylvania, School of Veterinary Medicine dairy facility. The Marshak Dairy is a green house free stall barn, with space for 200 cows. Alleys are cleaned by twice daily flushing with recirculated lagoon water. Currently the herd is composed of 135 milking cows. Dry cows are housed in a separate facility. Cows are milked twice a day, housed usually in four to five groups, and fed a total mixed ration.

To monitor N flow, we collected samples of flush water from the top of each alley at start of flush and at the bottom of each alley and at the collection pit. Solids are separated by conveyor and these were sampled after separation. Urine and fecal samples were collected for three sequential days each week from a random sample of cows in each group and composited for daily analysis. Samples were acidified on collection. Feed and fecal samples were analyzed for DM, CP, ADF, NDF, starch, lignin, fat, and minerals. Ammonia content of feces was also analyzed. Urine and blood samples were similarly analyzed for ammonia, urea, creatinine and potassium and phosphorus.

Table 1 presents mean values on a dry matter basis for feed and feces for the main groups of cows in the barn over the year. Table 2 presents the values for volatile losses if all the urea-N and ammonia were volatilized from the dairy. Twenty four to 29% of fed N was lost as ammonia in the lactating groups and 46% from the heifer group. Table 3 presents the urine and flush data for each group. Despite high concentrations in urine, little urea-N was collected at the bottom of each alley after flushing. Ammonia concentrations increased in bottom flush fluid, representing some collection of ammonia from alleys. In June – October no urea was collected in flush liquid in bottom of alleys (data not shown) and ammonia concentrations increased. Months when mean temperature was above 15°C urea at the bottom of flush collection was zero. This agrees with Muck (1985). The mean volatile loss of N was 27% (6% SEM) of feed input across the year based on collection of liquid at the separator. On a daily basis, the number of cows in the barn was 146.4; mean feed N each day was 79.2 kg; mean volatile N loss was 21.6 kg/day. This represents 53.8 kg/cow/year of volatile N.
Table 1. Mean (SEM) feed and fecal analysis for each group at the Marshak Dairy.

<table>
<thead>
<tr>
<th>Item (% DM)</th>
<th>Heifer Group</th>
<th>High Group</th>
<th>Middle Group</th>
<th>Low Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>53.5 (.1)</td>
<td>56.0 (.6)</td>
<td>56.3 (.8)</td>
<td>55.6 (.8)</td>
</tr>
<tr>
<td>CP</td>
<td>14.5 (.3)</td>
<td>17.0 (.2)</td>
<td>16.3 (.2)</td>
<td>15.9 (.2)</td>
</tr>
<tr>
<td>NDF</td>
<td>40.8 (.1)</td>
<td>38.1 (.6)</td>
<td>36.9 (.8)</td>
<td>41.4 (.8)</td>
</tr>
<tr>
<td>FAT</td>
<td>4.3 (.2)</td>
<td>5.8 (.1)</td>
<td>5.6 (.2)</td>
<td>5.2 (.2)</td>
</tr>
<tr>
<td>Starch</td>
<td>22.9 (.1)</td>
<td>21.6 (.6)</td>
<td>24.3 (.9)</td>
<td>21.2 (.9)</td>
</tr>
<tr>
<td>Lignin</td>
<td>4.5 (.2)</td>
<td>4.3 (.1)</td>
<td>4.2 (.1)</td>
<td>4.6 (.1)</td>
</tr>
<tr>
<td>P</td>
<td>.34 (.01)</td>
<td>.42 (.01)</td>
<td>.40 (.01)</td>
<td>.37 (.01)</td>
</tr>
<tr>
<td>Feces</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>16.8 (.5)</td>
<td>15.5 (.2)</td>
<td>16.2 (.4)</td>
<td>15.9 (.4)</td>
</tr>
<tr>
<td>CP</td>
<td>15.7 (.3)</td>
<td>17.0 (.2)</td>
<td>16.1 (.3)</td>
<td>17.2 (.3)</td>
</tr>
<tr>
<td>NDF</td>
<td>59.0 (.6)</td>
<td>55.5 (.3)</td>
<td>58.5 (.5)</td>
<td>56.4 (.5)</td>
</tr>
<tr>
<td>FAT</td>
<td>2.2 (.1)</td>
<td>2.7 (.1)</td>
<td>2.7 (.1)</td>
<td>2.4 (.1)</td>
</tr>
<tr>
<td>Starch</td>
<td>2.7 (.3)</td>
<td>2.9 (.1)</td>
<td>3.1 (.2)</td>
<td>2.4 (.1)</td>
</tr>
<tr>
<td>Lignin</td>
<td>9.8 (.2)</td>
<td>9.5 (.1)</td>
<td>9.3 (.2)</td>
<td>10.3 (.2)</td>
</tr>
<tr>
<td>P</td>
<td>.70 (.04)</td>
<td>.70 (.02)</td>
<td>.60 (.03)</td>
<td>.70 (.03)</td>
</tr>
</tbody>
</table>

Table 2. Nitrogen balance for each group. Mean (SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Feed N, g</th>
<th>Milk N, g</th>
<th>Fecal N, g</th>
<th>Urine N, g</th>
<th>Urea+NH3 N, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heifer</td>
<td>241.2 (38.0)</td>
<td>115.6 (20.0)</td>
<td>122.8 (13.7)</td>
<td>99.9 (11.1)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>581.4 (16.2)</td>
<td>176.5 (3.1)</td>
<td>264.2 (8.5)</td>
<td>212.1 (7.0)</td>
<td>165.5 (5.7)</td>
</tr>
<tr>
<td>Mid</td>
<td>594.5 (24.1)</td>
<td>154.8 (4.6)</td>
<td>283.9 (12.7)</td>
<td>190.4 (10.5)</td>
<td>140.3 (8.5)</td>
</tr>
<tr>
<td>Low</td>
<td>504.5 (24.1)</td>
<td>88.9 (4.6)</td>
<td>218.0 (12.7)</td>
<td>176.3 (10.5)</td>
<td>128.2 (8.5)</td>
</tr>
<tr>
<td>Herd</td>
<td>530.0 (80.0)</td>
<td>149.6 (15.4)</td>
<td>241.0 (42.0)</td>
<td>188.8 (34.7)</td>
<td>144.3 (28.1)</td>
</tr>
</tbody>
</table>

Balance, g If all Urea+NH3N volatilized : Loss for each group as % of intake N

<table>
<thead>
<tr>
<th>Group</th>
<th>Balance, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heifer</td>
<td>2.8 (1.2%)</td>
</tr>
<tr>
<td>High</td>
<td>-71.4 (12.2%)</td>
</tr>
<tr>
<td>Mid</td>
<td>-34.6 (5.8%)</td>
</tr>
<tr>
<td>Low</td>
<td>21.3 (4.2%)</td>
</tr>
<tr>
<td>Herd</td>
<td>-49.4 (9.0%)</td>
</tr>
</tbody>
</table>

.46 (.02) .29 (.02) .24 (.03) .26 (.03) .29 (.03)
Erickson et al. (2001) found using a mass balance approach on feedlot yearling steers that 63.1% of feed N was volatilized over a feeding period of 137 days on a conventional feed lot diet. Twenty point nine (20.9) kg of N was volatilized per steer. By using phase feeding, varying protein supply to meet changing requirements over the feeding period, volatile losses of N could be reduced to 14.2 kg of N/steer, 52.6% of intake N. Feeding less N reduced volatile losses, an observation consistent with Smits et al. (1995).

Table 3. Mean (SEM) urine concentrations and concentrations in flush water from the top and bottom of alley for each group of cows.

<table>
<thead>
<tr>
<th>Item</th>
<th>Heifer Group</th>
<th>High Group</th>
<th>Middle Group</th>
<th>Low Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH3, mg/dl</td>
<td>1.76 (.50)</td>
<td>1.40 (.15)</td>
<td>1.25 (.20)</td>
<td>1.74 (.19)</td>
</tr>
<tr>
<td>UreaN, mg/dl</td>
<td>707.6 (68.4)</td>
<td>666.4 (19.6)</td>
<td>610.1 (27.2)</td>
<td>717.2 (26.0)</td>
</tr>
<tr>
<td>Creat., mg/dl</td>
<td>109.3 (9.54)</td>
<td>78.4 (2.77)</td>
<td>86.1 (3.81)</td>
<td>101.2 (3.66)</td>
</tr>
<tr>
<td>Top flush</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH3, mg/dl</td>
<td>35.02 (2.34)</td>
<td>35.50 (.88)</td>
<td>35.13 (1.22)</td>
<td>35.29 (1.56)</td>
</tr>
<tr>
<td>UreaN, mg/dl</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bottom Flush</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH3, mg/dl</td>
<td>39.59 (1.66)</td>
<td>44.31 (.61)</td>
<td>41.03 (.87)</td>
<td>38.04 (2.33)</td>
</tr>
<tr>
<td>UreaN, mg/dl</td>
<td>2.64 (1.06)</td>
<td>4.00 (.39)</td>
<td>3.79 (.55)</td>
<td>4.56 (.71)</td>
</tr>
</tbody>
</table>

Erickson et al. (2001) found using a mass balance approach on feedlot yearling steers that 63.1% of feed N was volatilized over a feeding period of 137 days on a conventional feed lot diet. Twenty point nine (20.9) kg of N was volatilized per steer. By using phase feeding, varying protein supply to meet changing requirements over the feeding period, volatile losses of N could be reduced to 14.2 kg of N/steer, 52.6% of intake N. Feeding less N reduced volatile losses, an observation consistent with Smits et al. (1995).

Grazing cattle contribute significant losses of N as ammonia (Jarvis et al., 1989a, 1989b). The loss is associated with urinary N. About 8% of urinary N is lost from urine of grazing cattle. Losses are higher when pastures are grass with high rates of fertilization, which increases protein content of herbage. Intense rotational grazing also increases losses due to higher stocking rates and reduced canopy of grasses over soil.

Reducing Ammonia Losses

It has long been recognized that separating feces and urine at collection can minimize ammonia losses from animal waste. This removes the urea from the urease. However, this requires unique barn systems with holes in floors to collect liquid. Once liquid has be mixed with feces, storage needs covered with straw or more inert materials to prevent volatilization from the storage unit. And then slurry needs to be injected or incorporated with 4 hours of surface application to minimize ammonia losses.

Since urease activity is pH dependent, Muck found that lime addition to alleyways reduced urea conversion to ammonia by raising pH and reducing urease activity (Muck and Herndon, 1985). Acidification of slurry can also reduce volatilization by increasing the concentration of ammonium ion in slurry, since NH₃ is the volatile substance. Muck also suggested that rapid scrapping of barn floors could reduce ammonia losses (Muck and Richards, 1983).

Prevention of ammonia emissions can be done by controlling N content in manure, primarily urea in urine, control of pH in alleys and slurries, capping of slurry structures, and injection at time of land application. Drying manure can decrease activity of urease. Which measures prove fruitful will depend on cost and feasible application.


NUTRITIONAL FACTORS INFLUENCING REPRODUCTIVE SUCCESS
IN DAIRY CATTLE

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Introduction

Essential Fatty Acids (EFA) have been implemented as key nutrients in sustaining reproductive performance. In the early work of Burr and Burr (1930), rats were fed a fat-free diet resulting in cessation of growth and, in a majority of rats, cessation of or irregular ovulation. Rats were then supplemented with corn oil, olive oil, linseed oil, or coconut oil at approximately 1% of dietary DM. With the exception of coconut oil, consumption of the other oils resulted in a quick expression of heat (within 6 to 9 d of diet change). Coconut oil contained no C18:2. The other oils contain between 41% (corn oil) and 7% (olive oil) C18:2. Authors attributed this effect to “ovarian hormone” rather than to simply an improvement in overall animal well-being because “...the resumption of ovulation is so rapid that growth has hardly begun. Synthesis of ovarian hormone ceases when fatty acids are eliminated from the diet.” In a later study, EFA-deficient female rats could conceive but aborted before gestation was complete (Deuel et al., 1954).

The supplementing with some sources of fat to lactating dairy cows has improved reproductive performance. In several studies, lactating cows fed a basal diet containing whole cottonseed (~9% C18:2) and further supplemented with calcium salts of long chain fatty acids (CaLCFA; Arm and Hammer Nutrition, Princeton, NJ) (~8% C18:2) experienced a better rate of conception or pregnancy than cows fed the diet containing only whole cottonseeds (Staples et al., 1998). Lactating cows fed tallow (4.3% C18:2) at 3% of dietary DM tended to have a better conception rate by 98 days in milk than cows not fed tallow (Son et al., 1996). Grazing dairy cows supplemented with soybean oil soapstock (53% C18:2) at ~2% of dietary DM experienced a greater pregnancy rate than controls (62.5 vs. 22.2%) whereas those fed fat and housed in a freestall barn had lower pregnancy rates than controls (0 vs. 22.2%) (Boken, 2001). Primiparous beef heifers also have experienced greater pregnancy rates (94, 90, 91, and 79%) when fed rolled and cracked safflower seeds, soybeans, or sunflower seeds, all high in C18:2 concentration (Bellows et al., 1999). Protection of dehulled cottonseeds (~9% linoleic acid) with protein-aldehyde complexes (Protected Lipid, Rumentek Industries, Australia) delivered approximately 175 g/d of linoleic acid to the lower gut of lactating Hereford cows. Overall pregnancy rates were improved from 63 to 79% (Wilkins et al., 1996).

Integrated Nutritional and Reproductive Management

Successful management of lactating dairy cows requires integration of the disciplines of reproduction and nutrition with standard postpartum herd health programs to optimize both milk and reproductive performance. The achievement of high-energy intake, to bring cows out of a decreasing negative energy status as early as possible postpartum, is critical for both productivity responses. In the majority of lactating dairy cows, development of dominant follicles on the ovary occurs very early in the postpartum period. However, functional competence of these follicles varies in association with concentrations of insulin-like growth factor-1 (IGF-1) in plasma and energy status in which the majority of these follicles emerged after the nadir in energy status (Beam and Butler, 1997, 1998). The ability of
early dominant follicles to either ovulate, undergo turnover or to form follicular cysts influences length of the postpartum anovulatory period. Both regulation of IGF-1 and preovulatory surges of lutenizing hormone (LH) appear to be critical to the efficiency of this process. Subsequent timing of ovarian cycles, measured by formation of corpora lutea (CL), also is related to postpartum concentrations of IGF-1 and energy status. It is clear that the anestrous condition impacts reproductive efficiency to timed insemination systems such as Ovsynch and nutritional programs such as fat feeding may reduce the incidence of anestrus and thus benefit herd reproductive management.

Exciting strategies have developed to integrate nutritional and reproductive management. Fats (concentrated energy sources) can be incorporated into the diet of cows in early postpartum in order to try to minimize the differences between energy intake and energy output. Absorption of total fatty acids by the ruminant is linear up to 1200 g/day or about 6% of DMI. Typical nonfat-supplemented diets contain about 2 to 3% fat. Therefore it appears that there is significant room to increase the use of fat in diets without loss of efficiency (Staples et al., 1998). Because fat is an energy dense nutrient, it is natural to suppose that supplemental fat would improve energy status of the cow. However, this has not been the result in many cases. Oftentimes energy status is not affected by feeding fat because either DMI is depressed or milk production is increased. Nevertheless, feeding supplemental fat has proven effective in improving reproductive performance of lactating dairy cows. Conception rates were improved by feeding prilled fat or calcium salts of long chain fatty acids.

I would like to give an example of how fat feeding has interacted with high degradable intake protein (DIP) to influence reproductive responses in postpartum dairy cows (Garcia-Bojalil et al., 1998). The detrimental effects of feeding a high DIP diet on reproduction can be alleviated with supplemental fat feeding (CaLCFA). One possibility of how high protein feeding may adversely affect reproductive performance is the increased energy costs to the animal for detoxification of ammonia resulting in a "weakening" of the cow's energy state. This energy cost is likely to push early postpartum cows even further into negative or less positive energy states, thus delaying return to normal ovarian activity. To test the effects of intake of energy and DIP on reproductive performance of lactating dairy cows, 45 cows were assigned at calving to 20% CP diets containing either 15.7% or 11.1% DIP and 0 or 2.2% CaLCFA (Megalac®). Crude protein intake was 1100 g greater than required for milk produced. Treatments continued through 120 days in milk. Cows fed the highly degradable protein diets had greater blood urea nitrogen values (22.0 vs. 17.3 mg/dL). Based upon progesterone concentrations of blood samples taken three times per week, cows fed the 15.7% DIP diets experienced more days to first luteal phase postpartum than cows fed other diets (39 vs. 25 days). All cows on experiment were synchronized to estrus between days 50 and 57. Cows not cycling prior to synchronization were assigned 50 days to first luteal activity. If cows had not been synchronized, the number of days to first luteal activity likely would have been even greater for cows fed the 15.7% DIP diets. Four out of 10 cows fed 15.7% DIP diet without CaLCFA were anestruus at synchronization compared with only three out of 35 cows fed the other dietary treatments. These prolonged days to restoration of ovarian activity and the anestrus condition were matched with greater loss of body weight and body condition by these cows. Cows fed 15.7% DIP diets lost more body weight and for a longer period of time compared with cows fed 11.1% DIP diets. The absence of CaLCFA resulted in a 10 kg greater loss in body weight of cows fed 15.7% DIP diets. In addition, body condition loss was greater and more prolonged by cows fed the CaLCFA-free, 15.7% DIP diet. The additional energy costs of detoxifying ammonia from highly degradable dietary protein possibly led to a greater reliance on body energy stores for milk production. This resulted in a more severe energy state that delayed ovarian activity. By including CaLCFA in the diet, the energy shortage was somewhat alleviated, allowing cows to rely more on feed energy and less on body reserves for milk production. Days to first estrous was reduced by 6 days when CaLCFA was fed with 15.7% DIP diets. Accumulated progesterone concentrations throughout the postpartum period are depicted in Figure 1. The detrimental
effect of 15.7% DIP diets was alleviated markedly by supplementation of CaLCFA, but supplementation of CaLCFA to the 11.1% diet was not stimulatory. Results indicate that dynamics of postpartum ovarian activity can be suppressed indirectly by feeding of high DIP (15.7%), but this adverse effect can be alleviated partially by feeding of CaLCFA. Also of interest was the observation that pregnancy rate by 120 days postpartum was increased from 52.3% to 86.4% when CaLCFA was supplemented and evaluated as a main effect across diets. This study demonstrated the specific benefit of feeding by-pass fat to increase ovarian cycles and reduce the incidence of anestrus in the postpartum period which is a major impediment to herd reproductive efficiency as described above.

Figure 1. Regression curves of accumulated plasma progesterone concentrations from lactating Holstein cows fed diets containing 11.1% and 15.7% degradable intake protein and/or 0% and 2.2% CaLCFA.

**Whole cotton seed and bST.**

An additional example of how fat feeding can influence reproductive performance is a study of feeding of whole cotton seed (WCS) to lactating dairy cows and the interacting effects with bST that influence reproductive responses (Adams, 1998; Kassa et al., 2002). The study involved 186 cows that evaluated effects of WCS feeding and low doses of bST on reproduction during the postpartum period. Diets were total mixed rations (TMRs) formulated according to the requirements for lactating Holstein cows. Within 24 h after calving, cows received one of two experimental diets ad libitum. All cows that were on bST treatment received 208 mg (0.5 ml) of bST (Posilac®, Protiva Co., St. Louis, MO) subcutaneously every 2 wk starting within 7 d of calving. This dose of bST is 50% of the standard commercial dose rate. Since increases in IGF-1 appear to be stimulatory to follicle and ovarian development as described above, we were interested in administering bST at a low dose to evaluate ovarian activity and subsequent fertility. Healthy cows were assigned randomly to one of four treatments. Treatments were WCS diet group (15% of DM) with or without bST and no WCS diet groups with or without bST (Table 1). Although early ovarian activity may be associated with subsequent increases in fertility, we feel that it is important not to sustain a long period of progesterone exposure during the
period of uterine involution. Consequently, all cows received prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\), 25 mg i.m., Lutalyse®, Pharmacia-Upjohn Co., MI) at 30 ± 3 d postpartum to regress any CL and reduce progesterone concentrations. This stimulates turnover of CL and ovarian follicles, permits clearance of uterine contents, and reduces exposure to progesterone that may inhibit uterine defense mechanisms and predispose the uterus to infection. Blood samples were collected three times a week from calving until initiation of the Ovsynch protocol. The Ovsynch protocol was initiated on 65 ± 3 days postpartum, and cows were timed inseminated at day 75. On day 111 postpartum (36 days after insemination) cows were diagnosed for pregnancy by ultrasound examination. If cows were not pregnant the Ovsynch protocol was repeated and a second insemination was made at day 121 postpartum. Thus all cows received their first insemination on day 75 postpartum and both inseminations required no heat detection. Following second service, cows were monitored for heats for subsequent services.

Table 1. Least square means for pregnancy rates at Day 45 after timed artificial insemination (TAI) for cows fed diets of 0 or 15% WCS and injected with 0 or 208 mg of bST at 14 day intervals.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total cows</th>
<th>1(^{st}) TAI (%)</th>
<th>2(^{nd}) TAI (%)</th>
<th>1(^{st}) and 2(^{nd}) TAI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% WCS; no bST</td>
<td>50</td>
<td>37.1</td>
<td>23.6</td>
<td>51.3</td>
</tr>
<tr>
<td>15% WCS; no bST</td>
<td>45</td>
<td>33.6</td>
<td>26.4</td>
<td>51.8</td>
</tr>
<tr>
<td>0% WCS; +bST</td>
<td>43</td>
<td>27.1</td>
<td>35.8</td>
<td>51.1</td>
</tr>
<tr>
<td>15% WCS; +bST</td>
<td>48</td>
<td>27.3</td>
<td>26.2</td>
<td>46.8</td>
</tr>
</tbody>
</table>

Feeding WCS diets stimulated ovarian activity based upon a greater accumulation of progesterone during the postpartum period up to 62 days postpartum when the Ovsynch program was initiated. The increase in accumulated progesterone associated with WCS diets was associated with an earlier occurrence of a progesterone rise following PGF\(_{2\alpha}\) injection on day 30 (39.2 < 43.5 days), and a higher peak progesterone elevation during the rise after PGF\(_{2\alpha}\) injection (11.4 > 9.25 ng/ml). The increase in ovarian activity as measured by accumulated progesterone concentrations may have been associated with higher plasma concentrations of high density lipoprotein (HDL)-cholesterol in the WCS treatment group (107.4 vs. 83.5 mg/100ml). Cholesterol is essential for the synthesis of progesterone. Although ovarian activity differed significantly between diets with and without WCS, pregnancy rates did not differ following timed inseminations to either the first, second, or accumulative pregnancy rate to first and second service (Table 1). Pregnancy responses demonstrate the advantage of integrating a reproductive management program with nutritional management. Although the diet without WCS was associated with a lower level of ovarian activity, implementation of the Ovsynch protocol stimulated and controlled ovarian activity such that there was no dietary treatment effect on fertility. Indeed the Ovsynch protocol permitted a very precise first service for all cows, and the re-synchronized Ovsynch procedure for cows that did not conceive to first service guaranteed a second service within a 46-day period for all open cows.

Our field experiments with Ovsynch indicate a lower fertility rate in cows identified to be anestrus and in lower BCS. With our ability to guarantee that all cows can be inseminated precisely at a designated time postpartum with the use of Ovsynch, producers can lengthen the voluntary waiting period, since the time of first insemination is controlled more precisely. If all cows are cycling, a normal program of inseminating at detected estrus, assuming a 50% estrus detection rate, would have to be started at day 40 to ensure that mean time of insemination will be day 70 (range 40 - 100 days). However, an Ovsynch program permits all inseminations to be made at 70 ± 3 days if implemented on a weekly basis.
WCS and bST effects on ovarian follicular dynamics.

The effects of WCS diet and bST administration on ovarian follicular dynamics and plasma progesterone (P4) concentration were examined in a sub-sample of 28 cows during a period of synchronized follicular growth as part of the Ovsynch protocol (Kassa et al., 2002). Cows received gonadotrophin releasing hormone (GnRH) at 65±3 d postpartum (d 0), PGF2α (d 7), a second GnRH (d 9) and were inseminated 16 h later (d 10). Ovarian changes were monitored daily by ultrasonography from d 0 to 9. On d 9, 93% of cows had a preovulatory follicle and 86% ovulated.

The mean number of Class 2 follicles was influenced significantly by a treatment x day interaction (P<0.05). Further examination of the differences among the day trends detected a WCS x bST interaction (P<0.01), which is depicted in Figure 2A and B. The number of Class 2 (6 to 9 mm) follicles was influenced differentially by bST depending upon whether the diets contained WCS or not. For example, bST-treated cows receiving a WCS diet had a greater number of Class 2 follicles (Figure 2B), apparently due to presence of a dominant follicle with reduced dominance or fats increased ovarian responsiveness to stimulate follicle development in response to bST. In contrast, sustained recruitment of Class 2 follicles following GnRH injection was greatly reduced in bST-treated cows not fed WCS (Figure
indicating that bST stimulated the growth of a follicle with greater dominance; one that reduced recruitment and/or enhanced turnover of Class 2 follicles when WCS was not present in the diet. Previously, Wehrman et al. (1991) observed that a WCS diet stimulated the number of medium-sized follicles. Somatotropin administration in lactating dairy cows increased the population of Class 2 follicles during the first follicular wave (Lucy et al., 1993) and raised the concentration of IGF-1 in plasma (Bilby et al., 1999; Newbold, et al., 1997). The nutritional regulation of IGF-1 and GH and their effects on follicular and luteal function has been reviewed recently (Lucy et al., 1999). Thus, dietary- and bST-induced effects were the likely underlying causes for the alteration of follicular dynamics accompanying the use of bST in lactating cows, but this effect seems to be dependent upon increased dietary fat provided in the form of WCS. Such findings indicate that fats associated with WCS (i.e., C18:2 etc.) appear to influence sensitivity or responsiveness of the ovary to LH and follicle-stimulating hormone (FSH) induced by injection of GnRH in the presence of bST. Such observations imply a cross talk between lipids and hormonal (FSH, LH and bST) mediated receptor responses that influence ovarian function (e.g., follicular recruitment, dominance and possibly CL function).

In cows having CL during the experimental period, WCS increased plasma concentrations of P₄ (10.0 vs. 6.5 ± 1.5 ng/ml; P<0.10). This increase was evident after adjusting for number of CL during the experimental period. Grummer and Carroll (1991) postulated that HDL was the major lipoprotein fraction supplying cholesterol to luteal tissues for P₄ synthesis. Adding HDL with a high cholesterol:protein ratio, to luteal cell cultures in vitro increased P₄ production. However, no difference was noted between control and fat supplemented cows in their ability to stimulate P₄ production (Carroll et al., 1992) when pregnant lactating cows were used. In another study, Hawkins et al. (1995) demonstrated that early lactation cows fed a diet containing calcium soaps of fatty acids had approximately twice the concentration of cholesterol, HDL and P₄ in serum than cows on the control diet. Our study also focused on early lactation cows and the clear elevation in plasma HDL cholesterol in cows fed WCS diets (90.0 vs. 70.5 ± 4 mg/100ml; P<0.02) likely accounted for the higher P₄ in plasma of cows fed WCS. As reported by Hawkins et al. (1995), the increased concentration of P₄ may be associated with increased lipid accumulation within the CL and a slower rate of disappearance of P₄ from peripheral circulation. Beef heifers were fed either 0 or 0.57 kg/d of CaLCFA from 100 days prepartum through the third estrous cycle postpartum. Mean concentrations of plasma P₄ and cholesterol were elevated in heifers fed fat. On days 12 to 13 of the third cycle, heifers were ovariectomized. Higher concentrations of P₄ in repeated blood samples that were taken immediately before and after ovariectomy indicated a greater half-life of P₄ and suggested a slower clearance rate from blood of heifers fed CaLCFA. Two recent studies support this influence of fat on progesterone clearance (Sangritavong et al., 2002). Liver slices were incubated with P₄, estradiol, and several fatty acids including C18:2. When C18:2 was included in the media, the half-life of P₄ (50.7 vs. 31.7 min) and estradiol (37.3 vs. 25.9 min) were increased over that of media containing no fatty acids. This effect of C18:2 was confirmed in vivo using nonlactating Holstein cows. Progesterone and estradiol were infused intravenously with or without a soybean oil emulsion. Cows receiving soybean oil had greater serum concentrations of P₄ (3.83 vs. 2.42 ng/ml) and estradiol (379 vs. 287 pg/ml), strongly suggesting that the presence of soybean oil (possibly C18:2) reduced the clearance rate of these steroids. Progesterone not only prepares the uterus for implantation of the embryo but also helps maintain pregnancy by stimulating uterine secretions for nourishment of the developing conceptus. Increased concentrations of plasma P₄ have been associated with improved conception rates of lactating dairy cows (Butler et al., 1996).

Supplementation of dietary fats increased serum concentrations of GH, insulin and cholesterol (Thomas et al., 1997). The same workers observed that concentrations of cholesterol and IGF-1 in follicular fluid also were increased following fat feeding. In our study, the WCS diet (15% of DM) provided approximately 671 g of fat intake per cow per day. Based on estimated delivery of linoleic acid to the small intestine that escapes biohydrogenation in the rumen, up to 168 g of linoleic acid may be
absorbed (Staples et al., 1998). Thus, increased availability of fat (including esterified cholesterol) associated with the WCS diets may have permitted an increase in CL progesterone secretion.

Generally, feeding high-fat diets to cattle stimulates ovarian function (Hightshoe et al., 1991; Lucy et al., 1991b; Staples et al., 1998). During the postpartum period, the effects of fat supplementation have been attributed to the associated increase in energy intake and the accompanying change in energy balance (Butler and Smith, 1989), in elevated blood cholesterol levels and enhanced ovarian luteal activity (Hightshoe et al., 1991; Wehrman et al., 1991). Furthermore, studies showed that fat, in the form of calcium soaps of long chain fatty acids, and not increased energy intake, stimulated ovarian function (Sklan et al., 1991) and caused development of larger preovulatory follicles (Lucy et al., 1991a; Lucy et al., 1991b; Lucy et al., 1993b). Diets enriched in long chain fatty acids are suggested to modulate the production of P4 through increased availability of cholesterol, reduced synthesis of PGF2α in the uterus, possible alterations in growth hormone and IGF-1 secretion, and altered clearance rate of P4 (Grummer and Carroll, 1991; Mattos et al., 1999; Staples et al., 1998). Present results indicate that fat feeding may also influence ovarian tissue sensitivity to hormonal stimulation.

Omega-3 Fatty Acids

Three other long chain, polyunsaturated fatty acids may have an influence on reproductive performance; namely linolenic acid, eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). All three fatty acids have a double bond located between the third and fourth carbon counting from the methyl end of the molecule, thus classified as omega-3 fatty acids. These latter two fatty acids are found in marine products such as algae, fish meal, fish oil, and some seafood byproducts. These fatty acids are appearing more often in dairy cow diets due to an increased interest in feeding fish meal as a ruminally undegradable protein source (Kellogg et al., 2001). Linolenic acid is the main fatty acid found in some vegetable oils such as linseed and in pasture forages.

Linolenic acid may have been responsible for the improvement in conception rate (87.5 vs. 50.0%) of lactating dairy cows (n = 35) fed formaldehyde-treated whole flaxseed (17% of dietary DM) compared to those fed CaLCFA (5.6% of dietary DM) from 9 to 19 weeks postpartum (Petit et al., 2001). Supplementing diets of lactating dairy cows with fish meal has improved conception rates (Staples et al., 1998). In some of these studies (Armstrong et al., 1990; Bruckental et al., 1989; Carroll et al., 1994), fish meal partially replaced soybean meal resulting in a reduction of an excessive intake of ruminally degradable protein. Therefore the improved conception rates may have been due to the elimination of the negative effect of excessive intake of ruminally degradable protein. However, in a field study in which the concentration of ruminally undegradable protein was kept constant between dietary treatments, cows fed fish meal had better conception rates (Burke et al., 1996) suggesting that the positive response was due to something other than a reduction in intake of ruminally degradable protein. The unique polyunsaturated fatty acids in fish (EPA and DHA) may have been responsible for the improvement in fertility.

These fatty acids may improve overall pregnancy due to their influence on the synthesis of PGF2α. A quick review of the role of PGF2α during the postpartum period of resumption and reoccurrence of estrous cycles is in order here. Within the reproductive tract of cows, uterine tissue is a primary source of the F series prostaglandins (e.g., PGF2α) during the early postpartum period. Concentration of 13, 14-dihydro-15-keto-PGF2α metabolite (PGFM) in plasma rose dramatically to a peak of ~2200 pg/ml by 1 day postpartum (Mattos et al., 2001). This rise is associated with regression of the CL of pregnancy and postpartum regression of the uterus. (The PGFM is produced as the uterus and lung metabolize PGF2α.) Over the next 2 weeks, PGFM gradually returned to baseline concentrations. The uterus synthesizes and releases PGF2α regularly during the estrous cycle to regress each newly formed CL in order to initiate a
new estrous cycle if the cow is not pregnant. If the cow does conceive, PGF$_{2\alpha}$ release from the uterus is inhibited in order to preserve the CL on the ovary to allow it to synthesize P$_4$ to aid in the implantation and nutrition of the embryo. Because PGF$_{2\alpha}$ has an effect on the regression of the CL, concentrations of plasma P$_4$ are related inversely to PGF$_{2\alpha}$ concentrations during the period of CL regression in late diestrus.

The synthesis of PGF$_{2\alpha}$ from arachidonic acid (C20:4) is regulated by the key enzyme, prostaglandin endoperoxide synthase (PGHS) (Figure 3). The feeding of C20:5 may aid in the suppression of synthesis of PGF$_{2\alpha}$ by the uterus by competing for PGHS. Dihomo-$\gamma$-linolenic acid also competes for PGHS when it is converted to the series one prostaglandins. Although C22:6 is not a substrate for PGHS, it is a strong inhibitor of PGHS activity. Therefore when intake of C18:3, C20:4, or C22:5 increases, conversion of C20:4 to PGF$_{2\alpha}$ can be reduced, thus potentially increasing the chances of preserving the life of a newly formed embryo. In addition, the increased presence of C20:5 and C22:6 can inhibit the synthesis of C20:4 from C18:2 by inhibiting the desaturation and elongation enzymes required for that conversion (Figure 3; Bezard et al., 1994; Mattos et al., 1999). Linolenic acid also can compete with C18:2 for the desaturase enzymes so that more C20:5 and less C20:4 are synthesized (Figure 3). In addition, the omega-3 fatty acids can displace C20:4 in the phospholipids of cell membranes thus reducing availability of C20:4 (Howie et al., 1992). Therefore, increasing the dietary intake of the omega-3 fatty acids can reduce the production of PGF$_{2\alpha}$.

![Figure 3. Synthesis of the various prostaglandin (PG) series from fatty acid precursors.](image)

**Evaluation of Individual Fatty Acids.**

Which fatty acids are the most potent when it comes to suppression of synthesis of PGF$_{2\alpha}$? A series of in vitro experiments was performed at the University of Florida (Mattos, 2001) using bovine endometrial (BEND) cells from the uterus. The BEND cells were incubated with no fatty acids (control) or a variety of fatty acids that included C18:1, C18:2, C18:3, C20:4, C20:5, and C22:6 at a
concentration of 100 µM (Figure 4). Compared to the control, cells incubated with C20:4 tended to stimulate synthesis of PGF$_{2\alpha}$. This positive response was expected since C20:4 is the fatty acid precursor of PGF$_{2\alpha}$. Only the omega-3 fatty acids (C18:3, C20:5, and C22:6) suppressed synthesis of PGF$_{2\alpha}$ with C20:5 and C22:6 the most repressive. The fact that C18:2 did not affect secretion of PGF$_{2\alpha}$ somewhat contradicts previous reports indicating inhibitory activity of this fatty acid (Elattar and Lin, 1989; Pace-Asciak and Wolfe, 1968). Moreover, C18:2 has been considered a potential mediator of reduced endometrial PGF$_{2\alpha}$ secretion in the pregnant cow (Thatcher et al., 1995). Conversely, because C18:2 is the most abundant precursor for synthesis of C20:4 and PGF$_{2\alpha}$ it could be hypothesized that C18:2 would increase secretion of PGF$_{2\alpha}$ through increased precursor availability. This did not occur in the BEND cell system. One possible reason could involve lack of an efficient system for conversion of C18:2 to C20:4, which involves two steps of desaturation and one step of elongation. It is not clear why C18:3 was less inhibitory than DHA and EPA. Linolenic acid is the precursor for synthesis of DHA and EPA, and can be converted to these in a process that relies on activities of desaturase and elongase enzymes.

In a second study, BEND cells were co-incubated with C20:4 and C20:5 for 24 h. Figure 5 illustrates the competing effects of the two fatty acids. Arachidonic acid increased (P < 0.01) secretion of PGF$_{2\alpha}$ whereas C20:5 was inhibitory (P < 0.01). This illustrates the competition of precursors for processing by the PGHS enzymes involved in prostanoid synthesis. The reduced secretion of PGF$_{2\alpha}$ observed in cells incubated with C20:5 is likely a result of a shift of the PGHS pathway from synthesis of prostanoids from the 2 series to synthesis of prostanoids of the 3-series. In the presence of C20:5, less of the C20:4 present was converted to PGF$_{2\alpha}$.  

Figure 4. Synthesis of PGF$_{2\alpha}$ by bovine endometrial cells incubated with a variety of fatty acids. AA=arachidonic acid; OA=oleic acid; LA=linoleic acid; LNA=linolenic acid; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid. Difference between each fatty acid and control: *P<0.05; **P<0.01.
Application of these results to the dairy cow was tested. Our hypothesis was that feeding C20:5 and C22:6 through fish oil supplementation during the periparturient period would increase the proportion of these fatty acids in uterine tissue and reduce the spontaneous secretion of uterine PGF$_{2\alpha}$ of dairy cows at parturition. Pregnant Holstein cows (n = 17) and heifers (n = 9) were assigned randomly to diets containing fish oil (n = 13) (Arista Industries, Wilton, CT) or olive oil (n = 13) (Classico, Bertolli, Italy). A ration containing either fish oil or olive oil was supplied from 21 days before the expected calving date until parturition, when it was replaced by greater nutrient dense rations also containing either fish oil or olive oil that were fed until cows reached 21 days postpartum. Cows (n = 6) and heifers (n = 6) that had moderate to severe dystocia, or that were diagnosed with displaced abomasum, retained fetal membranes, or toxic metritis within 10 days after parturition were removed from the analysis.

Rations were formulated to provide approximately 2% oil prepartum and 1.8% oil postpartum. The fatty acids in olive oil were 61% C18:1, 16% C18:2, and 17% C16:0. The fish oil contained 36% C20:5 and 28% C22:6. The combined intake of C20:5 (68 g/d) and C22:6 (53 g/d) was 121 g/d pre and postpartum. Cows were milked three times daily. Blood was obtained once daily at 1730 h from 14 days prior to calving until parturition and from 14 to 21 d postpartum. Between the day of parturition and day 14 postpartum, blood samples were collected twice daily at 0800 and 1730 h. Blood was analyzed for PGFM, a product of PGF$_{2\alpha}$ metabolism. Caruncles were collected by manual extraction through the vagina within 12 h of parturition, frozen in liquid nitrogen, freeze-dried, and analyzed for fatty acid composition.

Concentrations of C20:5 (1.66 vs. 0.23 ± 0.02 g/100 g of fatty acids) and C22:6 (3.11 vs. 0.59 ± 0.37 g/100 g of fatty acids) in caruncular tissue were increased 5- to 6-fold in cows fed fish oil. The combined concentrations of caruncular C20:5 and C22:6 were correlated positively with the number of days that cows were supplemented with fish oil ($r^2 = 0.64$), suggesting that introduction of fish oil before 21 d prepartum may have increased the concentrations of C20:5 and C22:6 in the uterus even further. Cows fed fish oil had reduced concentrations of plasma PGFM during the period of maximum secretion in the early postpartum period compared to cows fed olive oil. Differences were significant (P < 0.05) at 0, 0.5, 2, and 2.5 days postpartum (Figure 6). The pattern of postpartum concentrations of plasma PGFM was similar to what was reported by Mattos et al. (2001). The increased concentrations of C20:5 and
C22:6 in caruncular tissue of cows fed fish oil suggest that these fatty acids may be the active compounds reducing secretion of PGF$_{2\alpha}$. However, a consistent difference in plasma PGFM concentrations between cows fed olive oil and fish oil was not observed throughout the experimental period. Plasma PGFM concentrations of cows fed olive oil and fish oil converged at about day 5 postpartum and remained similar until the end of the experiment. The reduction in plasma PGFM concentrations could be explained by the detachment and shedding of caruncular tissue with high PGF$_{2\alpha}$ synthetic activity that normally takes place in the postpartum period. Preferential shedding of caruncular tissue in cows fed olive oil could have reduced the apparent difference between plasma PGFM concentrations of cows fed olive oil and fish oil.

Figure 6. Pre- and postpartum plasma concentrations of prostaglandin F$_{2\alpha}$ metabolite (PGFM) of cows fed fish oil (▲) or olive oil (square) (LSM + SE). The PGFM concentrations were lower in cows fed fish oil at 0, 0.5, 2, and 2.5 days after parturition (*, P < 0.05).

Diet did not affect the number of days between the expected due date and actual calving date. It was anticipated that reduced uterine PGF$_{2\alpha}$ secretion could result in delayed parturition. Cows fed olive oil and fish oil calved 3.4 ± 1.8 and 3.3 ± 2.1 days before the due date, respectively. Additional evidence for an inhibition of uterine PGF$_{2\alpha}$ secretion is that lactating dairy cows fed fish meal at 0, 2.7, 5.2, and 7.8% of dietary DM had an attenuated plasma PGFM response to injections of estradiol-17β and oxytocin given on day 15 of the estrus cycle compared to cows not fed fish meal (Mattos et al., 2002).

Burns et al. (2000) at Colorado State University have demonstrated that nonlactating beef cows will store C20:5 and C22:6 in endometrial tissue when fed fish meal. Mature Angus cows were fed a corn silage-based diet containing either corn gluten meal (n=4) or Menhaden fish meal (n=3) at 8.7 and 5% of dietary DM, respectively. Diets were isonitrogenous and isocaloric. After 25 days of supplementation, estrous cycles of cows were synchronized. Cows were slaughtered at d 18 of the second estrous cycle and uteri were collected, frozen, and analyzed for C18:3, C20:5, and C22:6. The uterus of cows fed fish meal had greater concentrations of C20:5 (P<0.01) and tended to have greater concentrations of C22:6 (P = 0.12). Concentrations of C18:3 were similar between the two groups.

If the omega-3 fatty acids are able to decrease secretion of PGF$_{2\alpha}$ then embryo survival should be increased. Bonnette et al. (2001) fed 82 lactating, primiparous beef cows a corn silage-based diet containing either 5% fish meal or 8.7% corn gluten meal (DM basis). Diets were initiated at 25 days prior
to the breeding season and continued through the 90-d breeding season. Cows were artificially inseminated and pregnancy determined at 25-30 days post breeding using ultrasonography. First service conception rate tended to be greater for cows fed fish meal (75.6 vs. 61.5%; P = 0.14). Serum progesterone concentrations after insemination were similar between the two groups. In a study with Holstein cows (n=141), cows were allotted to one of three dietary treatments initiated at calving (Petit and Twagiramungu, 2002). Diets were isonitrogenous, isoenericgetic, and isolipidic. Diets contained either whole flaxseed, CaLCFA, or micronized soybeans. Flaxseeds contain ~32% oil with 57% C18:3, 14% C18:2, and 18% C18:1. The diameter of the CL of the cows fed flaxseed was larger than that of cows fed soybeans (19.7 vs. 16.9 mm) but not larger than that of cows fed CaLCFA (17.5 mm). Embryo mortality from day 30 to 50 after artificial insemination (AI) tended to be lower (P < 0.11) when cows were fed flaxseed (0%) compared to CaLCFA (15.4%) or soybeans (13.6%).

We have final results from a California field trial (Tulare, CA) conducted to determine if a diet supplemented with a protected source of fish oil stimulates pregnancy rate and/or reduces pregnancy losses in lactating dairy cows (Juchem et al., 2002). Multiparous cows at 2 d postpartum were assigned randomly to receive a diet containing 400 g of fatty acids from either a calcium salt of palm and fish oil (n = 368) or tallow (n = 370) from 21 to 150 d in lactation. Milk samples were collected from 44 cows (22/trt) to determine the fatty acid profile by gas chromatography. Cows were timed AI 71 days postpartum following the Presynch/Ovsynch protocol. Pregnancy was diagnosed by ultrasonography on days 28, 39, and 63 after AI. The experimental period Sept. 2001 to Oct. 2002 was divided into a Thermal Neutral (TN) and a Heat Stress (HS) season. Results indicate that feeding a calcium salt of fish oil compared to tallow increased concentrations of milk EPA (0.043 vs. 0.037%; P=0.03) and DHA (0.024 vs. 0.014%; P<0.001), and increased plasma progesterone concentrations on days 11 and 14 of the estrous cycle (P=0.05). A treatment × season interaction was detected for pregnancy rates at day 39 (TN: Ca Salt 39.2% vs. Tallow 33.5%; HS: Ca Salt 24.9% vs. Tallow 37.5%; P<0.03) and day 63 (TN: Ca Salt 32.4% vs. Tallow 28.5%; HS: Ca Salt 21.3% vs. Tallow 35.2%; P<0.07) of pregnancy. Pregnancy rates appeared to be higher with the calcium salt of palm and fish oil in the TN period whereas, during the HS period pregnancy rates were higher for tallow. Additional research is needed to understand the potential impact of selected fat feeding on reproductive performance. Results during the TN period support our hypothesis that specific fatty acids can reduce embryo mortality in dairy cows. However, the polyunsaturated complement of fatty acids appeared to have a negative effect during the HS period.

Summary

Growing evidence indicates that the design and delivery of supplemental unsaturated fatty acids to the lower gut for absorption (specifically linoleic acid, linolenic acid, EPA and DHA) may target reproductive tissues to alter reproductive function and fertility. Improvement in reproductive performance involves stimulation in ovarian activity (follicle development) in the postpartum period, and possibly improved pregnancy rates due to increased production and/or decreased clearance of progesterone. Changes in follicular dynamics can be affected by fat supplementation involving potential alterations in ovarian sensitivity to metabolic hormones such as bST, IGF-1 and gonadotrophins (LH and FSH). In addition, the suppression of uterine prostaglandin secretion by omega-3 fatty acids may improve pregnancy rate. However, nutritional interactions with thermal neutral and heat stress seasons on reproductive responses need to be considered.

References


Introduction

Rapid rearing of replacement dairy heifers has the potential to increase dairy profitability by bringing heifers to puberty and milk production at an early age. This reduces the time period during which the animal produces no revenue. However, rapid rearing during the prepubertal period can result in decreased milk production (Sejrsen and Purup, 1997) and calving difficulty (Hoffman, 1997). Data indicate that overfeeding during the prepubertal period causes deposition of mammary fat and impairment of mammary epithelial growth (Sejrsen et al., 1982). This has led to the hypothesis that impairment of prepubertal mammary growth causes a lifetime reduction in the number of mammary secretory cells and hence lactation potential. It has also been suggested that prepubertal overfeeding may cause a permanent adjustment in nutrient partitioning within the animal, resulting in a propensity toward fat deposition rather than lactational performance (Capuco et al., 1995; Gaynor et al., 1995). Finally, it has been suggested that lactational effects of prepubertal nutrition can be largely attributed to effects on skeletal growth and body weight on lactational performance (Clark and Touchberry, 1962; Hoffman, 1997; Markusfeld and Ezra, 1993). These hypotheses are not mutually exclusive and effects of prepubertal nutrition on lifetime production may be a consequence of more than one of these factors, which in turn may be influenced by other management factors. This review will summarize the nature of mammary growth during the prepubertal period and hypotheses formulated to explain the potential impact of prepubertal nutrition on milk production.

Mammary Growth Prepubertally

Outwardly, little mammary growth is apparent during the prepubertal period and one might assume that mammary epithelial cells are proliferatively quiescent during this period. However, from birth to puberty the number of mammary cells increases approximately 1,000-fold and the mammary growth is said to be allometric in nature because it exceeds the general rate of body growth during this time (Figure 1) (Sinha and Tucker, 1969; Sinha and Tucker, 1969). Recent studies have utilized in vivo labeling with bromodeoxyuridine (a thymidine analog) to label cells in the DNA synthetic phase (S-
phase) of the cell cycle. These studies have shown that the proliferative rate of the mammary epithelium in prepubertal heifers is quite high (Capuco et al., 2002). The percentage of mammary epithelial cells in S-phase ranges from approximately 3 to 6%, depending upon stage of prepubertal development (Ellis and Capuco, 2002). The proliferation appears most rapid during 2 to 3 months of age, declining slightly after about 3 months and increasing again around the time of puberty (Figure 2) (Capuco et al., 2002). These observations suggest that the allometric phase of mammary development is initiated by 2 months of age, one month earlier than the often-cited value. After puberty, the number of mammary epithelial cells appears to remain relatively stable in nonpregnant heifers, with cyclical changes in cell number and a degree of lobular-alveolar development during the estrous cycle. Some cumulative net isometric growth occurs during the first cycles (Figure 1). It is during the period from birth to puberty that the framework of mammary ducts within the mammary gland is established. During estrous cycle activity there is some development of rudimentary alveoli and secretion of milk products in response to hormonal changes. It is only with onset of pregnancy that this rudimentary network differentiates to produce the extensive lobular-alveolar structures that are necessary for milk production.

Regulation of prepubertal mammary growth is dependent upon a number of hormones and growth factors, so that regulation is both systemic and paracrine in nature. Early research indicated that mammary growth during the prepubertal period is primarily influenced by estrogens and growth hormone (GH) or somatotropin (Cowie et al., 1966; Lyons, 1958; Wallace, 1953).

Figure 1: Net mammary growth from birth to peripubertal period. Mammary growth was evaluated as total mammary DNA. Adapted from: Sinha and Tucker (1969)
Estrogen secretion by the ovary appears necessary for prepubertal mammary growth in heifers. Ovariectomy of calves prevents mammary growth and normal mammary growth can be restored by estrogen administration (Wallace, 1953). This estrogen effect has been reinforced by studies in rodents, which extended the observation and demonstrated that estrogens are required for growth and morphogenesis of mammary ducts. Classical endocrine ablation/replacement studies in rats (Lyons, 1958) and more recent estrogen receptor knockout studies in mice (Bocchinfuso and Korach, 1997) indicated that estrogens are essential for the normal mammary ductal growth that occurs from birth to sexual maturity. Two major forms of estrogen receptor (ER) exist, and the receptor that mediates ductal growth and morphogenesis appears to be the ERα isoform (Bocchinfuso and Korach, 1997) rather than the ERβ isoform (Couse and Korach, 1999). In prepubertal heifers, both forms of receptor exist but the predominant isoform is ERα (Capuco et al., 2002) (Capuco et al. unpublished data). Furthermore, the ER is expressed in a subpopulation of epithelial cells only. After estrogen stimulation of mammary growth, proliferating cells were almost exclusively (>99%) ER-negative. Data suggest that proliferation in response to estrogen treatment was initiated within ER-positive epithelial cells of the developing mammary gland and that the signal was propagated in paracrine fashion to stromal cells and ER-negative epithelial cells. A paracrine mediation of estrogen-induced mammary ductal growth similarly has been concluded for regulation in mouse mammary gland, where ER is expressed in a portion of epithelial and stromal cells. In mice, tissue transplantation studies of epithelium and stromal elements between ERα knockout mice and wild-type mice support the conclusion that ERα-positive cells in the stroma stimulate growth of mammary epithelial cells through paracrine mechanisms (Bocchinfuso et al., 2000).

Growth hormone is an essential hormone for normal mammary ductal growth in rats and mice; the minimal hormone requirement for ductal growth being a combination of GH, estrogen and adrenal glucocorticoid (Kleinberg, 1997; Lyons, 1958). In ruminants, GH appears to be similarly important for administration of exogenous GH stimulates prepubertal mammary growth in heifers and ewes (Purup et al., 1993; Sejrsen et al., 1986). Many effects of GH on the mammary gland appear to be mediated by the insulin-like growth factors (IGF), most prominently IGF-I. Insulin-like growth factor-I has mitogenic

Figure 2: Proliferation of mammary epithelial cells from weaning to the peripubertal period. Heifers were injected with bromodeoxyuridine (BrdU; a thymidine analog) approximately 2 hours before slaughter. The percentage of epithelial cells that had incorporated BrdU and thus were synthesizing DNA was determined by quantitative immunohistochemistry. Each bar represents the mean for 3 to 12 heifers. Preliminary data: Meyer, Capuco, Hummel and Van Amburgh.

Growth hormone is an essential hormone for normal mammary ductal growth in rats and mice; the minimal hormone requirement for ductal growth being a combination of GH, estrogen and adrenal glucocorticoid (Kleinberg, 1997; Lyons, 1958). In ruminants, GH appears to be similarly important for administration of exogenous GH stimulates prepubertal mammary growth in heifers and ewes (Purup et al., 1993; Sejrsen et al., 1986). Many effects of GH on the mammary gland appear to be mediated by the insulin-like growth factors (IGF), most prominently IGF-I. Insulin-like growth factor-I has mitogenic
effects on mammary epithelial cells of numerous species (Akers et al., 2000). Although the liver is the primary source of circulating IGF-I, locally produced IGFs may be extremely important. Indeed, the importance of locally produced IGF-I is indicated by the demonstration of normal body growth of mice that do not produce hepatic IGF-I and have very low circulating concentrations of IGF-I (75-80% reduction), due to deletion of the hepatic IGF-I gene (Yakar et al., 1999). Rather than systemic IGF-I, locally produced IGF-I may be of primary importance for paracrine regulation of mammary gland function. Additionally, IGF-regulated functions are modulated by local production of IGF-binding proteins. However, it is possible that there are direct, IGF-independent, effects of GH on prepubertal mammary growth, as low levels of GH receptor have been demonstrated in bovine (Glimm et al., 1990) and rodent (Lincoln et al., 1995) mammary tissues.

In addition to actions of estrogen and GH/IGF-I on prepubertal mammary growth, a number of peptide factors and novel steroid receptors may play a role in modulating mammary growth through direct and indirect effects. Members of the epidermal growth factor (EGF) family are known to be key regulators of rodent mammmogenesis. Stromal cells produce EGF and it serves as an important paracrine regulator of ductal elongation in mice, as indicated by the fact that ductal elongation does not occur in EGF-receptor knockout mice (Wiesen et al., 1999). Other EGF family members, such as transforming growth factor-α (TGFα) and TGFβ appear to serve in various capacities to stimulate or inhibit growth, ductal branching, and differentiation in rodents (DiAugustine et al., 1997) and heifers (Plath et al., 1997; Plaut, 1993). Other growth factors, such as hepatocyte growth factor/scatter factor (HGF), keratinocyte growth factor (KGF), may also serve as regulators of mammogenesis in cattle (Purup et al., 2000). The roles of these growth factors, their regulation and interactions with other regulators of mammogenesis remain to be elucidated. It is clear that potentially important interactions occur. For example, there is evidence for interactions between the GH/IGF-I and estrogen axes. Growth hormone fails to stimulate ductal growth in ovariecetomized heifers (Purup et al., 1993), estrogen enhances GH-induced production of IGF-I by mammary stroma (Kleinberg, 1997) and GH induces ER expression (Feldman et al., 1999; Kleinberg et al., 2000) in rodents. Because of the importance of both GH and estrogens in mammogenesis, greater understanding of these interactions is critical to a more thorough understanding of mammary growth.

**Nutritional Effects on Mammary Growth During the Prepubertal Period**

Rapid rearing of replacement dairy heifers to decrease the age at sexual maturity is potentially advantageous in that heifers can begin milk production younger or at an increased body size. However, milk production of heifers calving at an early age is low (Gardner et al., 1977; Little and Kay, 1977; Swanson, 1967) and may be related to high-energy intake to permit early breeding (Little and Kay, 1977). More recent studies indicated that high energy consumption prepubertally, but not postpubertally, reduces growth of mammary parenchyma (Sejrsen, 1978; Sejrsen et al., 1982). The prepubertal period of sensitivity appears to coincide with the phase of allometric mammary growth, suggesting a time-frame of critical mammary development. Examination of a number of hormones that are known to affect mammary growth and development led to the finding that serum concentrations of GH are reduced in prepubertal heifers on a high plane of nutrition (Sejrsen et al., 1982). Because GH appears to be required for ductal growth in rodents and prepubertal administration of GH increased the amount of mammary parenchyma (Sejrsen et al., 1986), these data strongly implicated decreased GH concentrations as the endocrine mechanism accounting for decreased mammary growth in rapidly reared heifers.

By extension, it has been hypothesized that decreased parenchymal tissue at puberty equates to reduced lifetime milk production. However, the validity of this relationship has not been rigorously tested. Although Harrison et al. (Harrison et al., 1983) demonstrated a permanent reduction in mass and morphology of mammary parenchyma as a consequence of rapid prepubertal weight gain; only one study (Capuco et al., 1995) has quantitatively assessed the influence of diet on both prepubertal mammary...
development and on subsequent milk production. Additionally, most studies dealing with effects of overfeeding dairy heifers prepubertally have utilized high concentrate diets. Potential effects of diet composition during the prepubertal period of mammary development have only recently received attention (Capuco et al., 1995; Radcliff et al., 2000; Sejrsen and Foldager, 1992; Waldo et al., 1998). In addition to feed and energy intake, diet composition may alter energy deposition, influence secretion of key hormones or tissue responsiveness, and produce permanent effects on development of mammary and adipose tissues.

A study at the Beltsville Agricultural Research Center by Waldo and coworkers evaluated the impact of two diets and rates of gain on mammary growth and first lactation milk yields (Capuco et al., 1995; Waldo et al., 1998; Waldo et al., 1997). The diets compared were alfalfa or corn silage based diets, which differed in protein and energy content. The alfalfa silage diet contained 22% crude protein and 3.1 Mcal of digestible energy/kg of DM, whereas the corn silage diet provided 16% crude protein and 3.4 Mcal of digestible energy/kg DM. Differences in fat deposition were clearly evident in both carcass and mammary gland composition (Table 1). More fattening occurred in corn silage- than alfalfa silage-fed heifers and puberty was accelerated in the corn silage group. Greatest fattening occurred in heifers fed the corn silage diet for accelerated growth, and mammary parenchymal growth was reduced in this group. Consistent with Sejrsen’s hypothesis, decreased mammary parenchymal growth was correlated with

<table>
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reduced concentrations of GH in the circulation. Concentrations of IGF-I in the circulation were greatest in the group with reduced GH and parenchymal growth (accelerated growth rate, corn silage-fed), arguing against IGF-I mediated regulation of mammary ductal growth. However, IGF binding proteins may serve to alter tissue response to circulating IGF-I or locally derived IGF-I may be the primary regulator rather than the circulating hormone. Alternatively, GH may have direct mammogenic activity in the prepubertal mammary gland. Greater understanding of processes involved in the regulation of mammary growth in cattle is needed. It is clear that paracrine factors, including IGFs, act in consort with circulating hormones to regulate mammary growth, however the full extent of interactions remains to be elucidated (Akers et al., 2000; Purup et al., 2000).

Although accelerated rearing of heifers on a corn silage based diet caused excessive body fattening and had a negative impact on mammary parenchymal growth, there was no decline in first lactation milk yield (Table 1). Assuming that impaired mammary growth prepubertally accounts for the reduced milk production accompanying accelerated rearing on some experiments, one might hypothesize that there was compensatory mammary growth in the Beltsville study. This is not inconceivable, for the mammary gland exhibits a degree of plasticity, as evidenced by the compensatory growth and development of glands adjacent to a blind quarter. In fact, data indicate that a degree of compensation occurs even in those experiments where a permanent effect on milk production has been observed. The inhibition of mammary growth and cell number during the prepubertal period has been of greater magnitude than the negative impact on milk production observed in later life. Effect of high energy intake during the prepubertal period has been reported to reduce mammary cell number at the end of the allometric phase of mammary growth by 30 to 45% (Capuco et al., 1995; Sejrsen and Foldager, 1992; Sejrsen et al., 1982), whereas the milk production deficit reported for non-contemporaneous heifers reared with similar accelerated rates are in the order of 7 to 14% for recent investigations using modern U.S. Holsteins (Lammers et al., 1999a; Radcliff et al., 2000). Although data are consistent with the concept that accelerated rearing of dairy heifers can induce excessive fattening and inhibition of mammary growth during a critical period of udder development, data relating to the impact of these prepubertal events on mammary cell number and function during ensuing lactations are needed.

It has been suggested that crude protein is a limiting factor for developing accelerated heifer rearing programs (Van Amburgh et al., 1991; VandeHaar, 1997). By supplying a diet of high protein and energy from 4 months of age until luteal phase of the fifth estrous cycle, Radcliff et al. increased growth rate to 1200 g/d (controls at 800 g/d) without negatively impacting mammary development, and reduced age at puberty without impact on BW or skeletal size at puberty (Radcliff et al., 1997). Administration of GH to heifers on either high-gain or control diets increased BW, skeletal size and mammary growth (47%). In a subsequent experiment (Radcliff et al., 2000), heifers were reared on analogous diets for BW gains of 800 vs. 1200 g/d. A third group of heifers reared on the high-gain diet were injected daily with GH (25 µg/d). Heifers were bred after BW exceeded 363 kg and treatments (dietary and GH) were continued until pregnancy was confirmed. Heifers in both high-gain groups were 90 d younger than control heifers at first breeding and parturition. Postpartum BW, body condition score and skeletal size did not differ among treatments. Milk production of heifers reared for high rate of gain produced 14% less milk than heifers reared at the standard rate of gain, even though the diet was formulated for high protein content. However, GH treatment prepubertally prevented the decline in milk production observed in the high-gain group. These results were contrary to expectations. In light of results from their first experiment, it was hypothesized that the high-gain group would not produce less milk than heifers in the low gain group and that GH injection would increase milk production beyond that of heifers on the standard diet.

Van Amburgh et al. (1998) evaluated the impact of body weight gain and different sources of protein (fed at 18% CP) during the prepubertal period. Three growth rates were employed from 90 to 320
kg of body weight: 600, 800 and 1000 g/d. Among diets, energy was balanced to achieve the target rates of gain, and protein was formulated to meet ruminal nitrogen requirements and to exceed the tissue requirements. Ages at first calving were 24.5, 22.0 and 21.3 months for heifers reared at 600, 800 and 1000 g/d, respectively. First insemination occurred when heifers weighed 340 kg. Without regression analysis, heifers reared at 1000 g/d produced 9% less milk than those reared at 600 g/d. However, when calving weight was taken into account, there was no difference in milk production due to rate of gain or protein source. Posttreatment factors, such as postcalving weight, accounted for more variation in milk yield than prepubertal BW gain. This of course emphasizes the importance of postpubertal heifer management. However, the impact of accelerated postpubertal body weight gain has provided conflicting results. Finally, no effects of prepubertal dietary treatments were detected for milk yield of cows in second lactation.

Another recent study evaluated the effects of accelerated gain (700 vs. 1000 g/d) and estrogen (estradiol) treatment prepuber tally (4.5 to 9.5 months of age) on mammary development and milk production (Lammers et al., 1999a; Lammers et al., 1999a; Lammers et al., 1999b). The experimental rationale was to determine if estrogen treatment could be used to increase prepubertal mammary growth and overcome potential negative effects of accelerated body growth on milk production. Only indirect, non-invasive measurements of mammary development were employed. Although, data suggested that estrogen treatment enhanced mammary development during the treatment period, the effects were subsequently lost. Consistent with its apparent increase in mammary gland growth, estrogen treatment increased circulating concentrations of GH and IGF-I during the treatment period. However, both accelerated rate of gain and estrogen treatment prepubertally decreased first lactation milk yield (7.1 and 5.2%, respectively), with heifers being of similar body weight at calving regardless of treatment.

Knowledge of the interactions between rate of gain, the GH/IGF axis and estrogen axis are essential to our understanding of prepubertal development. We and others have hypothesized that negative impact of accelerated heifer rearing on mammary development may be partly mediated by estrogen. As summarized earlier, GH stimulates ER expression. Consequently, decreased GH concentrations in high-gain heifers may cause decreased expression of ER in epithelial cells and a reduction in epithelial sensitivity to the mitogenic effects of estrogen. A recent study evaluating the impact of GH treatment on ER expression, found no change in the percentage of cells expressing ER by immunohistochemistry, but protein and transcript levels were not evaluated (Berry et al., 2003). We are currently evaluating ER expression during prepubertal development and the impact of accelerated rearing on this expression.

Experimental results suggest that early postnatal life is the most sensitive for producing effects that are manifested by decreased milk production during subsequent lactations. Perhaps if feeding for rapid weight gain is delayed for a few months, impaired mammary growth prepubertally is not irreversible and can be compensated by mammary growth during gestation so that negative effects on milk production are not evident. Conversely, although GH can increase prepubertal mammary growth by nearly 50%, this effect is seldom manifested by increased milk production (Sejrsen et al., 1999) and a similar relationship may be true for estrogen stimulation of prepubertal mammary growth (Lammers et al., 1999a). Are effects on mammary growth subtle, or are the observed prepubertal growth effects red herrings and the underlying cause of reduced lactation potential as yet to be discovered?

If underlying changes by which alterations in mammary growth prepubertally exert effects on subsequent lactation are subtle, perhaps greater insight can be gained by knowledge of the underlying proliferative population in the prepubertal bovine mammary gland. We recently characterized the proliferative epithelial populations on the basis of BrdU labeling and differential staining characteristics (Ellis and Capuco, 2002). The proliferative populations in the prepubertal bovine gland consist of two classes of lightly staining cells in histological sections that may reflect adult stem cells and their progeny.
of progenitor cells. Total number or lineage of these cells may be altered by nutritional “overfeeding” so that their ability to fully populate or maintain a cohort of fully differentiated secretory cells in the mammary gland throughout lactation is impaired. Further characterization of these progenitor cells and their regulatory mechanisms may provide opportunities to alter proliferation and cell renewal in prepubertal and mature cattle (Capuco et al., 2003).

Nutritional Programming

Prepubertal dietary regimen may influence secretion of key hormones or tissue responsiveness, thus producing permanent effects on development of mammary and adipose tissues or endocrine controls of lactogenesis and lactation. Certainly nutritional deficiencies can impact lifetime function of an organ, such as brain, by failure to supply necessary nutrients for complete development of the organ in question. However, it has been suggested that nutrients might serve as critical signals for more subtle developmental effects, such as by affecting stem cell proliferation and thereby permanently altering the cellular composition of a tissue or organ (Lucas, 1998). Lifetime metabolic patterns may be altered. For example, rats fed low protein diets during gestation gave birth to pups with greater gluconeogenic capacity (Desai et al., 1995), an early postnatal shift in energy supply to suckling rats from a fat-rich milk to carbohydrate rich caused metabolic programming that resulted in permanent hyperinsulinemia, adult onset diabetes and obesity (Srinivasan et al., 2003), and protein restriction of suckling dams caused a permanent alteration in lipid metabolism of the neonate, resulting in permanent reductions in cholesterol and triacylglycerol concentrations (Lucas et al., 1996). The critical windows for these effects are restricted to the period of gestation or early postnatal life.

Whether such metabolic programming occurs in cattle has received scant attention. An experiment was designed to evaluate the effects of rate of BW gain and type of silage before puberty on the partitioning of excess dietary energy between synthesis of milk and BW gain (Gaynor et al., 1995). Cows that had been fed at two rates of gain (725 vs. 950 g/d) from 175 to 325 kg of body weight on an alfalfa or corn silage based diet were used. Lactating cows were switched from a control to a high-energy diet according to a double-reversal experimental design with 6-week periods. Neither body weight gain nor diet influenced the magnitude of change in DMI, milk yield, milk composition or circulating hormone concentrations. These data argue against a metabolic programming induced by accelerated heifer rearing. However, because there was no effect of the prepubertal treatment on milk yield, the results do not fully address whether metabolic programming can explain potential reductions in milk yield from heifers subjected to accelerated prepubertal growth rates.

Body Growth Mediation of Lactational Effects Resulting from Prepubertal Nutrition

A goal of rearing replacement dairy heifers is to decrease the interval from birth to first calving, but to do so while promoting skeletal growth and minimizing negative impacts on lactation potential. It is well established that there is positive relationship between body weight at calving and milk production in first lactation dairy cows (Clark and Touchberry, 1962; Hardville and Henderson, 1966; Hoffman, 1997). First lactation milk yield appears to be maximal for Holstein heifers weighing between 590 and 635 kg at calving (Kewon and Everett, 1986). Furthermore, adequate skeleton size is needed to minimize dystocia during the first parturition and is more positively related to first lactation milk yield than body weight (Markusfeld and Ezra, 1993; Sieber et al., 1988). The majority of skeletal growth occurs during the prepubertal period (Heinrichs and Hargrove, 1987) when rates of withers and hip height growth are as much as 3-fold greater than after puberty (Barash et al., 1994). Relative rates of skeletal growth as measured by changes in withers or hip height decrease gradually over time from as much as 5 cm/month at 2 months of age to around 1 cm/month during the post-pubertal period (Heinrichs and Hargrove, 1987). This suggests that the greatest opportunity for enhancing skeletal growth is during the prepubertal period. Increases in rates of skeletal and body weight in prepubertal dairy heifers can be achieved by increasing
the energy density of diets. However increasing body weight gain above 1 kg/d reduces mammary parenchymal tissue and increases mammary fat deposition (Capuco et al., 1995; Sejrsen et al., 1982), and both factors are associated with lower milk production during the first lactation. Thus methods to increase skeletal growth rates without increasing fat deposition might provide appropriate strategies to accelerate growth without detrimental effects of accelerated growth on mammary development.

Somatotropin, particularly when used in the presence of increased intestinal protein such as abomasal infusion of casein (Houseknecht et al., 1992) has been shown to enhance N retention in Holstein steers, suggesting that lean tissue and possibly skeletal deposition may be enhanced by combined treatment with bST and dietary rumen undegradable protein (RUP). Previous studies demonstrated that prepubertal treatment with recombinant bovine somatotropin (bST) enhances mammary growth (Sejrsen, 1994; Tucker, 1987) and increases withers height at puberty (Grings et al., 1990; Radcliff et al., 1997). Thus, bST in combination with added RUP might provide a practical means to optimize skeletal growth rates during the prepubertal period without having negative impact on mammary development.

This provided the objective of a recent study at the University of Maryland involving 72 dairy heifers. Treatments consisted of a control (Control), 2% added dietary RUP (RUP), recombinant bST (100 µg/kg BW per day) and the combination of RUP and bST (RUPbST) that were applied from 90 days of age through onset of puberty. The added RUP diets contained 16.9% CP with 7.9% RUP (DM basis) compared with 14.9% CP and 5.9% RUP, respectively in the control diet. Thirty-two of the 72 heifers provided a subset of animals for slaughter measures. Eight heifers were killed at 3 months of age (beginning of treatment period) and 3 heifers per treatment were killed at 5 months and 10 months of age. The remaining 40 heifers provide body growth and milk production data.

There were significant treatment by age interactions for rates of body weight and skeletal growth as shown in Figures 3 to 4 (Moallem et al., 2001a; Moallem et al., 2001b; Moallem et al., 2001c). Average daily gain in body weight across treatments increased from 630 g per day at 90 days of age, to 1320 g/d at 330 days of age. There was a RUP by age interaction (P < .001) where growth rates at 90 days were maximized by RUP addition alone while at 330 days of age, the combination of bST and RUP were required to maximize body weight growth (Figure 3). Smaller responses were shown at 90 days for bST treated animals. After approximately 200 days of age, the combination of RUP and bST was the only treatment that resulted in significant increases in rates of body weight growth. During the treatment period, average daily body weight gains were increased by both RUP and bST, with the treatments having additive effects. Body weights at 330 days were 311, 318, 328 and 355 kg for control, bST, RUP and RUPbST, respectively. Effect of RUP on body weight gain was significant (P < .05). In comparison to rates of body weight gain, rates of withers height growth decreased with increasing age. Withers height growth rates (Figure 4) decreased from .15 to .11 cm per day as age increased from 90 to 330 days, respectively. There was an age by RUP × bST interaction (P < .001) for withers height growth rates, where RUP alone increased growth rates early but not at the end of the prepubertal period. At 330 days of age withers heights were 115.9, 117.4, 119.4 and 120.7 cm for control, bST, RUP and RUPbST, respectively. Effect of RUP on withers growth rate was significant (P < .05). Thus, both body weight and skeletal growth rates were increased at an early age by RUP addition to the diet while responses to RUP were small at later stages of the prepubertal period. In contrast, the effect of bST on rates of body weight and skeletal growth were small early but grew larger as the heifers matured. These data suggest that protein but not bST was limiting during the early post-weaning period of development while bST may have been limiting at 250 to 300 days of age, typically the time at or just prior to puberty.
Figure 3. Rate of body weight gain of heifers fed control (Control), injected with bovine somatotropin (bST), fed added rumen undegradable protein (RUP), or the combination of RUP and bST (bST-RUP) from 90 d until puberty.

Figure 4. Rates of withers height gain of heifers fed control (Control), injected with bovine somatotropin (bST), fed added rumen undegradable protein (RUP), or the combination of RUP and bST (bST-RUP) from 90 d until puberty.
RUP addition to the diet and bST administration successfully accelerated body and skeletal growth rates, without producing deleterious effects on mammary growth (P > .1)(Capuco et al., 2001). Consequently, milk production for all groups was equivalent (P > .1, Table 2). With decreased onset of puberty, heifers in treatment groups could be bred at an earlier age than controls. However, due to management problems, not all heifers were bred in timely fashion. Nonetheless, age at first calving was significantly reduced in RUPbST treatment group (Table 2). Data suggest that incorporation of dietary RUP and bST administration into an accelerated heifer-rearing program can provide for larger framed heifers without associated loss of milk production or increased dystocia. RUP supplementation should be initiated during early prepubertal growth, whereas bST treatment can be delayed until 8 months of age, with both treatments being continued through 11 months of age and onset of puberty.

Table 3. Least squares means for subsequent first lactation performance of heifers fed control (Control), injected with bovine somatotropin (bST), fed added rumen undegradable protein (RUP), or the combination of RUP and bST (RUPbST) from 90 d until puberty.

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<td>268</td>
<td>310</td>
<td>294</td>
<td>387</td>
<td>15.6</td>
<td>bST .233 RUP .892 RUP*bST .101</td>
</tr>
<tr>
<td>Fat %</td>
<td></td>
<td>3.94</td>
<td>3.71</td>
<td>3.72</td>
<td>3.88</td>
<td>.171</td>
<td>bST .826 RUP .883 RUP*bST .228</td>
</tr>
<tr>
<td>Protein %</td>
<td></td>
<td>3.01</td>
<td>3.00</td>
<td>3.08</td>
<td>2.96</td>
<td>.051</td>
<td>bST .212 RUP .810 RUP*bST .234</td>
</tr>
</tbody>
</table>

¹ Standard error of the difference.

Summary

Rapid rearing during the prepubertal period can result in decreased milk production and increased calving difficulty. Data suggest that mammary gland development is inhibited when excessive body fattening occurs. The mechanisms by which fattening prepubertally impacts milk production are uncertain but may be due to a reduction in the number of secretory epithelial cells during lactation, nutrient programming, or negative impact on body size. Factors that may impact development and proliferation of progenitor cells within the mammary gland may be key to understanding mammary gland biology of replacement heifers and to improving production efficiency through direct effects on the mammary gland. Accelerated body growth in the absence of excessive fattening can be achieved with appropriate nutritional management to provide sufficient protein and energy to ensure balanced growth. This can be aided by GH supplementation. Such regimens can be used to achieve early calving, maximal mammary development prepubertally, increased skeletal growth and maximal or near maximal milk production.
References


NATURAL PRODUCTS FOR MANIPULATION OF FERMENTATION IN RUMINANTS

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Introduction

Legislators in Europe have moved to prohibit the use of growth-promoting antibiotics in animal feeds from the end of 2005. This decision was based on public and political concerns that the heavy use of antibiotics in general can give rise to transmissible resistance factors that can compromise the potency of therapeutic antibiotics in man. Growth promotion was a clearly avoidable use. US legislators may soon follow suit. Whether many of the commonly used growth promoters present such a threat is the subject of intense debate, nevertheless livestock producers in many countries must face a future without antibiotic growth promoters. Problems may be more acute in pig and poultry production, but ruminants will also be affected in the sense that existing and potential new strategies for manipulating rumen fermentation must avoid selective antimicrobials. Organically produced meat and milk are increasing in demand by consumers, and organic farmers therefore face the same problems. Thus, there is increasing interest in exploiting natural products, which have no similar public health hazard, as feed additives to solve problems in animal nutrition and livestock production. The ‘natural’ products include probiotics, prebiotics, enzymes, organic acids, and secondary plant compounds or their nature-identical chemicals.

A huge variety of secondary compounds are produced by plants as natural protection against microbial and insect attack. Some are toxic to animals too, but others may not be, indeed many have been used in the form of whole plants or plant extracts for food or medical applications in man. The potential of one class of plant secondary compounds as beneficial feed additives in ruminant production are described in this paper, namely essential oils.

Essential oils are steam-volatile or organic-solvent extracts of plants, used traditionally by man for many centuries for the pleasant odor of the essence, or its flavor, or for its antiseptic and/or preservative properties. Although commonly thought of as being derived from herbs and spices, they are present to some degree in many plants for their protective role against bacterial, fungal or insect attack. They comprise mainly cyclic hydrocarbons and their alcohol, aldehyde or ester derivatives (Table 1).

Table 1. Some common essential oil compounds.

<table>
<thead>
<tr>
<th>Hydrocarbon monoterpenes</th>
<th>Oxygenated monoterpenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>Thymol</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>Eugenol</td>
</tr>
<tr>
<td>Limonene</td>
<td>Citronellol</td>
</tr>
<tr>
<td>Terpinene</td>
<td>Terpeneol</td>
</tr>
<tr>
<td>Cymene</td>
<td>Geranyl acetate</td>
</tr>
<tr>
<td>Myrcene</td>
<td>Linalool</td>
</tr>
<tr>
<td>Camphene</td>
<td>Fenchyl alcohol</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>Citronellal</td>
</tr>
</tbody>
</table>
As the power of antibiotics became apparent, research into the action of essential oils declined. However, interest has increased once again because essential oils are perceived to be natural alternatives to chemical biocides and, in some applications, antibiotics. Recently, for example, useful effects of essential oils have been demonstrated against pathogenic bacteria. *E. coli* O157:H7 was inhibited by oregano oil (Elgayyar *et al.*, 2001), peppermint oil (Imai *et al.*, 2001) and essential oils from other herbs (Marino *et al.*, 2001). Oils from *Cinnamomum osmophloeum* have been shown to possess antibacterial activity against *E. coli*, *Enterococcus faecalis*, *Staphylococcus aureus* (including the clinically problematic methicillin-resistant *S. aureus*), *Salmonella* sp. and *Vibrio parahemolyticus*; cinnamaldehyde was the main antibacterial component of the mixture (Chang *et al.*, 2001). Several aromatic plants, mainly *Eucalyptus* spp., indigenous to the Democratic Republic of Congo had potentially useful medicinal effects against *Pseudomonas aeruginosa*, although the effectiveness of different plants could not be correlated with the content of any major constituent of the oils (Cimanga *et al.*, 2002). *Helicobacter pylori* was highly sensitive to spearmint oil (Imai *et al.*, 2001). Essential oils are potent against a wide range of oral bacteria (Shapiro *et al.*, 1994), and they are used widely in antiseptic mouthwashes.

With this background of antimicrobial activity, we have evaluated essential oils for possible beneficial effects on ruminal fermentation. Essential oils from plants were examined many years ago with ruminal bacteria, assessing the possibility that essential oils contributed to poor palatability in some plant species (Oh *et al.*, 1968). General inhibitory activity was found across a range of plant materials. Oh *et al.* (1967) demonstrated that individual oils had different effects on mixed ruminal bacteria. Monoterpene hydrocarbons were less toxic and sometimes stimulatory to microbial activity compared to the corresponding oxygenated compounds, the monoterpene alcohols and aldehydes (Oh *et al.*, 1967). The sensitivity of ruminal bacteria to essential oils of *Artemisia tridentata* (big sagebrush) was the same in captive deer as it was in wild deer, which was suggested to mean that ruminal bacteria did not adapt to essential oils (Nagy & Tengerdy, 1968). Thus, essential oils were not necessarily toxic to ruminal bacteria, and if they had a beneficial effect, that effect might be expected to persist.

In order to determine the effects of essential oils on ruminal fermentation and ruminal microorganisms, we have undertaken three animal trials with essential oils, and also investigated their effects on individual rumen microbial species *in vitro* and to a limited extent *in vivo*. Many of these results were reported in Wallace *et al.* (2002).

**Experiment 1: Feeding Essential Oils to Sheep Receiving a Mixed 40:60 Concentrate:Grass Silage Diet.**

Four ruminally fistulated sheep receiving a maintenance diet comprising 40% concentrate and 60% grass silage. Each sheep received 100 mg per day of a specific blend of essential oils (CRINA Ruminants, Akzo Nobel Surface Chemistry, Hertfordshire, UK) or the control diet in a 6-week latin square design. Proteinase, peptidase and deaminase activities were measured as described elsewhere (Floret *et al.*, 1999). Nylon bag incubations were done according to Mehrez & Ørskov (1977).
Essential oils had no influence on VFA or NH$_3$ concentrations, on protozoal numbers, or on microbial protein flow. The rate of degradation of soybean meal tended to be decreased at 8 and 16 h (Figure 1), but there was no effect on rapeseed meal breakdown (not shown). The breakdown of ground barley suspended in nylon bags in the rumen was also inhibited by essential oils (Figure 2). The breakdown sequence of protein to NH$_3$ was measured by assaying the rates of the individual reactions in ruminal fluid \textit{in vitro}. Ammonia formation was affected only at the last step, namely the deamination of amino acids (Figure 3).

Figure 1. Influence of dietary essential oils on the loss of soybean meal from nylon bags suspended in the rumen.

![Figure 1](image1)

Figure 2. Influence of dietary essential oils on the loss of ground barley from nylon bags suspended in the rumen.

![Figure 2](image2)
Experiment 2: Feeding Essential Oils to Cattle Receiving a Mixed 40:60 Concentrate:Maize Silage Diet.

Four ruminally fistulated dairy cows received a total mixed ration made up of grass silage, maize silage and concentrate. Effects of essential oils on the sequence of protein catabolism were assessed as before, and the results were similar, in that only the final step, which is the breakdown of amino acids to NH$_3$, was inhibited in cows receiving dietary essential oils (not shown). The final step, deamination of amino acids, was assessed further by including incubations to which monensin had been added (Table 2). Again, dietary essential oils caused a decrease in the rate of NH$_3$ production. Monensin addition to the in vitro incubations caused a larger decrease in deaminative activity of ruminal fluid from both control and essential oil-supplemented cows; the decreased activity no longer showed a difference between the two groups, indicating that the species affected by dietary essential oils were also affected by monensin - an overlapping mode of action, with monensin being the more potent.

Table 2. Rates of NH$_3$ production from Trypticase in vitro of ruminal digesta from sheep receiving dietary essential oils. 5 µM monensin was added to one set of incubations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NH$_3$ production rate (nmol/mg microbial protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>None</td>
<td>410</td>
</tr>
<tr>
<td>Monensin</td>
<td>280</td>
</tr>
</tbody>
</table>

Several additional incubations were carried out in this experiment. In order to determine if the breakdown of different proteins was affected by essential oils in different ways because of their different structures, several pure proteins were labeled using $^{14}$C-formaldehyde (Wallace 1983) and incubated in ruminal fluid in vitro. It emerged that essential oils had no influence on the breakdown of the different proteins, whose rates of breakdown varied by two orders of magnitude (not shown). Thus, it was concluded that essential oils had no influence on the first step of protein breakdown, namely proteolysis.
Peptidase activity was assessed by the breakdown of Ala\textsubscript{5} and Ala\textsubscript{2} as before, and also by the hydrolysis of Ala\textsubscript{2}-p-nitroanilide and GlyArg-4-methoxynaphthylamide, synthetic substrates for the two main dipeptidyl peptidase activities in ruminal fluid (Wallace et al., 1999). Once again, neither activity was affected by the dietary treatment. There seemed, therefore, to be no role for essential oils in controlling peptide metabolism in the rumen, although acute effects \textit{in vivo} were subsequently shown to be a possibility (see below). Proteolysis was unaffected, even though there had been a slight indication from Experiment 1 that the breakdown of protein supplements – rather than pure proteins – might be affected.

**Experiment 3: Feeding Essential Oils to Sheep Receiving Diets of Differing Protein Content.**

In this experiment, two concentrate:grass silage diets were used, one with a high protein content (17.7\%), the other with a lower protein content (13.7\%). Additional measurements were made in which the rate of breakdown of different protein meals was measured both \textit{in vivo} and \textit{in vitro} and the colonization of feedstuffs incubated in nylon bags was assessed by attached enzyme activity (Silva et al., 1987). Of the protein meals tested, essential oils had a significant effect only on the breakdown of pea meal, the most rapidly degraded meal (Figure 4). Proteinase activity associated with the supplements, a measurement of the attached proteolytic microflora, decreased significantly with pea and rapeseed meals, but the decrease was small (Figure 5). There was no effect of essential oils in the same animals receiving a high-protein diet. Glutamate dehydrogenase activity, a measure of total microbial colonization, associated with grass hay suspended in the rumen was decreased by 47\% with essential oils, while colonization of the less degradable fibrous substrates, grass silage and barley straw, decreased, but the effect was small and not statistically significant. Carboxymethylcellulase (CMCase) activity, a measure of the fibrolytic population, was unaffected. These data suggest that essential oils may suppress the colonization and/or digestion of readily degraded substrates without affecting fiber digesters. Thus,
colonization of proteinaceous substrates may be affected by essential oils, leading to a consequent decrease in protein breakdown, independent of any direct effect on proteolysis.

In this trial, the total and so-called ‘hyper-ammonia-producing’ (HAP) bacteria (see below), which include *C. sticklandii* and *P. anaerobius* (Russell *et al.* 1991), were enumerated by their ability to grow on a tryptic digest of casein as sole source of C and N. The total viable count of bacteria was unaffected, but the numbers of HAP bacteria decreased by 77% in sheep receiving the low-protein diet. In these trials, essential oils had no significant effect on protozoal numbers or activity.

**Sensitivity of Ruminal Microorganisms to Essential Oils.**

Many of the predominant species of ruminal bacteria were tested in pure culture for growth in a range of concentrations of essential oils. *Butyrivibrio fibrisolvens, Clostridium aminophilum, Escherichia coli, Eubacterium ruminantium, Lachnospira multipara, Megasphaera elsdenii, Mitsuokella multiacidus, Prevotella albensis, Prevotella brevis, Prevotella bryantii, Ruminococcus albus, Ruminococcus flavefaciens, Selenomonas ruminantium, Streptococcus bovis and Veillonella parvula* were insensitive to 40 ppm essential oils in medium M2 (Hobson 1969). Only *Clostridium sticklandii, Prevotella ruminicola* and *Peptostreptococcus anaerobius* were prevented from growing at 40 ppm essential oils. *Methanobrevibacter smithii*, a methanogen related to those found in the rumen, was similarly unaffected. *Ruminobacter amylophilus* grew in the presence of 40 ppm essential oils, but the oils greatly enhanced its lysis in stationary phase, so it may be considered also to be sensitive to essential oils. Although growth of *Prevotella albensis*, another prominent proteolytic and peptidolytic *Prevotella* sp., was unaffected by essential oils, its rate of ammonia formation from peptides was decreased by 38%, suggesting an acute effect of essential oils – perhaps inhibiting certain aspects of metabolism without suppressing growth.

Thus, the bacteria most sensitive to essential oils were HAP species, *Prevotella* spp. and *R. amylophilus*. HAP bacteria have a high capability to generate NH$_3$ from amino acids (Russell *et al.*, 1991). They comprise only around 1% of the rumen bacterial population, however, and the fact that monensin inhibits only 32% of total NH$_3$-forming activity suggests that their role is not the main one in deamination, and that other, monensin-insensitive bacteria are mainly responsible. Nevertheless, even a small decrease in the rate of NH$_3$ production may be beneficial nutritionally, so the suppression of these
species would be expected to be significant to livestock production. Essential oils had less effect on
deamination than monensin, presumably because essential oils affect fewer bacterial species. *Prevotella*
spp. are involved in all of the steps of protein catabolism (Stewart et al., 1997). *R. amylophilus* is a highly
active starch and protein digester which proliferates on concentrate diets (Stewart et al., 1997). Therefore,
pure-culture results are consistent with observations which have been made in vivo. Whether these effects
translate into improved productivity will depend on animal and dietary factors, of which little
experimental evidence is as yet available.

**A Proposed Mode Of Action For Essential Oils In The Rumen**

The effect of dietary essential oils on NH₃ production from amino acids, on HAP bacteria in pure
culture, and on HAP numbers in vivo are all consistent with a primary effect of essential oils on HAP
bacteria. As HAP species vary from diet to diet and perhaps geographically (Attwood et al., 1998;
McSweeney et al., 1999; Eschenlauer et al., 2002), it may be important to look in more detail at the full
range of HAP bacteria affected; it is also important to identify which of the multiple components of the
commercial essential oils mixture is responsible for the effect.

The effects on proteolysis and the breakdown of protein supplements are less clear, although a
reasonable hypothesis can be advanced. The effects of essential oils on the protein supplements may be
mediated partly via *R. amylophilus*, but because *R. amylophilus* is amylolytic as well as proteolytic, it
may be the loss of the amylolytic activity from the consortium digesting the supplement that affects the
colonising microbial consortium more than the loss of the contribution of *R. amylophilus* to proteolysis.
That would explain why the degradation of rapidly degraded starchy protein meals was affected by
essential oils, but less rapidly degraded starchy meals or non-starchy protein supplements were not
affected. The effects of essential oils on *Prevotella* spp. indicated that growth might not be affected by
essential oils in pure culture, and peptidase activity was unaffected in the mixed culture, yet ammonia
production was inhibited when essential oils were added to cultures of *P. albensis* in vitro – an acute
rather than an adaptive effect.

Figure 6. A scheme which describes the mode of action of essential oils in terms of the species affected and whether the response is adaptive or acute.
The effects of essential oils on colonisation were clearer. The observed effects of essential oils on the breakdown of solid materials, particularly starchy or proteinaceous supplements, may stem from a decreased adhesion to readily digested solids; alternatively, the rate of development of solids-associated bacteria - once attachment has already occurred – may be inhibited. The exact cause is as yet unclear. Thus, essential oils may have several independent actions, depending perhaps on individual oils within the mixture, and they may be related to each other in their biochemical consequences (Figure 6).

Prospects for Essential Oils and Other Plant Secondary Compounds as Feed Additives

Essential oils form only one group of plant secondary compounds that might be useful in manipulating ruminal fermentation in a positive way. Saponins, like essential oils, cover a wide variety of chemical compounds and, also like essential oils, their properties have been used by man for centuries. A considerable amount of research has been carried out with saponins in ruminants. Potentially, their beneficial effects include suppression of ciliate protozoa (Diaz et al., 1994; Navas-Camacho et al., 1993; Newbold et al., 1997; Odeny et al., 1997) and the proliferation of S. bovis (Wallace et al., 1994; Wang et al., 2000). Among the polyphenolic compounds, tannins have received most attention in ruminant nutrition (Cheeke, 1998), because they bind to protein and may slow the degradation of those proteins which are degraded too rapidly in the rumen. Alkaloids, flavonoids, glycosides, amines and non-proteinic amino acids also provide defensive functions in plants, and their potential needs to be examined. Indeed, arguably the best route would be not to anticipate what the effective chemical components might be, but instead to examine a wide range of plant species, favouring those which have been used, for example, as herbal remedies, but not excluding others which have no such traditional use. In these investigations, care will have to be taken that saponins or other natural additives, do not damage the product. The plant materials must not damage the acceptability of milk or meat, by altering flavour or product quality. Above all, the safety of the additives to the animals themselves and to the consumer who eats the products must be beyond doubt.

Much of the emphasis with essential oils, saponins and the plants which contain these compounds has been directed in ruminants towards more efficient nitrogen retention. There are other objectives which should be addressed as well in order to maximise the usefulness of phytochemicals in ruminant nutrition. Antibacterial as well as antiprotozoal effects are worthy of future consideration. These include altering biohydrogenation, in order to decrease methane formation or to lower the saturated fatty acid content of rumen digesta and hence ruminant products; improving animal welfare, in terms of controlling bloat or lactic acidosis, which is also often a higher priority than increasing feed efficiency; finally, the control of pathogens in the food chain must be an objective that could be achievable with phytochemicals or suitable plants, and one that would meet with the approval of consumers whose faith in ruminant products has been shaken in the last decade.

Acknowledgment

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References


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A MODEL TO DESCRIBE RUMINAL METABOLISM AND INTESTINAL DIGESTION OF FATTY ACIDS

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Summary

Fats provide more than calories! They provide specific polyunsaturated fatty acids that participate in a host of metabolic reactions that can have an impact on dairy cow metabolism. No dairy nutrition model describes fat correctly. Fatty acids and not ether extract are the nutritional entities of importance. However, all models operate on ether extract and not fatty acids. Dairy-NRC (2001) attempts to estimate fatty acids empirically from ether extract. No model considers specific fatty acids. Instead, they consider dietary lipid as a single entity. In addition, they ignore the transformation processes that affect dietary fatty acids in the rumen and they generally have a very simplistic treatment of de novo production of fat within the rumen. Intestinal digestibility is also handled in a simplistic manner. We developed a lipid sub-model that operates within CPM-Dairy to describe ruminal metabolism and intestinal digestion of long chain fatty acids (LCFA). The fat model focuses on the major LCFA (C12:0, C14:0, C16:0, C16:1, C18:0, C18:1cis, C18:1trans, C18:2, C18:3). Ruminal lipolysis (defined as enzymatic cleavage of ester linkages and dissociation of salts of fatty acids) varies between feeds. Megalac (calcium salts of palm oil fatty acid distillate) was the only ingredient that showed appreciable protection against lipolysis. Lipolysis is a pre-requisite for ruminal biohydrogenation. Polyunsaturated fatty acids, especially C18:2, appear to have an energy independent effect on improving reproduction. However, less than 10% of the C18:3, C18:2 and C16:1 that are in the form of free fatty acids will escape biohydrogenation. C18:1t, which is associated with decreased mammary synthesis of fat, accumulates in the rumen when there is incomplete biohydrogenation to C18:0. De novo synthesis of fatty acids occurs, but as fatty acid intake increases, the extent of de novo synthesis declines as a result of increased uptake of LCFA by microbes. There is insufficient data to model the effect of rumen active fat on rumen digestion and fermentation so we suggest using the advisory of Jenkins (1997), which is based on unsaturation of LCFA and ration fiber, for the upper limit. Most of the LCFA flowing to the small intestine are free fatty acids but there are also non-lipolysed fatty acids in the form of glycerides and calcium salts and fatty acids in bacteria. In general, intestinal digestion of free fatty acids and non-lipolysed fatty acids in forages, grains, proteins, whole cottonseed, and cracked or ground soybeans and other oil-seeds are similar. Digestion of non-lipolysed fatty acids in tallow, hydrogenated tallow, grease, vegetable oils, animal/vegetable blends, whole-intact soybeans and other whole-intact oil-seeds is less than digestion of free fatty acids. In particular, digestion of non-lipolysed C18:0 in some feeds, like hydrogenated tallow, is zero. Digestion of non-lipolysed fatty acids in the form of calcium salts is greater than digestion of free fatty acids. To increase absorbed amounts of LCFA like C18:2 and C18:3, they must either be in a form that protects them from lipolysis or large amounts must be fed. The latter, however, can lead to accumulation of C18:1t that is associated with decreased mammary synthesis of fat.

Introduction

Fats provide more than calories! They provide specific polyunsaturated fatty acids that participate in a host of metabolic reactions that can have an impact on dairy cow metabolism (Jenkins, 2002; Sanchez
and Block, 2001). This is the most exciting development in ruminant nutrition since we recognized that proteins provide amino acids.

For some years now, it has been evident that dairy cow nutrition and nutrient management models are vital to the continued success of the dairy industry. We have computer programs like CPM-Dairy (Boston et al. 2000), CNCPS (Fox et al. 2000) and NRC-Dairy (2001) that allow us to balance rations on the basis of amino acids but no dairy nutrition model describes fat correctly. For example, CPM-Dairy and CNCPS operate on ether extract and not fatty acids. Dairy-NRC (2001) estimates fatty acids empirically from ether extract. No model considers specific fatty acids. Instead, they consider dietary lipid as a single entity. In addition, they ignore the transformation processes that affect dietary fatty acids in the rumen and they generally have a very simplistic treatment of de novo production of fat within the rumen. Intestinal digestibility is also handled is a simplistic manner. In CPM-Dairy and CNCPS, absorbed fat is 95% of the ether extract entering the small intestine. The NRC-Dairy model employs different digestion coefficients for total LCFA from different sources and discounts digestibility as dry matter intake increases above maintenance. However, NRC-Dairy assumes that for diets containing 3% or less ether extract, the digestibility of total LCFA is 100%.

### Table 1. Acronyms, their units and definitions used in the fat sub-model

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Unit</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>decimal</td>
<td>Adjustment factor for the effect of RFLCFA on biohydrogenation</td>
</tr>
<tr>
<td>BHL(CFA)</td>
<td>g/d</td>
<td>Amount of specific LCFA produced by biohydrogenation</td>
</tr>
<tr>
<td>C12:0</td>
<td></td>
<td>Lauric acid</td>
</tr>
<tr>
<td>C14:0</td>
<td></td>
<td>Myristic acid</td>
</tr>
<tr>
<td>C16:0</td>
<td></td>
<td>Palmitic acid</td>
</tr>
<tr>
<td>C16:1</td>
<td></td>
<td>Palmitoleic acid</td>
</tr>
<tr>
<td>C18:0</td>
<td></td>
<td>Stearic acid</td>
</tr>
<tr>
<td>C18:1c</td>
<td></td>
<td>Oleic acid</td>
</tr>
<tr>
<td>C18:1t</td>
<td></td>
<td>Vaccenic acid</td>
</tr>
<tr>
<td>C18:2</td>
<td></td>
<td>Linoleic acid</td>
</tr>
<tr>
<td>C18:3</td>
<td></td>
<td>Linolenic acid</td>
</tr>
<tr>
<td>COther</td>
<td></td>
<td>LCFA other than those listed above and with more than 12 carbon atoms.</td>
</tr>
<tr>
<td>DIETLCFA</td>
<td>g/d</td>
<td>Intake of LCFA from feeds</td>
</tr>
<tr>
<td>DMI</td>
<td>kg/d</td>
<td>Dry matter intake of feeds</td>
</tr>
<tr>
<td>EE</td>
<td>%</td>
<td>Ether extract in the dry matter of feed</td>
</tr>
<tr>
<td>FTCHO</td>
<td>g/d</td>
<td>Fermentable carbohydrate in feeds</td>
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<tr>
<td>K</td>
<td>%/h</td>
<td>Maximum rate of lipolysis</td>
</tr>
<tr>
<td>Kb</td>
<td>%/h</td>
<td>Maximum rate of biohydrogenation</td>
</tr>
<tr>
<td>KbRFLCFA</td>
<td>%/h</td>
<td>Rate of biohydrogenation adjusted for unsaturated RFLCFA</td>
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<tr>
<td>Kd</td>
<td>%/h</td>
<td>Rate of rumen digestion</td>
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<td>Kilip</td>
<td>%/h</td>
<td>Rate of lipolysis of adjusted for LCFA in feeds</td>
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<tr>
<td>Kp</td>
<td>%/h</td>
<td>Rate of passage rate feeds</td>
</tr>
<tr>
<td>L</td>
<td>decimal</td>
<td>Adjustment factor for the affect of DIETLCFA on lipolysis</td>
</tr>
<tr>
<td>LCFA</td>
<td>g/d</td>
<td>Long chain fatty acid</td>
</tr>
<tr>
<td>LCFA%</td>
<td>%</td>
<td>Percentage of the total diet that is LCFA</td>
</tr>
<tr>
<td>LCFASYN</td>
<td>g/d</td>
<td>De novo production of specific LCFA per gram of FTCHO</td>
</tr>
<tr>
<td>LCFAUUP</td>
<td>g/d</td>
<td>Adjustment factor for the uptake of RFLCFA by microbial cells</td>
</tr>
<tr>
<td>RD</td>
<td>decimal</td>
<td>Rumen digestibility of feeds</td>
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<tr>
<td>RFLCFA</td>
<td>g/d</td>
<td>Free fatty acids that are produced by lipolysis in the rumen</td>
</tr>
<tr>
<td>RNLCFA</td>
<td>g/d</td>
<td>Fatty acids that were not lipolysed in the rumen</td>
</tr>
<tr>
<td>RPLCFA</td>
<td>g/d</td>
<td>De novo production of LCFA in the rumen</td>
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</tbody>
</table>
In this report, a new lipid sub-model (Moate et al. 2000a,b,c; 2001) is used to illustrate ruminal metabolism and digestion of LCFA. The fat model focuses on the major LCFA (C12:0, C14:0, C16:0, C16:1, C18:0, C18:1cis, C18:1trans, C18:2, C18:3). Acronyms used in the fat sub-model are defined in Table 1.

Major issues of the fat sub model include:

1. intake of fatty acids
2. ruminal lipolysis of dietary fats
3. biohydrogenation of fatty acids in the rumen
4. de novo production of fatty acids in the rumen
5. effects of fat on rumen digestion and fermentation
6. intestinal digestion of fatty acids

Data used to develop this model came from 8 published experiments that reported intakes and flows of LCFA to the duodenum and to feces. Dairy cows were fed a diverse range of feeds with a wide range in intakes (mean, 878 g; range, 197 to 1339 g) of LCFA. These experiments contained 27 common dietary ingredients in 36 diets.

**Intake of Fatty Acids**

Fatty acids and not ether extract are the nutritional entities of importance. In dealing with this problem, NRC-Dairy (2001) advocates measuring LCFA content of feeds or using the equation: LCFA = EE-1 (Allen, 2000), and assuming that LCFA = 0 if EE is less than 1. Data in Figure 1 shows that the Allen (2000) equation predicts the LCFA content of some feeds well but it may not be accurate for lush grasses and legumes that contain high amounts of pigment that are extracted by ether.

![Figure 1. Relationship between ether extract and total fatty acids.](image-url)

Many references list fatty acid profiles but not total LCFA. There are over 200 references that contain information on fatty acid composition of feeds but this only includes about 40 different feeds. When the major fatty acids in feed ingredients (C16:0, C18:0, and C:18:2) account for 20% or more of the
total LCFA, coefficients of variation are usually less than 20% (Moate, 2001). We are assembling a bank of feeds that will be analyzed for fatty acids and ether extract by Dr. Tom Jenkins at Clemson University.

**Ruminal Lipolysis and Biohydrogenation**

Nutrients entering the rumen can only disappear from the rumen by two routes; by digestion or by passage. CPM-Dairy and CNCPS employ the model of Waldo et al. (1972) to define rumen digestibility (RD) as the specific rate (%/h) of rumen digestion (Kd) divided by the specific rate (%/h) of disappearance due to digestion and passage (Kd + Kp):

\[
RD = \frac{Kd}{Kd + Kp}
\]

Variable passage rates provide a method for estimating variations in ruminal digestibility as feed intake changes. As feed intake increases, rates of passage increase and the extent of ruminal digestion is reduced (Sniffen et al. 1992).

In this model, rate of lipolysis (Klip) and rates of biohydrogenation (Kb) of individual fatty acids replace Kd in the above equation.

**Lipolysis of dietary fat**

We use the term “lipolysis” to refer to the liberation in the rumen of LCFA in feed ingredients. This includes enzymatic hydrolysis of acylester linkages in triacylglycerols, phospholipids, galactosylglycerides and sterol esters and the dissociation of calcium salts of fatty acids. We define fatty acids arising from lipolysis as rumen free long chain fatty acids (RFLCFA) and fatty acids that were not lipolysed as rumen non-lipolysed long chain fatty acids (RNLCFA).

The approach of Waldo et al. (1972) was extended to describe the extent of lipolysis. The following equation calculates the amounts of LCFA in feeds that are “lipolysed.”

\[
RFLCFA = DIETLCFA \times \frac{Klip}{Klip + Kp}
\]

Where RFLCFA (g/d) is the amount of fatty acids in feeds that are lipolysed or converted to a free form in the rumen, DIETLCFA (g/d) is the intake of fatty acids, Klip (%/h) is the rate of lipolysis and Kp (%/h) is the rate of passage.

There is evidence that, for some lipids (tallow in particular), the rates of lipolysis may depend upon the concentration of LCFA in the rumen (Beam et al. 2000). We take account of this by using moderating factors to allow the Klip to be adjusted in response to different levels of total fatty acids from specific feeds in the total diet:

\[
Klip = K \times \text{Exp}(-L \times \text{DIETLCFA})
\]

Where Klip (%/h) is the rate of lipolysis adjusted for LCFA in feeds, K (%/h) is the maximum rate of lipolysis, L is a lipolysis adjustment factor for the affect of DIETLCFA on lipolysis and DIETLCFA is the percentage of the total diet that is LCFA from a specific feed. When L = 0, Klip = K.

Rates of ruminal lipolysis vary depending on the feed ingredient (Table 2). Most feeds have high rates of lipolysis and are therefore extensively lipolysed in the rumen. The lipolysis rate of tallow decreased as the amount of tallow in the ration increased but even at a level of 5% of ration dry matter,
92% of tallow fatty acids are lipolyzed. Megalac (calcium salts of palm oil fatty acid distillate) was the only ingredient that showed appreciable resistance to lipolysis (53%).

<table>
<thead>
<tr>
<th>Feed</th>
<th>Expts</th>
<th>Diets</th>
<th>K</th>
<th>L</th>
<th>Klip</th>
<th>Lipolysis(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>6</td>
<td>28</td>
<td>500</td>
<td>0</td>
<td>500</td>
<td>99</td>
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<tr>
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<td>500</td>
<td>0</td>
<td>500</td>
<td>99</td>
</tr>
<tr>
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<td>12</td>
<td>479</td>
<td>0</td>
<td>479</td>
<td>99</td>
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<td>5</td>
<td>65</td>
<td>0</td>
<td>65</td>
<td>93</td>
</tr>
<tr>
<td>Pasture hay</td>
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<td>9</td>
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<td>9</td>
<td>64</td>
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<tr>
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<td>309</td>
<td>0</td>
<td>309</td>
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<td>Barley</td>
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<td>500</td>
<td>99</td>
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<td>2</td>
<td>29</td>
<td>0</td>
<td>29</td>
<td>81</td>
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<td>8</td>
<td>15</td>
<td>0</td>
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<td>68</td>
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<tr>
<td>Soybean (whole-intact, raw)</td>
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<td>1</td>
<td>9</td>
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<td>9</td>
<td>56</td>
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<tr>
<td>Soybean (whole-intact, roasted)</td>
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<td>1</td>
<td>16</td>
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<td>16</td>
<td>70</td>
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<tr>
<td>Soybean (cracked, roasted)</td>
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<td>1</td>
<td>26</td>
<td>0</td>
<td>26</td>
<td>79</td>
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<tr>
<td>Soybean (ground, roasted)</td>
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<td>1</td>
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<td>0</td>
<td>35</td>
<td>83</td>
</tr>
<tr>
<td>Fish meal</td>
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<td>0</td>
<td>23</td>
<td>77</td>
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<tr>
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<td>500</td>
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<td>79-500</td>
<td>92 - 99</td>
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<tr>
<td>Animal Vegetable</td>
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<td>0</td>
<td>392</td>
<td>98</td>
</tr>
<tr>
<td>Hydrogenated fat</td>
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<td>1</td>
<td>18</td>
<td>0</td>
<td>18</td>
<td>77</td>
</tr>
<tr>
<td>Megalac</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>47</td>
</tr>
</tbody>
</table>

1. Number of experiments in data set with this feed
2. Number of diets in data set with this feed
3. K is the lipolysis rate (%/h) without correction for the effects of level of fatty acid from the ingredient
4. L is the constant that describes the effect of level of fatty acid from the ingredient on K
5. Klip (%/h) = K * Exp (-L * TLCFA%) where TLCFA% is the % of the total diet that is LCFA from the feed ingredient
6. Klip/( Klip + Kp ) assuming Kp = 7%/h for concentrates and 5%/h for forages
7. Tallow fatty acids at 0.1 to 5.0% of dry matter intake

**Biohydrogenation**

The model assumes that lipolysis is a prerequisite for biohydrogenation. Thus, only rumen free unsaturated fatty acids are biohydrogenated. Unsaturated LCFA not liberated by lipolysis escape biohydrogenation.

Biohydrogenation is a complex process that involves the formation of many isomers (Jenkins, 2002). We adopted a simplified depiction of biohydrogenation of the major unsaturated RFLCFA that describes the essential features of these pathways. In our model, RFLCFA are biohydrogenated in stepwise processes: C18:3 \( \rightarrow \) C18:2 \( \rightarrow \) C18:1t \( \rightarrow \) C18:0; C18:1c \( \rightarrow \) C18:0; C16:1\( \rightarrow \) C16:0. The model considers the biohydrogenation of the RFLCFA derived from each feed separately but we assume that the
biohydrogenation rates ($K_b$) are independent of the feeds from which the RFLCFA were derived. At each step, there is opportunity for the specific fatty acid to either pass out of the rumen or to be further biohydrogenated. In this model, the ruminal passage rates of RFLCFA are the same as the feed ingredients from which they were derived.

A model based on that of Waldo et al. (1972) was used to calculate biohydrogenation of unsaturated RFLCFA.

$$BHLCFA = RFLCFA \times \left( \frac{K_b}{K_b + K_p} \right)$$

Where $BHLCFA$ is the amount (g/d) of specific fatty acids that are produced by biohydrogenation, $RFLCFA$ is the amount (g/d) of specific free fatty acids that are produced by lipolysis, $K_b$ (%/h) is the rate of biohydrogenation and $K_p$ (%/h) is the rate of passage.

After examining the flows of specific LCFA to the duodenum, we observed that the ‘estimated concentration’ of RFLCFA appeared to influence the rates of biohydrogenation. Therefore, we again employed exponential moderating equations to allow for adjustment of biohydrogenation rates:

$$K_{bRFLCFA} = K_b \times \exp\left(\frac{-B \times RFLCFA}{DMI}\right)$$

Where $K_{bRFLCFA}$ (%/h) is the adjusted rate of biohydrogenation, $K_b$ (%/h) is the theoretical maximum rate of biohydrogenation, $B$ is the adjustment factor for the effect of RFLCFA on biohydrogenation, $RFLCFA$ (g/d) is the amount of rumen free LCFA and $DMI$ (kg/d) is dry matter intake.

**Figure 2. Effect of level of rumen-free long chain fatty acids on the rate of biohydrogenation.**

Rates of biohydrogenation are in Figure 2. There are considerable differences in rates with C18:3>C16:1>C18:2>C18:1t>C18:1c. The amount of rumen free LCFA affected the biohydrogenation rates of C18:3, C16:1 and C18:1t but had no affect on biohydrogenation rates of C18:2 or C18:1c.

Data in Figure 3 present the extent of ruminal biohydrogenation and, by difference, the percentage of rumen free unsaturated fatty acids that will escape biohydrogenation and pass to the small
intestine. Polyunsaturated fatty acids, especially C18:2, appear to have an energy independent effect on improving reproduction in the dairy cow. However, more than 90% of the rumen free C18:3, C18:2 and C16:1 will be biohydrogenated so that 10% or less will escape the rumen. Increased ruminal outflow of C18:1t appears to be associated with decreased synthesis of milk fat (Chalupa and Sniffen, 2000; Jenkins, 2002). Trans-10, cis 12 CLA rather than C18:1t is probably the fatty acid that inhibits mammary synthesis of milk fat (Bauman et al. 2001) but there was insufficient data to include CLA in our model. It may be that ruminal accumulation of C18:1t is a marker for situations that lead to increased amounts of trans-10, cis 12 CLA. Feeds contain little C18:1t. It is produced through biohydrogenation of C18:3 and C18:2. Because the rates and extents of biohydrogenation of C18:3 and C18:2 are greater than the rate and extent of biohydrogenation of C18:1t, it is easy to see how there will be increased absorption of C18:1t when diets contain polyunsaturated fatty acids in forms that have high rates of lipolysis. It is interesting that as the level of rumen free C18:1t increases, biohydrogenation decreases so that more C18:1t will flow to the small intestine.

Figure 3. Effect of level of rumen-free long chain fatty acids on the extent of biohydrogenation \([K_b/(K_b+K_p))*100 \text{ where } K_b \text{ is from Figure 2 and } K_p = 7 \%/h\]. The extent of rumen escape is \([100 – \text{biohydrogenation}]\).

**De Novo Production of LCFA in the Rumen**

Jenkins (1993) reviewed the literature on the factors affecting the balance of LCFA across the rumen of sheep and cattle. He concluded that the flow of LCFA to the duodenum is generally closely related to, but is usually slightly higher than the dietary LCFA intake. De novo synthesis of fatty acids occurs, but at high Fatty acid intakes, the extent of de novo synthesis may decline as a result of enhanced uptake of exogenous LCFA by microbial cells.

In this model we assume that each feed containing fermentable carbohydrate has the potential to induce in the rumen, through growth of ruminal bacteria, de novo production of LCFA. The main LCFA that are produced de novo are C18:0, C16:0, C16:1 and COther. In order to take into account this negative effect of fatty acids on de novo synthesis of LCFA, we again employed exponential moderating factors to adjust the rate of de novo synthesis of LCFA in relation to the amount of the relevant RFLCFA produced in the rumen as a result of lipolysis and biohydrogenation. The generalized formula is
RPLCFA = LCFASYN*FTCHO * EXP\left(\frac{-LCFAUP*RFLCFA}{DMI}\right)

Where RPLCFA (g/d) is the de novo production of LCFA, LCFASYN is the de novo production of specific LCFA per gram of FTCHO, FTCHO (g/d) is fermentable carbohydrate in feeds, LCFAUP is the adjustment factor for the uptake of RFLCFA by microbial cells, RFLCFA (g/d) is the amount of rumen free LCFA and DMI (kg/d) is dry matter intake.

The impact of dry matter intake and level of dietary fatty acids is shown in Figure 4. As dry matter intake increases there is more FTCHO (and bacterial growth) so that de novo synthesis increases. However, as the amount of RFLCFA increases, bacteria take up more LCFA and de novo synthesis decreases.

![Figure 4. Effect of level of dietary fatty acids and dry matter intake on predicted de novo production of total LCFA. The diet contained (DM basis) 26%alfalfa silage, 26% corn silage, 22% steam-flaked corn, 14% soybean meal, 2% blood meal and 10% mineral mix /fatty acid supplement. The dietary concentration of total LCFA was varied by adjusting the proportions of mineral mix and fatty acid supplement](image)

Effects of Fatty acids on Rumen Digestion

While we can predict the level of rumen free LCFA in the rumen, there is insufficient data to model the effect of rumen active fat on rumen digestion and fermentation. We suggest using the advisory of Jenkins (1997), which is based on unsaturation of fatty acids and ration fiber, for the upper limit of rumen active fat.

Unprotected fat (%DM) = (6*ADF)/UFA or (4*NDF)/UFA

Where ADF and NDF are expressed as a percentage of ration DM and UFA are unsaturated fatty acids (C18:1 + C18:2 +C18:3) expressed as a percentage of total fatty acids.
Intestinal Digestion of Fatty Acids

Most of the LCFA flowing to the small intestine are in the form of rumen free fatty acids but there are also non-lipolysed fatty acids and fatty acids in bacteria.

In general, there are strong linear relationships between the flow of LCFA to the duodenum and intestinal absorption. However, as shown in Figure 5, diets that contained hydrogenated tallow and whole-intact soybeans had lower digestibilities. It is likely that the intestinal digestibility of duodenal free fatty acids derived from different ingredients is the same but the digestibility of fatty acids that escaped ruminal lipolysis could differ.

\[
Y = 17.7 (16.7) + 0.74 (0.02) \times D_{\text{TotalLCFA}} \\
N = 27, R^2 = 0.99
\]

**Figure 5. Relationship between duodenal flow and absorbed total LCFA.** Different symbols represent different diet types: most diets (solid triangles) contained corn silage, corn and a protein source; diets (dot) contained Megalac. Diets containing hydrogenated fat (hollow diamonds) or intact soybeans (hollow squares) were excluded from the regressions.

We used optimization techniques to derive digestibilities for RFLCFA (including bacterial fatty acids) and six classes of rumen non-lipolysed LCFA: (1) forages, grains, proteins, whole cottonseed, and cracked or ground soybeans and other oil-seeds; (2) tallow, grease, vegetable oils and animal/vegetable blends; (3) hydrogenated tallow, (4) whole-intact soybeans and other whole-intact oil-seeds with the exception of cottonseed; (5) fish meal supplements and (6) calcium salts of fatty acids.

In general, the digestion coefficients (Table 3) for RFLCFA and rumen non-lipolysed LCFA in feeds in category 1 are similar. The digestion coefficients for all of the rumen non-lipolysed fatty acids from feed categories 2 to 5 are less than, and in many cases, substantially less than the corresponding coefficient for RFLCFA. In particular, the digestion coefficients for rumen non-lipolysed C18:0 in categories 2, 3 and 4 are zero. In contrast, Børsting et al. (1992) reported that when cows were fed emulsified vegetable fat protected by means of formaldehyde-casein, the digestion coefficient for C18:0 was 0.92. Thus it seems that the often reported low digestibility of C18:0 from hydrogenated fat sources may be more likely due to low or inefficient intestinal emulsification instead of an ineffective lipase system.
Table 3. Optimized digestion coefficients for rumen free LCFA (RFLCFA) and non-lipolysed LCFA

<table>
<thead>
<tr>
<th>Category of non-lipolysed LCFA</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCFA</td>
<td>RFLCFA</td>
<td>Feeds&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Fats&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Hydrogenated Tallow</td>
<td>Whole-intact oil-seeds&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Fish Meal</td>
</tr>
<tr>
<td>C12:0</td>
<td>0.95</td>
<td>0.95</td>
<td>0.95</td>
<td>0</td>
<td>0</td>
<td>0.82</td>
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<tr>
<td>C14:0</td>
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<td>0.49</td>
<td>0</td>
<td>0</td>
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<tr>
<td>C16:0</td>
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<td>0.72</td>
<td>0</td>
<td>0.18</td>
<td>0.73</td>
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<tr>
<td>C16:1</td>
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<td>0.64</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>0.56</td>
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</table>

1. Non-lipolysed LCFA from forages, grains, proteins, whole cottonseed, and cracked or ground soybeans and other oil-seeds
2. Non-lipolysed LCFA from tallow, grease, vegetable oils and animal/vegetable blends
3. Non-lipolysed LCFA from whole-intact soybeans and other whole-intact oil-seeds with the exception of cottonseed
4. Whole-intact soybeans and other whole-intact oil seeds with the exception of cottonseed
5. Fish meal supplements
6. Calcium salts of fatty acids

The digestion coefficients for the major rumen non-lipolysed fatty acids in the form of calcium salts are substantially greater than the coefficients for RFLCFA. This is consistent with the findings of Enjalbert et al. (1997) and Moller (1988) where the apparent digestibilities of C16:0 and unsaturated C18 fatty acids were elevated in diets containing calcium salts of palm or rapeseed fatty acids.

Model Validation

Data used to validate the model came from 8 published experiments that reported intakes and flows to the duodenum and feces of the major LCFA (Moate, 2001). Due to the scarcity of published experiments with the requisite in vivo data, there were only two experiments with lactating Holstein*Friesian dairy cows while the remaining experiments involved steers of various ages and breeds. Thus, intakes of LCFA were less (mean, 479 g; range, 72 to 1040) than intakes in the data used to develop the model (mean, 878 g; range, 197 to 1339 g).

From data in Figure 6 and Tables 4 and 5, it is apparent that there is close concordance between measured and predicted flows of total LCFA to the duodenum (R<sup>2</sup>=0.99; bias=5%) and measured and predicted absorption of total LCFA from the intestine (R<sup>2</sup>=0.98; bias<1%).

Data in Table 4 show that there was a high correlation (R<sup>2</sup>&gt;0.91) between measured and predicted flows of C16:0, C18:0, C18:1<sup>t</sup>, C18:1<sup>c</sup>, C18:2, C18:3 and COther to the duodenum. The predicted bias was 13% or less. The low correlation (R<sup>2</sup>=0.61) and high bias (75%) between measured and predicted flows of C16:1 probably reflects the low flow (mean flow=2 g/d) of C16:1.

Data in Table 5 show that there was a high correlation (R<sup>2</sup>&gt;0.86) between measured and predicted absorption of C12:0, C14:0, C16:0, C18:0, C18:1<sup>t</sup>, C18:1<sup>c</sup>, and C18:2. The predicted bias was12% of less for C12:0, C14:0, C16:0 and C18:0 and C18:2. For C18:1<sup>t</sup> and C18:1<sup>c</sup>, the bias was about 20%. Absorption of C16:1, C18:3 and COther was predicted poorly. However, only small amounts (2 to 3 g/d)
of C16:1 and C18:3 were absorbed and COther is a “mixed bag” of LCFA not always reported in all experiments.

![Graph showing Duodenal Total LCFA and Absorbed Total LCFA](image)

**Figure 6.** Measured and predicted duodenal and absorbed total long chain fatty acids.

<table>
<thead>
<tr>
<th>LCFA</th>
<th>n</th>
<th>Mean</th>
<th>STD</th>
<th>Intercept</th>
<th>Coefficient</th>
<th>R²</th>
<th>Bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
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1. Intercept in original regression was not significantly (P>.05) different from 0 so the subsequent regression was forced through 0

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<th>Coefficient</th>
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1. Intercept in original regression was not significantly (P>.05) different from 0 so the subsequent regression was forced through 0
Application of the Model

To demonstrate the application of the model, we fed a 650 kg cow 25 kg of a diet that contained (DM basis) 26% alfalfa silage, 26% corn silage, 22% steam-flaked corn, 14% soybean meal, 2% blood meal and 10% mineral mix/fatty acid supplement. 400 g of LCFA were provided by adjusting the proportions of mineral mix and fatty acid supplement (Table 6).

Table 6. Intestinal flows and absorption of LCFA (g/d) predicted by the CPM-Dairy lipid sub model

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Basal</th>
<th>Meaglac</th>
<th>Meaglac R</th>
<th>Energy Booster</th>
<th>WCS</th>
<th>RSB</th>
<th>Tallow</th>
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<tr>
<td>Fat Supplement (g)</td>
<td>0</td>
<td>474</td>
<td>474</td>
<td>404</td>
<td>2395</td>
<td>2222</td>
<td>460</td>
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<tr>
<td>Klip (%/h)</td>
<td>6</td>
<td>6</td>
<td>500</td>
<td>500</td>
<td>37</td>
<td>277</td>
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<tr>
<td>Non-lipolysed LCFA (%Intake)</td>
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<td>54</td>
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<td>16</td>
<td>2</td>
<td></td>
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<tr>
<td>Total long chain fatty acids</td>
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<td></td>
<td></td>
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<tr>
<td>Duodenum</td>
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<td>400</td>
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<td>400</td>
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<td>De novo production</td>
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<td>0</td>
<td>4</td>
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<tr>
<td>Absorbed</td>
<td>468</td>
<td>327</td>
<td>337</td>
<td>291</td>
<td>300</td>
<td>298</td>
<td>293</td>
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<td>Intestinal Digestion (%)</td>
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<td>84</td>
<td>73</td>
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<td>73</td>
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<td>104</td>
<td>201</td>
<td>291</td>
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<td>Absorbed</td>
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<td>56</td>
<td>78</td>
<td>146</td>
<td>211</td>
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<tr>
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<td>1.9</td>
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</table>

1. 25 kg of a diet containing (DM basis) 26% alfalfa silage, 26% corn silage, 22% steam-flaked corn, 14% soybean meal, 2% blood meal and 10% mineral mix/fatty acid supplement. 400 g of fatty acids was provided by adjusting the proportions of mineral mix and fatty acid supplement.

Total long chain fatty acids.

The basal diet provided 500 g LCFA (2% DM basis). 400 g of each fatty acid supplement raised dietary LCFA to 3.6%.

De novo production of LCFA only occurred on the basal diet because the fatty acid supplements provide no or little fermentable carbohydrate for microbial growth. When fatty acid supplements are added to rations, bacterial cells will take some of the RFLCFA up with a concomitant decrease in de novo production.
Intestinal digestibility of Megalac and Megalac R is higher than the basal diet because rumen non-lipolysed fatty acids in the form of calcium salts have higher intestinal digestibilities than rumen non-lipolysed fatty acids in the form of glycerides.

**C18:0.**

With the exception of Energy Booster, intakes of C18:0 are low but substantial amounts of C18:0 reach the small intestine. This reflects the intense biohydrogenation of C18:unsaturated free fatty acids in the rumen.

**C18:1t.**

As noted before, C18:1t may not directly inhibit mammary synthesis of fat but it is associated decreased milk fat synthesis. There is little C18:1t in feeds but because of biohydrogenation of C18:3 and C18:2 to C18:1t and incomplete biohydrogenation of C18:1t to C18:0, C18:1t can accumulate in the rumen. The amounts of C18:1t absorbed from Megalac, Megalac R, Energy Booster and tallow are small. Feeding 400 g of LCFA in the form of whole cottonseed and roasted soybeans doubled the amount of C18:1t absorbed.

**C18:2.**

As noted before, C18:2 appears to have an energy independent effect on improving reproduction in the dairy cow. The basal ration in Table 6 contained 225 g C18:2, but because of extensive biohydrogenation in the rumen, only 58 g reached the duodenum with 48 g absorbed. On an energy basis, the basal ration would support production of 40 kg milk with 3.7% fat. Cow’s milk contains approximately 2 to 6% of the fatty acid content as C18:2 (Sanchez and Block, 2001) so our example cow would secret 30 to 89 g C18:2 in milk. It is thus possible that today’s high producing cows may be deficient in this essential fatty acid. To increase amounts absorbed, C18:2 must either be in a form that protects it from ruminal lipolysis (Megalac R) or the feed ingredient must contain high amounts of C18:2 (soybeans). With an ingredient like soybeans, however, there is also an increase in absorbed C18:1t which might lower milk fat test.

**C18:3.**

Feeds contain C18:3 but little reaches the intestine because of rapid biohydrogenation in the rumen. As with C18:2, dietary C18:3 must either be in a form that protects it from lipolysis or large amounts must be fed to increase absorbed amounts.

References


FORAGE BREEDING TO IMPROVE NUTRIENT CONTENT
FOR RUMINANTS

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Summary

The most limiting factor in forage quality is quantity of digestible energy. As such, forage breeders have focused on selection for greater fiber digestibility (and higher energy content). To date, forage quality improvement has received more attention from alfalfa breeders than any other forage species. It is estimated that approximately 90% of all public and private funding for forage breeding in the U.S. is targeted at alfalfa improvement. Despite this, alfalfa usage has stagnated in the U.S. over the last 10 years, primarily because higher-energy alternatives (e.g. corn silage) have become more widely utilized in ruminant feeding programs. To address this issue, alfalfa breeders have begun working to develop a “new” type of alfalfa plant that will deliver significantly greater levels of digestible energy when fed to ruminants.

The cell wall fraction of alfalfa makes up 30-60% of the dry matter and represents a major source of nutritional energy for ruminants. However, generally less than 50% of this fraction is digested and utilized by the animal. If the digestibility of this cell wall fraction were increased, there would be considerable positive economic impact. It is estimated that a 10% increase in alfalfa cell wall digestibility would result in an additional $380 million in milk and meat sales while reducing manure solids by 2.3 million tons. To achieve these objectives, breeders have utilized both conventional and (more recently) genetic engineering approaches to increase forage cell wall digestibility.

Conventional selection for improved forage quality has been effective. Techniques employed consist primarily of selection from large (> 2000 elite genotypes) breeding nurseries utilizing Near Infrared Reflectance (NIRS) or wet laboratory IVTD criteria. Breeders in all forage crops have had to utilize selection protocols that allow improvement for forage quality parameters without reduction in yield potential in the resulting lines. Alfalfa lines selected for improved IVTD characteristics produced 4 lbs more milk per animal per day (vs. unselected alfalfa) in a University of Wisconsin lactation study. In forage grasses, limited resources have prevented breeders from achieving the levels of gain observed in alfalfa. However, bromegrass breeders have achieved a 1.4% increase in IVTD through conventional selection. Selection for improved forage quality (primarily utilizing NIRS to measure IVTD) is now an integral part of breeding programs in most forage species. However, conventional selection for higher quality and greater fiber digestibility now appears capable of taking forage breeders only so far towards the ultimate goal of significantly higher energy content. It now appears that breeders must go outside of traditional gene pools to obtain the new variation needed to take forage digestibility to the next level. In these cases, genetic engineering provides the opportunity to incorporate genes from outside sources (or knock-out activity of existing genes at the molecular level) that might enable breeders to make significant advance not otherwise possible.

One of the most exciting developments in genetic engineering to improve forage fiber digestibility is the recent “knock-out” of a gene(s) controlling lignin biosynthesis in alfalfa. Lignin cross-linking with cell wall polysaccharides limits both the rate and extent of fiber fermentation and
degradation in the rumen. As such, forage cell wall digestibility is negatively correlated with lignin content in forages. Research (Dixon et al., 2001) has shown that genetic engineering to down-regulate key enzymes in the lignin biosynthetic pathway has produced significant changes in lignin content and lignin composition in transgenic plants. Preliminary results show significant increases in IVTD of transgenic alfalfa lines with decreased lignin content. These low-lignin transgenics, based on “knock-outs” of various enzymes in lignin biosynthesis, are demonstrating significant improvements in digestibility characteristics when compared to conventional alfalfa lines. Of particular interest is the observation that in many of these alfalfa transgenics, down-regulation (knock-out) of specific enzymes in lignin biosynthesis has resulted in significant increases in digestibility with only modest decreases in lignin content. Agronomically, this is exciting, since lignin plays an important role in forage (alfalfa) yield and standability; driving lignin to very low levels would most likely produce unacceptable agronomic performance in the resulting cultivars. If these new technologies allow forage breeders to modify lignin in the plant and improve fiber digestibility without driving lignin levels to agronomically unacceptable levels, the resulting cultivars would move much closer to the “perfect forage plant” ideal discussed earlier.

Breeders (and molecular biologists) are looking at a number of additional targets where genetic engineering might significantly improve nutritional value of forage for ruminants. Commercial forage varieties incorporating genetically engineered traits may become available to producers in the later half of this decade. These new technologies will allow breeders to make improvements in forage nutrient content for ruminants not possible with current conventional breeding techniques and with currently available levels of natural variation.

**Increased stability of alfalfa protein in the rumen.**

Several genetic engineering strategies are being explored to increase the amount of protein that bypasses rumen fermentation, thus decreasing amounts of required supplemental feed protein and reducing the amount of N lost to the environment.

**Increased stability of alfalfa protein in silage.**

Alfalfa silage often contains very high concentration of non-protein nitrogen, significantly limiting the efficiency of protein utilization of alfalfa silage by dairy cows. Genetic engineering may provide breeding tools to limit the rate and extent of post-harvest protein losses in alfalfa.

**Bloat-free alfalfa.**

The rapid fermentation of alfalfa protein in the rumen is implicated as one cause of legume pasture bloat. Condensed tannins complex with protein, reducing the stable rumen foaming that occurs when proteins are rapidly digested, and thus potentially reducing the incidence of pasture bloat in ruminants. Genes for condensed tannins have been isolated from *Medicago truncatula* (Xie et al., 2002) and work is underway to introduce these genes into alfalfa.

**Increasing levels of sulfur-rich amino acids for wool production.**

Low levels of sulfur-rich amino acids are a major limitation to wool growth in sheep. Alfalfa has been transformed with genes coding for sulfur-rich amino acids (Higgins et al., 1989). This research is ongoing, with a goal to significantly improve the nutrient content of alfalfa specifically for wool production in sheep.
As the forage breeding community begins to develop, evaluate, and feed these new, genetically
engineered cultivars, it is very important to carefully examine all pertinent food safety issues associated
with these new products. A number of transgenic forage feeding studies (Faust, 2001) have investigated
milk, eggs, chicken, pork, and beef for the presence of transgenic genes, gene fragments, or protein. In all
cases, no plant transgenic DNA or protein has been detected in products from animals fed transgenic
forages. Transgenic proteins in feed are completely broken down when incorporated into animal products.
However, forage breeders must still make a dedicated effort to educate the public on the food safety of
products derived from animals fed transgenic forage plants.

The forage breeding community, both public and private, has seen funding for conventional
research stagnate and decline over the last decade. Despite these resource limitations, conventional efforts
have made significant advances in breeding for improved nutrient content in forages. Recent
developments in genetic engineering of forages have now produced dramatic improvements in fiber
digestibility and have re-energized forage breeding efforts in the U.S. Hopefully, we will see these
potential new “output” traits, with their significant commercial potential, attract additional sources of
funding to forage breeding activities in both the public and private sectors.

Introduction

Ruminants are able to consume large quantities of forage and convert the fiber components
(cellulose and hemicellulose) into useable energy. The most limiting factor in forage quality is quantity of
digestible energy. As such, this paper will emphasize recent efforts by forage breeders to select for greater
fiber digestibility (and higher energy content). In addition, we will discuss several other breeding targets,
including selection for greater protein bypass potential and reduced bloat potential.

Most Breeding Resources Targeted at Alfalfa

Alfalfa (Medicago sativa), often called the “Queen of the Forages”, is the most important forage
legume grown in the United States. It is the only forage grown in virtually every state and it has the
highest feeding value of all the commonly grown forages (Marten et al., 1988). Alfalfa has potential to
produce more protein per acre than grain or oil crops due to multiple harvests per year. In addition, alfalfa
has the advantage of fixing nitrogen from the atmosphere into forms useable by the plant, thus reducing
fertilizer requirements. In the U.S, approximately 90% of all public and private funding for forage
breeding is targeted at alfalfa improvement.

Over the past 50 years significant advances have been made in the development of new, higher-
yielding alfalfa cultivars with much-improved winter hardiness and pest resistance (insects, diseases, and
nematodes) characteristics. Crop rotations utilizing alfalfa have a major, positive impact in terms of
stabilizing soils, decreasing nutrient inputs, and providing rotational benefits to subsequent grain crops.
Despite these outstanding characteristics, alfalfa usage in the U.S. has stagnated over the last 10 years.
There are several important limitations inherent in current alfalfa cultivars that make this forage less
competitive with alternatives (e.g. corn silage) in modern dairy and beef feeding systems. As such, alfalfa
breeders have begun working, through both conventional and biotechnological approaches, to develop a
“new” type of alfalfa plant that will increase the use of alfalfa in sustainable dairy and beef production
systems.

The Perfect Alfalfa Plant

An effort to design the “perfect” alfalfa plant was recently launched by a joint venture among
Forage Genetics International (a private alfalfa breeding firm), the Noble Foundation (Ardmore, OK), and
the U.S. Dairy Forage Research Center (Madison, WI). The primary objective of this venture is to
develop alfalfa lines with both physical and biochemical properties that fit the needs of the high producing dairy cow (greater cell digestibility, less protein degradation during ensiling, increased by-pass protein, increased yield without quality loss). This group identified two key areas where current alfalfa cultivars are limiting in animal production systems:

- **Energy limitations.** The cell wall fraction of alfalfa makes up 30-60% of the dry matter and represents a major source of nutritional energy for ruminants. However, generally less than 50% of this fraction is digested and utilized by the animal. If a greater percentage of this potential energy were made available to the animal (i.e. the digestibility of the cell wall fraction was increased) there would be considerable positive economic impact. The USDFRC estimates that a 10% increase in alfalfa cell wall digestion would result in an additional $380 million in milk and meat sales while reducing manure solids by 2.3 million tons. The alfalfa breeder’s goal? To significantly increase fiber digestibility while maintaining acceptable agronomic performance.

- **Protein too readily degradable.** Alfalfa has a major advantage over other forage because of its high content of available protein (20-24%). However, the full benefit of this protein in alfalfa is not realized due to poor utilization by the ruminant animal. Ruminal microbes degrade alfalfa protein too rapidly resulting in excretion of excessive amounts of nitrogenous waste by the animal. The breeding goal? To make the protein in alfalfa less rapidly degradable (increase levels of RUP).

**Conventional Breeding for Improved Forage Quality**

For over 15 years, the alfalfa breeding community has been selecting alfalfa for improved forage quality. Early, but minimal, improvements were accomplished by incorporating the multifoliate trait. Greater advances have been made by selecting for altered chemical composition via either Near Infrared Reflectance Spectroscopy (NIRS) or *In Vitro* True Digestibility (IVTD) utilizing wet-lab analyses. Each year, private breeding programs analyze thousands of samples, utilizing NIRS, for fiber content (NDF), fiber digestibility (CWD or dNDF), and protein content. To a lesser extent, IVTD analyses (utilizing rumen liquor taken from fistulated animals) is used to select directly for improved digestibility.

Conventional selection for improved forage quality, utilizing the techniques discussed above, has been effective. A University of Wisconsin lactation study (Table 1 below) showed that quality-selected alfalfa produced 4 lbs more milk per animal, per day when compared to unselected alfalfa (Combs *et al.*, 1992). Milk composition was essentially identical from cows fed either the quality-selected or unselected alfalfa. In addition, the quality selected line demonstrated greater intake potential and lower rumen fill when compared to the unselected line.

**Table 1. Impact of Selection for High Quality on Milk Production Potential in Alfalfa**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alfalfa Variety</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quality-Selected</td>
<td>Unselected</td>
<td></td>
</tr>
<tr>
<td>Milk Yield (Lbs/cow/day)</td>
<td>75</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Milk Fat (%)</td>
<td>3.4</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Milk Protein (%)</td>
<td>2.9</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Hay Intake (Lbs/cow/day)</td>
<td>47</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Rumen Fill (Lbs/cow/day)</td>
<td>185</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>Relative Feed Value of Hay (RFV)</td>
<td>153</td>
<td>146</td>
<td></td>
</tr>
<tr>
<td>Crude Protein of Hay (%)</td>
<td>18.5</td>
<td>17.5</td>
<td></td>
</tr>
</tbody>
</table>

(Combs *et al.*, 1992)
Limited Resources Devoted to Forage Grass Breeding

Improving forage quality in forage grasses has received little attention from breeders. Casler et al. (2000) states that breeding has tended to actually reduce the forage quality of temperate grasses. This is probably due to long term selection for agronomic-fitness traits such as high forage yield, high seed yield, and increased concentration of cell wall components that would confer desirable lodging resistance on the forage grass. Despite these resource limitations, efforts to improve digestibility in certain forage grasses (e.g. smooth bromegrass) have met with some success (1.4% improvement in IVDMD). However, limited resources have prevented grass breeders from making selection gains for improved nutritional characteristics similar to those achieved in alfalfa.

Breaking the Negative Correlation Between Quality and Yield

One of the greatest challenges that forage breeders have faced when attempting to select for improved nutritional content is the relatively strong negative correlation between forage yield and quality. Invariably, selection for higher quality and improved feed value in any forage, without concurrent selection to maintain yield, results in a lower-yielding cultivar. Clearly, this result is commercially unacceptable. To combat this undesirable yield/quality linkage, forage breeders have resorted to several techniques:

- Recognize that desirable plants (high yield and high feed value) occur in very low frequencies within breeding populations, and design selection nurseries that are much larger and more uniform (to reduce non-genetic variation)
- Develop more sophisticated NIRS calibrations, unique to the population under selection
- Be prepared to conduct multiple cycles of selection on a single population to achieve the desired level of improvements in digestibility while maintaining competitive yield potential
- Accept moderate levels of improvement in forage quality parameters, while maintaining competitive yield potential

Selection for improved forage quality (primarily utilizing NIRS to measure IVTD, dNDF) is now an integral part of an overall selection strategy that incorporates many of the breeding techniques described above. Forage yield, persistence, and forage quality parameters are all used to identify elite parents. These selections programs have enabled the development of new alfalfa cultivars with both improved yield potential and significantly higher forage quality and feed value (see Table 2).

Table 2. Forage Quality at West Salem, Wisconsin

<table>
<thead>
<tr>
<th>Line</th>
<th>FD</th>
<th>% CP</th>
<th>RFV</th>
<th>IVTD</th>
<th>Yield (3 yr total-tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality-Selected (A)</td>
<td>2.8</td>
<td>22.1</td>
<td>166</td>
<td>72.5</td>
<td>22.81</td>
</tr>
<tr>
<td>Quality-Selected (B)</td>
<td>4.3</td>
<td>21.9</td>
<td>163</td>
<td>73.1</td>
<td>22.79</td>
</tr>
<tr>
<td>Magnum IV (CK)</td>
<td>4.2</td>
<td>19.3</td>
<td>139</td>
<td>69.5</td>
<td>20.03</td>
</tr>
<tr>
<td>Innovator +Z (CK)</td>
<td>3.2</td>
<td>19.6</td>
<td>141</td>
<td>70.2</td>
<td>20.58</td>
</tr>
<tr>
<td>Vernal</td>
<td>2.1</td>
<td>20.2</td>
<td>143</td>
<td>70.8</td>
<td>17.33</td>
</tr>
</tbody>
</table>

Increasing Fiber Digestibility: The Link Between Conventional Breeding and Genetic Engineering

The primary role of alfalfa in the ruminant diet is as a source of protein and fiber. All of the quality-selected alfalfa cultivars currently on the market have decreased fiber (NDF) content when
compared to unselected check cultivars. However, forage breeders have found that decreasing NDF content below some minimum threshold appears to have a negative impact on yield (see above) and standability of the forage. In addition, since proper levels of dietary fiber are required for maximum milk/beef production and good animal health, forage breeders must be careful to not drive fiber levels too low when selecting for greater digestibility. In fact, conventional selection programs are probably approaching the lower threshold of fiber in selected forages. Conventional breeding techniques appear to be capable of taking breeders only so far towards the ultimate goal of greater fiber digestibility. How then can breeders take alfalfa nutritional value to the next level: much higher fiber digestibility (and energy content) while maintaining some minimum level of overall fiber for proper rumen function?

In traditional plant breeding, natural genetic variation within a species is the sole source of new genes for making improvements in forage quality. However, there are several valuable traits (including forages with significant improvements in fiber digestibility) for which genes have not been found within traditional gene pools. In these cases, genetic engineering provides the opportunity to incorporate genes from outside sources that might enable breeders to make significant advances not otherwise possible.

Genetic engineering also offers the potential to “knock-out” alfalfa genes that negatively impact crop quality. The recent “knock-out” of a gene controlling one of the enzymes in lignin biosynthesis has resulted in lower lignin content and improved digestibility in alfalfa.

**Down-Regulated Lignin Transgenics**

Alfalfa is a primary source of fiber in many ruminant diets. Lignin cross-linking with cell-wall polysaccharides limits both the rate and extent of fiber fermentation and degradation in the rumen. As such, forage cell wall digestibility is negatively correlated with lignin content in forages. In some grasses, low lignin mutants (BMR) have been identified that display significant improvements in digestibility. In alfalfa, recent research at the Noble Foundation shows that genetic engineering to down-regulate key enzymes in the lignin biosynthetic pathway has produced significant changes in lignin content and lignin composition in transgenic plants (Guo et al., 2001). Preliminary results show significant increases in *in situ* DMD of transgenic alfalfa lines with decreased lignin content (Guo et al., 2001). More recent results demonstrate that low-lignin transgenics, based on “knock-outs” of various enzymes in lignin biosynthesis, are being produced in alfalfa and are producing lines with significantly improved digestibility characteristics (Table 3). Of particular interest is the observation that in many of these alfalfa transgenics, down-regulation (knock-out) of certain enzymes in lignin biosynthesis has resulted in significant increases in digestibility with only modest decreases in lignin content. Agronomically, this result is exciting, since lignin plays an important role in forage (alfalfa) yield and standability; driving lignin to very low levels would most likely produce unacceptable agronomic characteristics in the resulting cultivar.
Table 3. Increased Stem Digestibility of Transgenic Lines (T1, T2) at Nampa ID.

Other Output (Quality) Traits Currently Targeted By Genetic Engineers

Breeders (and molecular biologists) are looking at a number of current targets where genetic engineering might significantly improve the nutritional value of forage for ruminants. Genetic engineering may allow plant breeders to make basic changes to forages that increase the value of these forages to both dairy and beef producers. Commercial forage varieties incorporating genetically engineered traits will likely be available to producers in the later half of this decade.

**Increases stability of alfalfa protein in the rumen.**

Alfalfa protein is very rapidly fermented in the rumen, limiting the efficiency and extent of alfalfa protein utilization in ruminants. Several genetic engineering strategies are being explored to increase the amount of protein that bypasses rumen fermentation, thus decreasing amounts of required supplemental feed protein and reducing the amount on N lost to the environment.

**Increased stability of alfalfa protein in silage.**

Alfalfa protein rapidly degrades post-harvest, especially in the early stages of ensiling. Alfalfa silage often contains very high concentrations of non-protein nitrogen, significantly limiting the efficiency of protein utilization of alfalfa silage by dairy cows. Genetic engineering strategies are being examined to limit the rate and extent of post-harvest protein losses in alfalfa.

**Bloat-free alfalfa.**

Grazing fresh alfalfa can cause ruminants to bloat. The rapid fermentation of alfalfa protein in the rumen is implicated as one cause of pasture bloat. It may be possible to use genetic engineering to decrease both the rate and the extent of protein degradation in the rumen, decreasing the likelihood of
bloat when grazing alfalfa. Condensed tannins are secondary metabolites that complex with protein, reducing the stable rumen foaming that occurs when proteins are rapidly digested, and thus reducing the incidence of pasture bloat in ruminants (Li et al., 1996). Adding condensed tannins to alfalfa forage reduces protein solubility (Julier et al., 2002). Genes for condensed tannins have been isolated from Medicago truncatula, an annual medic closely related to alfalfa (Xie et al., 2002). Isolation of these genes is the first step towards production of bloat-safe alfalfa.

*Increasing levels of sulfur-rich amino acids for wool production.*

Low levels of sulfur-rich amino acids are a major limitation to wool growth in sheep. Alfalfa has been transformed with genes coding for sulfur-rich amino acids (Higgins et al., 1989). This research is ongoing, with a goal of significantly improving the nutritional content of alfalfa specifically for wool production.

**Are Genetically-Engineered Forages Safe?**

As forage breeders utilize genetic engineering to improve nutritional content for ruminants, it is important to step back and carefully examine the safety of transgenic forages as components in animal feed. Numerous transgenic-feeding studies (Faust, 2001) have investigated milk, eggs, chicken, pork, and beef for the presence of transgenic genes, gene fragments, or protein. The result? In no case has plant transgenic DNA or protein been detected in products of animals fed transgenic forages. As expected, transgenic proteins in feed are completely broken down when incorporated into animal products. However, the public’s response to genetic engineering turns as much on emotion as on science, and plant breeders must work to educate the public on the safety of products derived from animals fed transgenic forages.

**Conclusions**

Selection for greater fiber digestibility (and higher energy content) is the primary focus of current forage quality breeding programs. Conventional selection in alfalfa, bromegrass, and BMR sorghums has produced new cultivars with improved fiber digestibility characteristics. In alfalfa, these selected lines have been shown to produce significantly more milk (vs. unselected lines) when fed to high-producing dairy cattle.

Genetic engineering of forages provides a unique opportunity to make significant advances in fiber digestibility and bypass protein potential, not currently possible through conventional breeding. Recent research has shown that genetic engineering to down-regulate (“knock-out”) gene(s) controlling enzymes in the lignin biosynthetic pathway in alfalfa have resulted in dramatically improved digestibility profiles. Other genetic engineering targets currently under investigation include selection for increased stability of protein in the rumen and development of bloat-free alfalfa cultivars through incorporation of condensed tannins.

The forage breeding community, both public and private, has seen funding for conventional research stagnate and decline over the last decade. Despite these resource limitations, conventional efforts have made significant advances in breeding for improved nutrient content in forages. Recent developments in genetic engineering of forages have produced dramatic improvements in fiber digestibility and re-energized the forage breeding community in the U.S. Hopefully, we will see these potential new “output” trails, with their significant commercial potential, attract additional sources of funding to forage breeding activities in both the public and private sectors.
References


MARYLAND EQUINE UPDATE: THE MARYLAND HORSE INDUSTRY BOARD,
THE MARYLAND HORSE INDUSTRY FEED FUND,
AND THE MARYLAND EQUINE CENSUS

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Summary

Since the invention of the combustion engine the public acceptance of the equine industry as a viable economic force has declined. However, the industry remains a strong economic factor in the State of Maryland. In order to establish proper representation and recognition of this industry the State of Maryland has taken bold steps, which have resulted in a renaissance of its horse industry. In 1998, Agricultural Article 2-703 established the Maryland Horse Industry Board (MHIB). The MHIB has several duties that include licensing boarding stable operations, promotion of the Maryland horse industry, and awarding financial grants to non-profit and not-for-profit organizations in Maryland to support educational, promotional, and youth activities, as well as equine research. Additionally, the MHIB was established to advise the Secretary of Agriculture and the Governor of Maryland of matters affecting the horse industry in the State. In 2002, Agricultural Article 6-107.2 established the Assessment of Commercial Equine Feed, which serves to generate money from feed sold in Maryland intended to be fed to equine. The first collections of the assessment were processed in late January of this year. The projected revenue from the equine feed assessment for the 2004 fiscal year is estimated at $300,000. Another landmark for the Maryland horse industry occurred in 2002 with the completion of an equine agricultural inventory census as a result of a joint effort between the MHIB, the Maryland Department of Agriculture (MDA) and the Maryland Agricultural and Statistics Service (MASS). The equine census provided Maryland with beneficial information on the number, type, use and value of horses in the state and some aspects of the industry’s economic impact.

The Maryland Horse Industry Board

Purpose

The MHIB was officially established in 1998 by Agricultural Article 2-to fund the promotion, education, and research of the Maryland horse industry. The MHIB is part of the MDA and functions as a commodity board to:

- Promote the horse industry;
- Provide grants to non-profit and not-for-profit groups for equine related educational, promotional and research activities;
- Create public awareness of the benefit of the equine industry to green space preservation;
- Acquire and disseminate information concerning the equine industry;
- Advise the Secretary of Agriculture regarding matters affecting the industry;

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• License, inspect, and enforce provisions of Agricultural Article 2-701 (Licensing and regulation of equine operations);
• Collect and distribute revenue from Agricultural Article 6-107.2 (Assessment on Commercial Equine Feed).

The MHIB consists of eleven non-paid members, not including the Secretary of Agriculture, who are appointed by the Governor of Maryland to serve and represent various aspects of the Maryland Equestrian community. Positions of the MHIB are listed in Table 1.

The MHIB, via the MDA, receives part of its budget from general funds and fees for services performed through the MDA. Additionally, revenue is generated from the Assessment on Commercial Equine Feed (see below).

Table 1: Maryland Horse Industry Board Members.

<table>
<thead>
<tr>
<th>Group Represented</th>
<th>Voting Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Academic Equine Community</td>
<td>Yes</td>
</tr>
<tr>
<td>Trails &amp; Recreational Riding</td>
<td>Yes</td>
</tr>
<tr>
<td>Maryland Thoroughbred Industry</td>
<td>Yes</td>
</tr>
<tr>
<td>Organized Competition &amp; Shows Industry</td>
<td>Yes</td>
</tr>
<tr>
<td>Equine Trade &amp; Support Industries</td>
<td>Yes</td>
</tr>
<tr>
<td>Licensed Veterinarian</td>
<td>Yes</td>
</tr>
<tr>
<td>Maryland Horse Council</td>
<td>Yes</td>
</tr>
<tr>
<td>Public Member</td>
<td>Yes</td>
</tr>
<tr>
<td>Maryland Standardbred Industry</td>
<td>Yes</td>
</tr>
<tr>
<td>Officer of a County Humane Society</td>
<td>Yes</td>
</tr>
<tr>
<td>Licensed Stable Owner</td>
<td>Yes</td>
</tr>
<tr>
<td>Maryland Department of Agriculture Secretary</td>
<td>Yes</td>
</tr>
<tr>
<td>Or Designee</td>
<td>Yes</td>
</tr>
<tr>
<td>Administrative Assistant</td>
<td>No</td>
</tr>
<tr>
<td>Executive Director</td>
<td>No</td>
</tr>
<tr>
<td>Stable Inspector</td>
<td>No</td>
</tr>
<tr>
<td>Stable Inspector</td>
<td>No</td>
</tr>
</tbody>
</table>

Adapted from Agricultural Article 2-703

Maryland Horse Industry Feed Fund

In 2001, Agricultural Article 6-107.2 established the Commercial Equine Feed Assessment, which generates $2.00 per ton on feed sold in Maryland with the intent to be fed to equine. All monies collected are pooled into a single account established through the MDA to be distributed by the MHIB. This 5-year feed fund program was proposed to benefit Maryland’s equine industry by providing the MHIB with the money to support its mission of promotion, education, and research of the Maryland equine. The term equine feed is meant to include feeds that are labeled or intended for an equine, but does not include unprocessed feed such as hay, oats, corn, or any feed that is exempt from the registration requirements. Feed manufacturers may pass the assessment on to consumers and consumers may request a reimbursement for monies paid by them as a result of the assessment. By generating funds from equine feed sold in Maryland, horse owners from all disciplines and breeds are able to support promotional and research programs fairly, without biasing one breed, sport, or program. Revenue generated from the feed fund is distributed to not-for-profit organizations each year in the form of grants and a portion of the revenue funds costs associated with the mission and operation of the MHIB.
Collection and Payment

There are 26 equine feed manufacturers licensed by the State of Maryland, which must comply with the Assessment on Commercial Equine Feed. The MDA collects the assessment from the feed manufacturers on a quarterly basis, with a month allowed for sales reporting following each quarter. Collection from the feed manufacturers rather than the feed distributors was established to minimize the amount of paperwork and possible errors in collection. This year (2003) is the first fiscal year in which the Assessment on Commercial Equine Feed was enforced. The MHIB has estimated over $70,000 of the revenue generated from the assessment to be distributed in the form of grants to the horse industry. Estimated revenue from the Commercial Equine Feed Assessment is $300,000 for the 2004 fiscal year. Non-payment of the Assessment on Commercial Equine Feed may result in the revocation of the manufacturer’s license to sell feed within Maryland.

Reimbursement

The Assessment on Commercial Equine Feed may be passed on to the equine feed consumer. Therefore, the consumer may submit their receipts to the MDA detailing that they have in fact paid the Assessment on Commercial Equine Feed. The MDA has supplied all equine feed retail stores in Maryland with signs to be clearly posted that describe the assessment reimbursement and contact information.

2002 Maryland Equine Census

Purpose

The purpose of the 2002 Maryland Equine Census was to serve as an agricultural inventory census, which would more fully characterize the equine population in Maryland and some aspects of the industry’s economic impact in the State. The definition of an equine included all horses, mules, donkeys, and burros. The MASS conducted the census with funding provided by the MHIB and the MDA. The census information was collected through a survey that was mailed to equine property and horse owners in Maryland with the final cost amounting to over $90,000. Address information of Maryland equine owners were obtained from equine organizations, equine practitioners, and equine publications in Maryland.

Summary Results

There were a total of 87,100 horses, mules, donkeys, and burros in the State of Maryland in 2002. Light Horse Breeds accounted for about 48% of the total, followed by Thoroughbreds with 33% of the total. Ponies and Standardbreds each accounted for about 7%, followed by draft breeds and mules, donkeys, and burros. The value of the equine inventory at the time of the census was a little over $680 million, putting the average value per animal at about $7,810. There were a total of 20,200 equine places in the State in 2002. This includes racing, breeding, and all sizes of boarding facilities as well as private residences where horses are being kept for recreational purposes. On these places, there were 38,000 people actively involved with the equine. These equine operations accounted for a total of 685,000 acres, of which 206,000 acres were used primarily for equine related purposes. The value of all equine related assets totaled $5.2 billion, including the value of the inventory. The value of land, fencing, and buildings made up about 76% of the total assets. The value of inventory accounts for 13% and equipment and supplies made up about 11% of total reported assets. Equine related expenditures amounted to nearly $766 million, of which 62% were operating expenditures and 38% capital expenditures. There were 8,400 horses sold during the past year with an average value of sales of $14,196. Less than 100 mules, donkeys, and burros were sold (MASS, 2002).
**County Data**

Maryland equine were concentrated in central and northern counties. The counties having the largest inventory of equine Baltimore (12%), Montgomery (10%), Frederick (10%), Prince George’s (9%) and Harford (8%). The 10 counties with the highest inventory of equine, all in northern and central Maryland, account for 79% of the total inventory. The average inventory value by county was influenced greatly by the racing and breeding operations located in each county. In addition, the counties with large concentrations of show horses tended to have higher average values. The five largest counties in terms of number of equine places and their respective percentages of the total number of places were: Montgomery (13%), Frederick (11%), Baltimore (10%), Washington (8%), and Harford (7%). The average number of people in each county (excluding hired labor) involved in equine activities varied from 2.6 people per place in Washington county to 1.5 people per place in Dorchester county. The acreage devoted to equine by county is concentrated in Baltimore, Frederick, Harford, Montgomery, and Cecil counties where 51% of the total acreage devoted to equine was reported (MASS, 2002). Table 2 provides a summary of the equine inventory, value, places, acreage, and number of people involved in each county.

**Table 2: Equine Inventory, Value, Places, Acreage and Number of People Involved by County**

<table>
<thead>
<tr>
<th>Counties</th>
<th>Total Equine Inventory</th>
<th>Total Value of Equine Inventory</th>
<th>Number of Equine Places</th>
<th>Total Equine Related Acres</th>
<th>Total Number of People Involved*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(number)</td>
<td>(thousand dollars)</td>
<td>(number)</td>
<td>(acres)</td>
<td>(number)</td>
</tr>
<tr>
<td>Allegany</td>
<td>270</td>
<td>685</td>
<td>120</td>
<td>1,300</td>
<td>240</td>
</tr>
<tr>
<td>Garrett</td>
<td>1,410</td>
<td>2,900</td>
<td>370</td>
<td>3,400</td>
<td>820</td>
</tr>
<tr>
<td>Baltimore</td>
<td>10,630</td>
<td>121,800</td>
<td>2,100</td>
<td>31,200</td>
<td>4,200</td>
</tr>
<tr>
<td>Carroll</td>
<td>5,730</td>
<td>31,735</td>
<td>1,290</td>
<td>14,700</td>
<td>2,430</td>
</tr>
<tr>
<td>Frederick</td>
<td>8,290</td>
<td>47,310</td>
<td>2,180</td>
<td>22,000</td>
<td>3,570</td>
</tr>
<tr>
<td>Harford</td>
<td>7,390</td>
<td>73,115</td>
<td>1,360</td>
<td>18,400</td>
<td>2,160</td>
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<tr>
<td>Howard</td>
<td>5,190</td>
<td>61,265</td>
<td>1,200</td>
<td>11,200</td>
<td>2,280</td>
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<tr>
<td>Montgomery</td>
<td>8,470</td>
<td>60,555</td>
<td>2,590</td>
<td>17,700</td>
<td>4,070</td>
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<tr>
<td>Washington</td>
<td>4,460</td>
<td>12,295</td>
<td>1,560</td>
<td>10,200</td>
<td>4,090</td>
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<tr>
<td>Anne Arundel</td>
<td>4,590</td>
<td>27,035</td>
<td>1,330</td>
<td>8,900</td>
<td>2,320</td>
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<td>Calvert</td>
<td>1,510</td>
<td>9,980</td>
<td>420</td>
<td>3,500</td>
<td>750</td>
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<td>Charles</td>
<td>1,640</td>
<td>7,375</td>
<td>490</td>
<td>4,300</td>
<td>1,110</td>
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<tr>
<td>Prince George's</td>
<td>7,420</td>
<td>63,610</td>
<td>1,170</td>
<td>11,700</td>
<td>1,860</td>
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<td>Saint Mary's</td>
<td>2,710</td>
<td>7,540</td>
<td>620</td>
<td>8,600</td>
<td>1,510</td>
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<td>Caroline</td>
<td>1,310</td>
<td>7,290</td>
<td>260</td>
<td>3,300</td>
<td>530</td>
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<tr>
<td>Cecil</td>
<td>6,580</td>
<td>68,345</td>
<td>900</td>
<td>15,300</td>
<td>1,580</td>
</tr>
</tbody>
</table>
### Breed Data

Table 3 provides a summary of the total number of equine and the value by breed. Light breeds and racing breeds dominated the breed categories across Maryland, accounting for 48 and 40% of the total inventory, respectively. The top 5 breeds with the highest numbers and the respective percent of total were Thoroughbreds (33%), Quarter Horses (14%), Standardbreds (7%), Arabian and Anglo Arabian (5%), and Other Warmbloods (4%). This breed ranking excludes the unknown light breed category, which accounted for 8% of the inventory and all breeds of ponies category which accounted for 7% of the total. The numbers of equine were reported by landowners who kept equine on their operations.

Of the 87,100 equine owned and housed in Maryland, landowners and family members owned 61,900 or 71%. Equine boarded for other owners totaled 25,200 or 29%. For the major breed categories, 34% of the racing breeds were boarded and 28% of the light breeds were boarded. The value of the equine inventory at the time of the census was estimated to total more than $680 million or an average of $7,810 per animal. The most valuable breeds were from the Other Warmbloods category with an average value of $16,883. This category grouped several warmblood breeds or cross breeds together and included a large concentration of show horses. The racing breeds ranked next in average value with standardbreds at $13,653 and thoroughbreds at $13,446. There was a wide range of values reported for most breeds with retired animals at the low end of the scale and show or racing animals at the top end of the value scale (MASS, 2002).
<table>
<thead>
<tr>
<th>Equine Breeds</th>
<th>Total Inventory</th>
<th>Total Inventory Owned*</th>
<th>Total Inventory Boarded for Others**</th>
<th>Total Inventory Value (thousand dollars)</th>
<th>Average Inventory Value (dollars)</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Saddlebred</td>
<td>680</td>
<td>520</td>
<td>160</td>
<td>1,185</td>
<td>1,743</td>
</tr>
<tr>
<td>Appaloosa</td>
<td>2,790</td>
<td>2,120</td>
<td>670</td>
<td>8,540</td>
<td>3,061</td>
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<tr>
<td>Arabian and Anglo Arabian</td>
<td>4,040</td>
<td>2,870</td>
<td>1,170</td>
<td>16,005</td>
<td>3,962</td>
</tr>
<tr>
<td>Miniature Horses</td>
<td>720</td>
<td>670</td>
<td>50</td>
<td>1,085</td>
<td>1,507</td>
</tr>
<tr>
<td>Morgan</td>
<td>1,660</td>
<td>980</td>
<td>680</td>
<td>5,520</td>
<td>3,325</td>
</tr>
<tr>
<td>Paint / Pinto</td>
<td>2,660</td>
<td>2,110</td>
<td>550</td>
<td>10,890</td>
<td>4,094</td>
</tr>
<tr>
<td>Quarter Horses</td>
<td>12,060</td>
<td>9,170</td>
<td>2,890</td>
<td>39,180</td>
<td>3,249</td>
</tr>
<tr>
<td>Tennessee Walker</td>
<td>1,700</td>
<td>1,180</td>
<td>520</td>
<td>5,120</td>
<td>3,012</td>
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<tr>
<td>Other Crossbreds</td>
<td>3,280</td>
<td>2,320</td>
<td>960</td>
<td>17,435</td>
<td>5,316</td>
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<tr>
<td>Other Warmbloods</td>
<td>3,600</td>
<td>2,170</td>
<td>1,430</td>
<td>60,780</td>
<td>16,883</td>
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<tr>
<td>Other Light Breeds</td>
<td>1,880</td>
<td>1,390</td>
<td>490</td>
<td>10,290</td>
<td>5,473</td>
</tr>
<tr>
<td>Unknown</td>
<td>6,930</td>
<td>4,800</td>
<td>2,130</td>
<td>11,320</td>
<td>1,633</td>
</tr>
<tr>
<td><strong>Total Light Breeds</strong></td>
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<td>30,300</td>
<td>11,700</td>
<td>187,350</td>
<td>4,461</td>
</tr>
<tr>
<td>Standardbreds</td>
<td>5,800</td>
<td>4,640</td>
<td>1,160</td>
<td>79,190</td>
<td>13,653</td>
</tr>
<tr>
<td>Thoroughbreds</td>
<td>28,800</td>
<td>18,250</td>
<td>10,550</td>
<td>387,235</td>
<td>13,446</td>
</tr>
<tr>
<td>Other Race Breeds</td>
<td>200</td>
<td>110</td>
<td>90</td>
<td>715</td>
<td>3,575</td>
</tr>
<tr>
<td><strong>Total Race Breeds</strong></td>
<td>34,800</td>
<td>23,000</td>
<td>11,800</td>
<td>467,140</td>
<td>13,424</td>
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<tr>
<td>Belgian</td>
<td>690</td>
<td>650</td>
<td>40</td>
<td>1,580</td>
<td>2,290</td>
</tr>
<tr>
<td>Percheron</td>
<td>420</td>
<td>330</td>
<td>90</td>
<td>1,985</td>
<td>4,726</td>
</tr>
<tr>
<td>Clydesdale</td>
<td>110</td>
<td>70</td>
<td>40</td>
<td>520</td>
<td>4,727</td>
</tr>
<tr>
<td>Other Draft Breeds</td>
<td>980</td>
<td>850</td>
<td>130</td>
<td>3,775</td>
<td>3,852</td>
</tr>
<tr>
<td><strong>Total Draft Breeds</strong></td>
<td>2,200</td>
<td>1,900</td>
<td>300</td>
<td>7,860</td>
<td>3,573</td>
</tr>
<tr>
<td>Ponies, All Breeds</td>
<td>5,900</td>
<td>4,700</td>
<td>1,200</td>
<td>16,480</td>
<td>2,793</td>
</tr>
<tr>
<td>Mules, Donkeys, &amp; Burros</td>
<td>2,200</td>
<td>2,000</td>
<td>200</td>
<td>1,410</td>
<td>641</td>
</tr>
<tr>
<td><strong>Total All Equine</strong></td>
<td>87,100</td>
<td>61,900</td>
<td>25,200</td>
<td>680,240</td>
<td>7,810</td>
</tr>
</tbody>
</table>

*Total inventory owned by the landowner and/or family.
**Equine inventory is owned by someone other than the landowner
Source: MASS, 2002
Other Data

Table 4 provides the number of equine by breed and primary function of the operation. Most of the Light Breeds (45%) were kept at private residences, while 31% were kept at boarding, training, riding, or show facilities. As would be expected, most of the race breeds (39%) were kept at race related facilities while 27% were kept at breeding facilities. The largest percent of Draft Breeds, 41%, were kept on crop or livestock farms. Ponies were mainly kept at private residences and boarding, training, riding, or show facilities. Mules, donkeys, and burros were mainly kept at private residences. Of the total number of equine places, approximately 11,800, or 58% indicated that they had rode through trails some time during the past year. An overwhelming majority of respondents indicated that they rode on private land (94%), while 60% indicated they rode on State, National, or Regional Parks. Respondents could choose multiple entries if they rode in multiple areas (MASS, 2002).

Table 4: Number of equine by breed and primary function of the operation.

<table>
<thead>
<tr>
<th>Type of Equine Operation</th>
<th>Number of Equine By Breed Category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light Breeds</td>
</tr>
<tr>
<td>Boarding, Training, Riding or Show Facility</td>
<td>13,000</td>
</tr>
<tr>
<td>Commercial or Private Breeding Facility</td>
<td>3,870</td>
</tr>
<tr>
<td>Racing or Race Related Facility</td>
<td>430</td>
</tr>
<tr>
<td>Private residence with Equine</td>
<td>18,850</td>
</tr>
<tr>
<td>Crop/Livestock Farm</td>
<td>4,310</td>
</tr>
<tr>
<td>All Other Facilities</td>
<td>1,540</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>42,000</strong></td>
</tr>
</tbody>
</table>

Trail Riding Preferences

- Total Number of Places Reporting Trail Riding* = 11,800
- Percent Using State, National, or Region Parks = 60%
- Percent Using State, or National Forest Land = 26%
- Percent Using Wildlife Management Areas = 24%
- Percent Using Private Land = 94%
- Percent Using Other Locations = 8%

*Respondents could indicate multiple trail riding locations
Source: MASS, 2002

Conclusion

With the establishment of the MHIB, revenue has been created through the Assessment of Commercial Equine Feed sold in the Maryland. This money directly benefits promotion, education, and research of the horse and the industry in Maryland. One such promotional tool was the creation of the 2002 Maryland Equine Census, which has received widespread media, legislative, and public attention. The MHIB continues to investigate additional sources of funding in order to further its mission. The existence of the MHIB has contributed to an awareness of the horse’s vital role in the state economy.
References

Annotated Code of Maryland: Agricultural Article 2-703
Annotated Code of Maryland: Agricultural Article 6-107.2
GERIATRIC HORSE NUTRITION
AN UPDATE ON CARE FOR THE OLDER HORSE: DIET AND HEALTH

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Summary:

The aging equine population is large, with horses over the age of 20 making up approximately 15% of the population. Many of these horses continue to be active both athletically and reproductively. As with their human counterparts, older horses can be afflicted with age-related conditions such as arthritis, malabsorption of nutrients and/or diseases that can be ameliorated with proper management and dietary manipulation.

However, not all aged horses require special dietary care. Horses that can maintain good body condition on traditional feeds do not need to be switched to “senior” rations just because they have reached a certain age. Healthy aged horses in adequate body condition do not gain further benefits from “senior” rations.

From a managerial standpoint, aged horses should continue to receive routine vaccinations and veterinary care and remain on a good rotational deworming program, even if they are “retired.” Their rations should include good quality roughage, free choice water and salt, and concentrates or other supplements only when needed to help maintain body condition or when known deficiencies are present. If fed in groups, insure that all horses have adequate access to their feed.

Older horses do have a lower tolerance for extreme weather (i.e. heat and cold). In extremely cold conditions, older horses need shelter, additional forage or concentrates and perhaps blanketing. There should be free access to fresh, unfrozen water. To help prevent impaction colic, feeding hay cubes or beet pulp soaked in water may further increase water intake. In extreme heat, shelter or at least shade is needed, with unlimited access to fresh water and salt. Aged horses with pituitary dysfunction that retain long hair coats in the summer, should be clipped.

Exercise is extremely important in aged horses. Provide as much time and space as possible for aged horses to move about freely. However, there is an age-related decline in thermoregulation and maximal aerobic capacity as well as an altered endocrine response to exercise. Therefore, exercise regimens must be appropriately matched to the horse’s fitness level and ability. Aged horses used for athletic endeavors should be monitored carefully for signs of distress such as excessively high heart and/or respiration rates as well as prolonged recovery times.

To combat the arthritic conditions that plague older horses, nutraceutical therapies such as glucosamine, chondroitin sulfate and methylsulfonylmethane may help. Acupuncture and herbs or nutrients such as yucca, willow bark and omega 3 fatty acids can also be considered as alternative...
therapies to the commonly prescribed non-steroidal anti-inflammatory drugs (NSAIDS) that can have deleterious side affects with prolonged use. Arthritic horses especially should be given as much access to free exercise as possible.

Older horses may lose body condition and muscle mass but this is usually reversible with proper management. If an older horse starts to lose weight, the underlying cause should be determined before merely changing the ration. Some “senior” feed formulations may actually exacerbate pre-existing kidney, liver and endocrine dysfunctions. The horse’s teeth also should be carefully examined for abnormalities. Sometimes appropriate dental correction is all that is needed for the horse to gain weight.

Horses with non-correctable dental problems can maintain good body condition if fed hay cubes, beet pulp and “complete” pelleted or extruded feeds soaked in water. One half gallon of water should be added per pound of feed. Horses with missing or misaligned incisors cannot utilize pasture well and should be provided with hay cubes, beet pulp or chopped hay for the roughage portion of their ration.

If an older horse in poor condition is not suffering from disease, inadequate access to feed, or poor dentition, the weight loss may be due to malabsorption or maldigestion. Such horses may benefit from a “senior” ration formulated to contain at least 12% protein, restricted calcium (<1.0%) and slightly increased phosphorus (0.3-0.5%). The calcium to phosphorus ratio should remain at least 1:1. The fiber portion of the ration should ideally be greater than 10% and no less than 7%. Processing the feed by pelleting, extrusion or pre-digestion will increase the digestibility of the ration. Adding edible fats to the ration (5-10% of total) will further increase the digestible energy intake. Supplements such as yeast products and B-vitamins may also help increase digestion and appetite, respectively.

Obesity (excessive accumulation of body fat) is also commonly seen in older horses. Obesity puts the horse at risk for laminitis, lipoma strangulation colic, heat and exercise intolerance and adds strain on arthritic joints. Overweight horses (body condition score of 8 or greater) should lose weight through a reduction in feed and an appropriate increase in exercise. Fat horses, however, should not be “starved” due to the risk of hypertriglyceridemia. They should be fed 75% of the amounts recommended for maintenance of their ideal (not current) body weight. The concentrate portion of the ration should be reduced first. Significant weight loss should take several months to achieve.

Endocrine dysfunctions are common in aged horses. Insulin resistance, characterized by hyperinsulinemia and/or hyperglycemia after a meal of grain, can be due to pituitary or thyroid dysfunction and/or obesity and is especially prevalent in old horses. Pituitary dysfunction is also characterized by muscle wasting, excessive thirst and urination, high cortisol and failure to suppress secretion with a dexamethasone challenge, increased susceptibility to infections and laminitis and low plasma vitamin C. Hypothyroidism leading to obesity and increased risk of laminitis is not uncommon. Dietary management of such endocrine dysfunctions includes feeding a primarily roughage based ration and avoiding “sweet feeds” that contain >3% molasses. Concentrates, preferably high fat (>5%) and fiber (>7%), should only be offered if necessary, and should be offered in relatively small meals to help control blood glucose levels. Exercise has also been shown to help increase insulin sensitivity. If excessive drinking and urination are present, free access to water and adequate bedding, if the horse is stalled, are necessary. Vitamin C supplementation may also help horses with chronic infections such as “rain rot,” hoof abscesses, etc.

Reduced kidney and liver function must be identified by appropriate blood analyses. Though kidney disease is usually irreversible, diets with restricted calcium, protein and phosphorus will improve the well being of affected horses. Good quality grass hay, edible oils and concentrates formulated for mature (not aged) horses are recommended. Beet pulp, wheat bran and legume hays should be avoided.
Liver failure also can be managed to some extent by dietary manipulations. High carbohydrate feeds such as corn and barley or sweet feed mixes are recommended. Adequate fiber should still be offered in the form of grass hay or beet pulp to maintain gastrointestinal function. Protein and fat supplements should be avoided. Vitamin B and C supplementation may also be beneficial.

In conclusion, geriatric horses may be afflicted with age-related conditions that can be partially relieved by dietary manipulation. Proper diagnosis of the condition is paramount for determining which recommendation to follow if an aged horse is in ill form. Implementation of proper management and nutritional changes can help these elder horses live longer, healthier lives.

Approximately 15% of the equine population is over the age of 20, with many of these horses remaining active and healthy and even reproductively and athletically sound (Hintz, 1995; Rich, 1989). As horses get older, however, they may be affected by age-related conditions that require specific management and dietary practices. The basic management of older horses will be discussed, as well as conditions requiring special care through dietary and managerial alterations.

General Management of the Older Horse

Most horse feed companies now have special feeds designed specifically for older horses. However, it is important to note that not all aged horses require special dietary care. Older horses that are able to maintain good body condition (score of 5 or higher based on the Henneke et al., 1983 system), and remain healthy and active on traditional rations do not require special dietary supplementation or alterations. Old horses at a large “retirement” facility that were in good body condition did not further improve their condition or health parameters when switched to a “senior” feed instead of the standard sweet feed mix that had been used previously (Ralston, 1989). As with all horses, aged horses should receive good quality roughage, free access to salt and water with supplementation of concentrates only when necessary to maintain body weight or to correct known deficiencies. It is important that aged horses have adequate access to their feed if fed in groups and that they have shelter from extreme environmental conditions. Routine annual vaccinations and veterinary examinations should be given and regular rotational deworming schedules should be maintained even if the horse is retired.

Many older horses are still used for athletic performance well into their mid to late twenties and beyond (Hintz, 1995; Rich, 1989). Regardless of use, all aged horses should be provided with adequate space and time to exercise. However, aged horses do have a lower maximal aerobic capacity, reduced ability to thermoregulate and an altered endocrine response to exercise compared to their younger counterparts (McKeever and Malinowski, 1997, 1999; McKeever et al., 2000). Therefore, exercise regimens should be designed to appropriately match the fitness capacity of the horse and the horses monitored more carefully for signs of distress (excessively high heart and/or respiration rates and prolonged recovery rates).

Arthritis is a common ailment in aged horses, as is to be expected from years of wear and tear on their joints. Mild arthritis, which the horse easily “warsms-up” out of, is very common. Such horses should not be confined to stalls unless absolutely necessary, since restricted activity will exacerbate their stiffness. Severe arthritis should be treated appropriately. Non-steroidal, anti-inflammatory drugs (NSAIDS) can provide relief, but long-term use can have adverse side effects such as an increased risk of gastric ulcers. Hence, alternative therapies such as nutraceutical joint supplements, acupuncture and anti-inflammatory herbs and nutrients such as yucca, willow bark and omega-3 fatty acids should also be considered.

Older horses also have a lower tolerance to severe weather- both heat and cold, compared to younger horses (Ralston et al., 1988). In cold weather, more feed (25-30% above maintenance
requirements) should be given. Special care must also be taken to provide plenty of clean, unfrozen water to encourage drinking and help prevent impaction colic. If horses are unwilling to drink adequate amounts of water in cold weather, soaking pelleted or extruded feeds and hay cubes in water (1/2 gallon/lb of dry feed) will help to increase fluid intake. Furthermore, aged horses should be provided with adequate shelter and blanketing, if necessary, in cold weather conditions. Blankets must fit the horse properly and be checked frequently for signs of rubbing or dermatitis. In extreme heat, aged horses should have access to shade and cooler areas out of direct sunlight, and have unlimited access to salt and clean water. If the horse fails to shed it’s winter coat, as is common in pituitary dysfunction (see below), it should be kept clipped in the summer months.

**Nutritional Considerations in Aged Horses**

Chronic weight loss or poor body condition is a frequent ailment affecting old horses. The most common causes are usually poor dentition, debilitating diseases such as hepatic and renal failure, and/or inadequate anthelmintic administration (Ralston, 1999), most of which can be at least partially ameliorated with proper nutrition and management. On the other hand, obesity is another familiar problem in older horses. Obesity is correlated with insulin resistance and increases the risk of laminitis, colic and other metabolic disorders such as hyperlipidemia (Jeffcott et al., 1986; Field and Jeffcott, 1989; Milne et al., 1990; Lewis, 1995). Regardless of body condition, before instituting any dietary change, the older horse should have blood analyses done to check for kidney and liver dysfunction. The nutritional recommendations for these conditions differ from those for otherwise healthy older horses suffering weight loss, and feeding a horse with kidney or liver dysfunction a “senior” feed may exacerbate clinical signs. Older horses have been found to have a tendency for mild microcytic, hypochromic anemia, but all other blood parameters should be within normal range for adult horses (Ralston et al., 1988; McFarlane et al., 1998).

In addition to the recommendations below, please refer to Tables 1 and 2 for a quick reference of management considerations and dietary suggestions for aged horses with specific problems.

**General Weight Loss**

If an older horse’s weight loss is not due to poor dentition, inadequate access to feed or chronic disease, the horse may be suffering from malabsorption. A study in the 1980’s reported that horses over 20 years old had a reduced digestibility of protein, fiber and phosphorus compared to younger horses, a profile that was similar to horses that had extensive large colon resection (Ralston and Breuer, 1996). Similar studies conducted 10 years later on old horses, however, did not reveal the same results (Ralston et al., 2001). It has been hypothesized that the difference could be due to chronic parasitic damage to the large intestines. In the earlier study the horses used had not had the advantages of more effective deworming agents and protocols developed in the 70’s and 80’s that had been available to the horses studied in the 1990’s since their birth.

If chronic disease has been ruled out (see below), concentrates formulated specifically for “senior” horses may help thin old horses to gain weight and overall condition. These concentrates should contain at least 12% protein, with restricted calcium (<1.0%) and slightly increased phosphorus (0.3-0.5%). The calcium to phosphorus ratio, however, should be greater than 1:1. The crude fiber percentage of the ration should also be at least 7%, with greater than 10% being ideal, especially if it is to be used as a “complete” feed without access to hay. The “senior” feed should be highly digestible and easily chewed due to processing techniques such as pelleting, extrusion or pre-digestion. Increasing the edible fat content of the ration (5-10% of total) is also an excellent way to increase the digestible energy intake, without increasing the size of individual meals (Potter et al., 1990).
An example of an ideal diet for the average size older horse (1100 lbs) that is having trouble maintaining body condition but is in otherwise good health would consist of free choice, good quality grass or grass/legume mix hay, two to eight pounds of “senior” feed split into several feedings and free choice water and salt. Straight alfalfa should be avoided, as its high calcium content may contribute to kidney and bladder “stones” and kidney dysfunction. *Saccharomyces cerevisiae* yeast products may improve digestion in some horses (Medina *et al.*, 2002), and may help the failing, older horse to regain body condition. Brewer’s yeast, used as a source of B-vitamins, can be added to the ration (2 to 4 ounces/day) to improve appetite. Make all dietary changes slowly over 4-5 days.

**Poor Dentition**

Aged horses should have their teeth checked for abnormal wear and/or tooth loss every 6 months. It is common for aged horses to suffer from abnormally large points and wave mouth secondary to the loss of teeth. The resultant inability to adequately chew feed, as reflected by the horse “quidding” or dropping feed or boluses of hay from their mouth, usually results in weight loss if the horse is fed only dry hay and textured grains. Furthermore, the inability to properly chew and subsequent reduced salivation lead to an increased risk of “choke” in aged horses.

To accommodate the horse with poor or missing teeth, “soups” can be made by soaking hay cubes and/or beet pulp with added pelleted or extruded feeds, preferably “complete” feeds formulated for old horses. At least ½ gallon of water should be used for each pound of feed in order to reduce the risk of choke and to increase water intake. These “soups” should also be made immediately before feeding to help prevent fermentation in the summer and freezing in the winter. They may need to be fed 3-4 times per day to maintain proper body condition if the horse can no longer effectively eat hay or other forages. Good quality pasture is an excellent source of nutrients for aged horses, provided they can “clip” the grass with their teeth. Horses that are missing their incisors or have incisors that are badly aligned cannot easily utilize pasture for nutrition. Hay cubes, beet pulp and/or chopped hay (1.0 to 2.0% of the horse’s body weight) should be provided in this situation.

**Endocrine Dysfunction**

Endocrine dysfunction in the form of pituitary and thyroid abnormalities, are common in geriatric horses. In one study 70% of the horses tested over the age of twenty had pituitary or thyroid dysfunction with reduced glucose tolerance and excessive cortisol secretion (Ralston *et al.*, 1988). In addition, aged mares with pituitary dysfunction had lower blood levels of vitamin C than young horses maintained in the same conditions and fed the same feeds (Ralston *et al.*, 1988).

Relative insulin resistance (also characteristic of obesity) is the apparent cause of the impaired glucose tolerance seen so frequently in aged horses. This results in excessive insulin secretion following ingestion of grain or other high carbohydrate sources and is correlated with an increased risk of laminitis (Jeffcott *et al.*, 1986; Field and Jeffcott, 1989; Stull and Rodiek, 1988). It is easy to document insulin resistance by feeding the horse 2 to 3 lbs of grain in the morning after an overnight fast and taking a blood sample for glucose and insulin 60-90 min later. Insulin concentrations >200 µIU/ml are considered to be abnormal (Ralston, 2002). Hyperglycemia (blood glucose >200 mg/dl) is not always seen, but, if present, usually causes polyuria and polydipsia (excessive drinking and urination).

For dietary management of insulin resistant horses, high sugar/starch feeds such as textured “sweet” feeds (>3% molasses) should be avoided and higher fat (>5%) and fiber (>7%) concentrates should be offered to reduce hyperinsulinemia/hyperglycemic responses. The ration should consist of primarily good quality roughage with only as much concentrate as absolutely necessary to maintain good body weight. Concentrates should be offered in relatively small meals (<2 to 3 lbs/meal). If
polyuria/polydipsia is present be sure to provide free choice access to clean, fresh water and provide adequate clean bedding to keep the horse comfortable if stalled.

Exercise may also help the older horse with endocrine dysfunction control its glucose and insulin metabolism. Aged horses that were previously unfit and demonstrating impaired glucose tolerance had lower insulin responses to an oral glucose tolerance test after 12 weeks of exercise training (Malinowski et al., 2002). Obese and insulin resistant mares also were able to improve their insulin sensitivity with forced exercise, however nine days after exercise ceased, the improvement in sensitivity had disappeared (Powell et al., 2002). It is necessary, however, to consider the horse’s fitness level, soundness and recovery capacity when implementing an exercise regimen.

There are pharmaceutical treatments for pituitary dysfunction available, but the most effective drugs, cyproheptadine and pergolide, have not been approved for use in horses and can also be prohibitively expensive. The dosage protocols also are still being refined. Pergolide may also cause inappetence or anorexia at the higher doses. It is important to work with the veterinarian to establish the correct dosage of medication (Levy et al., 1999).

Abnormal glucose and cortisol metabolism, coupled with lower antioxidant status and lower lymphocyte counts, may also explain why older horses are more susceptible to infection and seem to have a weaker immune system than their younger counterparts (McFarlane et al., 1998, Ralston et al., 1988). In the case of chronic infections such as “rain rot” or chronic hoof abscesses, ascorbic acid (vitamin C: 0.005 to 0.01gm/lb BW) can be added to the feed twice a day, although it should be gradually discontinued when the infection clears up or continued without interruption for the life of the horse. Long-term supplementation (>5 days) with high levels of ascorbic acid may impair endogenous synthesis and result in deficits if abruptly discontinued (Ralston, 2002).

Reduced Kidney Function

Although not as common in aged horses as in old cats and dogs, reduced kidney function or kidney disease can occur, causing inappetence, lethargy and weight loss. To diagnose kidney failure, appropriate blood work must be done (blood urea nitrogen and creatinine). Unfortunately, kidney disease in old horses is usually progressive and irreversible, but some clinical signs can be reduced by dietary manipulations.

Due to horses’ unique excretion of excess dietary calcium in the urine, decreased kidney function can cause renal and bladder “stones” of calcium oxalate, as well as a potentially lethal increase in blood calcium (Elfers et al., 1986) if the ration contains excess amounts of the mineral. Therefore, horses with kidney failure should be placed on a low calcium diet (0.4 to 0.6% calcium on a dry matter basis). Based on data from other species, protein should also be restricted to 8 to 10% and phosphorus restricted to 0.25% in the ration. Beet pulp and legumes such as alfalfa and clover should be avoided due to their high calcium contents, as well as wheat bran for its high level of phosphorus. Good quality grass hay and concentrates formulated for mature, inactive horses (10%-12% protein, depending on the hay quality) are fine choices for horses with failing kidneys. Fat supplements (any of the edible oils) may be used to increase the caloric intake if necessary.

Reduced Liver Function

Liver failure must also be diagnosed by blood analysis (hepatic specific enzymes and bile acids) and can result in weight loss, lethargy, jaundice, loss of appetite and intolerance of fat and protein in the diet. In severe cases, hepatoencephalopathy, which causes abnormal behavior and depression, may be present (McGorum et al., 1999). Dietary management includes increased simple carbohydrate sources to
help maintain blood glucose levels and lower protein and fat in the diet. In cases of
hepatoencephalopathy, protein sources should be higher in the branched chain amino acids (leucine,
iseoleucine, valine) and lower in the aromatic amino acids (phenylalanine, tyrosine, tryptophan) (Ralston,
1991). Oats, soybean meal and wheat bran are all relatively high in the aromatic amino acids and should
be avoided if possible. Although the ration should emphasize starch intake with grains such as corn and
barley, fiber sources such as hay and/or beet pulp are still necessary to avoid further gastrointestinal
dysfunction. Grass hay, low protein sweet feeds, and corn are recommended components of the ration.
The liver is also the site of vitamin C and vitamin B synthesis in the horse, and therefore daily oral
supplementation with B-complex and ascorbic acid may be beneficial.

**Obesity**

In contrast to the weight loss seen in some geriatric horses, many older horses are severely
overweight and therefore at risk for many health problems such as laminitis, lipoma strangulation colic,
heat and exercise intolerance and increased stress on arthritic joints. The most common cause is that as
older horses’ physical activity levels decrease, their owners continue to feed them their usual rations.
However, there is also a dynamic between the impaired glucose tolerance or insulin resistance commonly
seen in older horses and obesity (Ralston, 2002). Regardless of the cause of excessive body fat, weight
loss is necessary if an older horse’s body condition is an 8 or greater using the Henneke scoring system
(Henneke et al., 1983). Overweight horses should not be starved due to an increased risk of
hypertriglyceridemia, which can cause severe liver damage. Feed should be gradually decreased to 75%
of the recommended amounts for maintenance based on the horse’s ideal (not current) body weight, in
addition to making sure the horse is adequately and appropriately exercised. The concentrate portion of
the ration should be reduced first. When the feed is decreased, or if the horse is maintained only on dry
forage, a vitamin/mineral supplement may be necessary. It could take several months for the horse to
achieve desired body condition.

**Summary**

In conclusion, there are several instances in which geriatric horses require special dietary
considerations. Conditions of weight loss, poor dentition, endocrine dysfunction, kidney or liver failure
and obesity can be improved with the knowledge and implementation of appropriate dietary
manipulations. Owners of aged horses should be aware and considerate of their horses’ age-related
delay in weather tolerance, aerobic capacity as well as their arthritic tendencies. Not all aged horses
require special dietary care, however all horses should receive balanced rations, regular veterinary and
dental care, along with a routine deworming program. With proper management and dietary modifications
when necessary, older horses can live longer, more useful lives than deemed possible less than 15 years
ago.
### Table 1. Conditions requiring special attention in aged horses. See text for more details

<table>
<thead>
<tr>
<th>Condition Considerations</th>
<th>Clinical Signs</th>
<th>Causes/Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis</td>
<td>Chronic lameness, Bone deformity around joints, Inflexible joints</td>
<td>Chronic wear and trauma, Shoeing or trimming, adequate bedding and footing, avoid obesity, anti-inflammatory therapy, Judicious exercise</td>
</tr>
<tr>
<td>Weight Loss</td>
<td>Inability to maintain good body condition despite good teeth, and a ration that is adequate for mature horses</td>
<td>Dental abnormalities, inadequate ration or shelter, Liver or Kidney failure, Tumors, Malabsorption/Dental correction, shelter, Diet*</td>
</tr>
<tr>
<td>Inadequate Dentition</td>
<td>Sharp points on molars, loss of teeth, Inability to chew feed &quot;quidding&quot; of hay</td>
<td>Missing or poorly aligned teeth/dental correction, if possible Diet*</td>
</tr>
<tr>
<td>Pituitary/Thyroid Dysfunction</td>
<td>Failure to shed winter coat in the summer, Recurrent viral infections, Chronic founder (laminitis), Increased water intake and urination, Excessive weight loss (pituitary) or gain (thyroid)</td>
<td>Tumors in the pituitary and/or thyroid glands, Grooming and clipping, Diet*, routine vaccination, adequate water access, Vitamin supplementation (?), Drug therapy(?)</td>
</tr>
<tr>
<td>Kidney/Liver Failure</td>
<td>Weight loss, Lethargy, Poor appetite, Difficult or frequent urination (kidney), Jaundice (liver)</td>
<td>Multiple causes, Diet*</td>
</tr>
<tr>
<td>Grey hair appearing around ears, eyes, and forehead</td>
<td>This is not a problem, merely a sign of aging</td>
<td></td>
</tr>
</tbody>
</table>

*See Diet Recommendations in Table 2.*

### Table 2. Dietary management of conditions associated with aging in horses

<table>
<thead>
<tr>
<th>Condition</th>
<th>Recommended Diet Characteristics</th>
<th>Feeds/Supplements</th>
</tr>
</thead>
</table>
| Weight loss not due to liver or kidney failure | 12 to 14% protein  
7 to 10% fat  
High digestibility  
Easily chewed | Grass or grass mix hay  
Complete pelleted or extruded feeds  
Good quality pasture  
1/4 to 1 cup vegetable oil/day  
Yeast culture products  
Brewers yeast  
Beet pulp (soaked)  
Soybean meal (1/4 to 1/2 lb per day)  
Avoid poor quality or high fiber hay |
|------------------------------------------------|-------------------------------------------------|
| Inadequate Dentition | Easily chewed and digested | ‘Soups’ of complete pelleted or dentition extruded feeds.  
Soaked hay cubes or beet pulp  
Avoid coarse hay and dry pelleted feeds |
| Pituitary/thyroid tumors | Reduced starch  
Highly digestible fiber sources  
Increased vitamin C if chronic infections | Low molasses, high fat/fiber feeds  
Good quality hay or pasture  
5 - 10 gm vitamin C twice a day |
| Kidney failure | Restricted calcium, protein, phosphorus | Grass hay, corn, barley,  
Complete feeds designed for inactive adult, not aged, horses  
Avoid legumes, wheat bran, beet pulp |
| Liver failure | Restricted protein  
Increased starch  
Increased B-vitamins  
Increased vitamin C | Grass hay, corn, 10% protein sweet feeds  
Sweet feeds designed for maintenance B-complex supplement  
10 gm vitamin C daily  
Avoid soybean meal, oats, wheat bran, high fat rations. Limit legumes. |

**References:**


Introduction

Feeding young growing horses is of major concern to owners of future performance prospects. Nutritional imbalances and rapid growth rates have been associated with some forms of developmental orthopaedic disease (DOD) in young horses. Developmental orthopaedic disease has been defined as any abnormality in the normal conversion of cartilage to bone (McIlwraith, 1986). It is a blanket term that includes such conditions as physitis, osteochondrosis, osteochondritis dissecans (OCD) and others. These diseases are largely multi-factorial; however, growth rate and nutritional plane may have an effect on the occurrence or severity of the diseases. A possible cause-effect relationship among rapid growth, nutrition and DOD has been established in dogs (Hedhammar, 1974; Dämmrich, 1991), poultry, and pigs (Olsson and Reiland, 1978). However, the exact relationship among rapid growth, nutrition and DOD in horses is unclear.

Young horses achieve 90% of their mature body weight by age two while 90% of their skeletal growth (height) is attained by age 12 months (Lewis, 1995)! Due to this rapid rate of skeletal growth, it becomes very important to manage the nutrition of the young horse to enable it to meet its full genetic potential for size and athletic ability, while providing sufficient nutrition for healthy bone development. Much research in the area of nutrition of the growing horse has been focused on the effects of nutrition on sound bone development. The National Research Council’s (NRC) Nutrient Requirements for Horses (1989) provides minimum guidelines for nutrient intakes of all classes of horses including those in various stages of growth. It should be emphasized that these are minimum, not optimum recommendations.

Dietary Nutrients for the Growing Horse

Digestible Energy (DE)

Digestible energy, measured in megacalories (Mcal), is the primary controller of growth rate. However, energy imbalance is one of the most common dietary problems in young horses (NRC, 1989). Several researchers have suggested that there are detrimental effects of feeding unbalanced rations to young horses, specifically to bone growth and metabolism (Hintz and Kallfelz, 1981; Savage et al., 1993a,b). Increases in DE without proportional increases in crude protein (CP), vitamins or minerals have been shown to be detrimental to bone growth (Hintz and Kallfelz, 1981). When DE density is increased without accompanying increases in other nutrients, bone growth may be rapid; however the quality of the bone may be negatively impacted due to the absence of other nutrients necessary for optimal bone growth (Kronfeld et al., 1990; Ralston, 1997).
Many research reports have linked excess nutrition (high intake) to DOD in young growing horses. Hintz et al. (1976) reported that four out of six weanling horses fed a diet designed to restrict ADG developed flexural deformities due to rapid compensatory gain after being switched to ad libitum feeding. Weanlings fed ad libitum throughout the same study had no skeletal abnormalities (Hintz et al., 1976). However, the ADG results described as “rapid compensatory gain” were not different from those weanlings fed ad libitum throughout the study (Hintz et al., 1976). This suggests that the growth rate itself may not have been a cause of the flexure deformities. The diet fed to both groups of weanlings met or exceeded 1973 NRC recommendations for CP, Ca, P, and Vitamin A; however the energy density was not reported (Hintz et al., 1976). A similar study used balanced diets fed either ad libitum or to meet NRC (1978) minimum requirements (Cymbaluk et al., 1990). Ad libitum-fed weanlings in this study had significantly higher ADG during two different periods of the trial than did limit-fed weanlings. Those same ad libitum-fed foals also had higher occurrences of conformational and musculoskeletal abnormalities than did the limit-fed foals (Cymbaluk et al., 1990). The researchers in the aforementioned study pointed out that, though weight distribution on either the fore or hind limbs was not different between groups, the ad libitum-fed horses were more active during turn out which may have contributed to increased stress on the growing joints (Cymbaluk et al., 1990).

Savage et al. (1993a) conducted a study with weanling horses aged two to six months fed one of three rations: excess DE (129% NRC recommendation), excess CP (126% NRC recommendation) or a control diet (100% NRC for both DE and CP). Post-mortem examination of the joints showed that all of the weanlings fed the high DE diet had articular/epiphyseal lesions of osteochondrosis., Only two control horses had lesions and four out of six weanlings fed high protein had minor (and usually single) lesions in the metaphyseal growth plate.

In another study, weanlings fed a high energy diet (150% NRC requirements) had greater growth rates than weanlings fed a diet that met 100% NRC requirements for all nutrients or the same diet with only 35% of NRC recommendations for calcium (Thompson et al., 1988). Those weanlings fed the high DE diets or controls fed recommended energy levels (100% NRC) had greater increases in lateral radiographic bone density of the third metacarpal bone, indicating a greater growth rate in the long bones (Thompson et al., 1988). High energy-fed horses also had greater increases in medial radiographic bone density of the third metacarpal bone (Thompson et al., 1988). Increased radiographic bone density has been positively correlated with greater bone strength (Chapuy et al., 1992; Välimäki et al., 1999). The increases in radiographic bone density seen by Thompson et al. (1988) may have been a result of the higher body weights leading to increased loading on bones (Wasserman, et al., 1993).

Ott and Asquith (1986) conducted a study using Quarter Horse and Thoroughbred yearlings fed either ad-libitum (1.5 hours per day) or restricted amounts, of a grain supplement with adequate CP and DE. The results showed no significant differences in bone growth and development. Radiographic bone mineral density of the third metacarpal bone also showed no effect of diet on bone development. However, restricted-feeding of the grain mix led to lower weight and girth gains than in the adequate and non-restricted horses. While the ad-libitum fed horses tended to show greater increases in radiographic bone density characteristics as compared to restricted-fed yearlings, the results were not statistically significant.

**Crude Protein (CP)**

Excess dietary CP was once thought to be the major culprit in the pathogenesis of equine DOD. Studies have shown, however, that excess CP does not appear to negatively affect the development of the equine long bones (Frape, 1998). Amino acids, the building blocks of protein, are the main components of muscle tissue, enzymes and several body hormones (NRC, 1989) and are needed in high quantities during
growth stages. Protein and digestible energy are closely linked in their control of growth rate. In other words, when dietary DE content increases, protein requirements also go up. (NRC, 1989).

Protein deficiency is a more realistic problem when it comes to feeding the growing horse. Foals fed a diet deficient in CP (9% CP) had decreased growth (0.06 kg/d), impaired bone turnover, lower dry matter intake and inefficient feed utilization as compared to foals fed 20% CP or 14% CP diets (0.63 and 0.69 kg/d, respectively) (Schryver et al., 1987). When the protein deficient foals were switched to the high or moderate protein diet, they had rapid compensatory gain (1.01 kg/d) with no apparent skeletal problems (Schryver et al., 1987).

Of major importance in the growing horse’s diet is the quality of the protein source of the feed. High quality protein sources will have high levels of the amino acids lysine, threonine and methionine. Lysine is the first-limiting amino acid for growth (Ott and Asquith, 1986). If it is not in sufficient quantity, the horse’s own protein-producing ability will be compromised, thus decreasing growth. When threonine was supplemented at two different levels to yearling horses fed a diet containing 12% protein, those yearlings receiving the higher levels of threonine had higher ADG than yearlings not receiving any supplemental threonine. This result suggests that threonine is the second-limiting amino acid for growth (Graham et al., 1994). Weanlings and yearlings fed a low protein (9% CP) diet fortified with additional soybean meal, lysine and threonine utilized the available protein more efficiently for growth and development than similar aged horses on a control supplement with no added lysine or threonine (Staniar et al., 2001). These results suggest that horses fed low protein diets with additional high quality amino acids added will not have impaired growth performance.

**Calcium & Phosphorus**

Calcium is one of the major components of bone mineral. It is obviously needed in large quantities by the growing horse, and is most readily absorbed by young horses. Calcium absorption declines as the horse ages, with a range of 50-70% absorptive efficiency (NRC, 1989). Phosphorus accounts for 14-17% of the equine skeleton and is also required for energy production. It is also required in high amounts by the growing horse.

As nutritionally important as total Ca intake is the Ca:P ratio. The ideal dietary Ca:P ratio is considered to be approximately 2:1 – a number that reflects the relationship of these two minerals to each other in the bones. Ratios should never be lower than 1:1 because when dietary P is in greater quantity than dietary Ca, the result is the removal of Ca from the bones leading to osteomalacia or rickets (see Figure 1). A study conducted by Savage et al. (1993b) looked at the effects of diets with excess P (388% NRC recommendations) on bone quality (Savage et al., 1993b). A significant increase in cortical bone porosity was reported in horses fed the high P diet (Savage et al., 1993b), meaning that high P led to a weakening of the bones. Post-mortem lesions of osteochondrosis were high in the high P groups, as well (Savage et al., 1993b).

*Figure 1. Photo of a 2-year old Quarter Horse colt suffering from rickets.* Note the small stature, crooked forelimbs, enlarged joints as well as a previous fracture site on the medial left front cannon bone. Photo by Erin Petersen, taken 1995 at Colorado Horse Rescue.
One of the problems in equine nutrition is a lack of specific mineral recommendations; the NRC (1989) provides minimums, not optimums. In contrast, the RDA (Recommended Daily Allowance) for humans suggests a similar or greater increase in intake of vitamins and minerals when the energy density for growing children increases (RDA, 1989). However, the NRC (1989) does not recommend increases for most of the vitamins and minerals important for bone growth and development (see Table 1; adapted from Kronfeld et al. (1990)).

Table 1. Percentage Comparisons of Select Nutrient Recommendations for an Adult Male (70 kg):Child (13 kg) as Compared to an Adult Horse:Four-Month Old Weanling. These figures demonstrate an increase in energy density with little or no increases in other nutrients as recommended by the NRC (1989).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>RDA, as percentage of adult requirement</th>
<th>NRC, as percentage of adult requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Density</td>
<td>160%</td>
<td>150%</td>
</tr>
<tr>
<td>Cu</td>
<td>160%</td>
<td>No increase</td>
</tr>
<tr>
<td>I</td>
<td>160%</td>
<td>No increase</td>
</tr>
<tr>
<td>B-vitamins</td>
<td>160%</td>
<td>No increase</td>
</tr>
<tr>
<td>Fe</td>
<td>720%</td>
<td>125%</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>980%</td>
<td>167%</td>
</tr>
<tr>
<td>Zn</td>
<td>270%</td>
<td>No increase</td>
</tr>
<tr>
<td>Mn, Se</td>
<td>115%</td>
<td>No increase</td>
</tr>
</tbody>
</table>

Copper and Zinc

Copper is an important component of lysyl oxidase, an enzyme that plays an important role in the cross-linking of cartilage during endochondral ossification (Kronfeld et al., 1990). Copper deficiency can seriously affect cartilage growth and metabolism and has been implicated as a cause of osteochondrosis and OCD (Eamens et al., 1984; Hurtig et al., 1990). Foals fed a diet low in copper had increased incidence of OCD lesions, physitis and limb deformities than foals fed recommended levels of copper (Hurtig et al., 1990). Zinc and copper are closely related in their absorption in the digestive tract of the horse. As the dietary concentration of zinc increases, the absorption of copper decreases (Frape, 1989). Eamens et al. (1984) reported on a case study of four horses pastured in an industrial area. The pasture contained toxic amounts of Zn, which caused enlarged bony growths on the knee, hock, and front and rear fetlocks, an increased incidence of OCD in the knee, hock, stifle, and elbow, as well as a softening of the proximal portion of the cannon bones. Toxic amounts of Zn in the pastures led to a Cu deficiency and clinical signs of OCD. With proper Cu supplementation, these horses improved within two months. (Eamens et al., 1984).

Rapid Growth and Developmental Orthopaedic Disease

The rate of gain can be manipulated through nutrition (NRC, 1989). A possible cause and effect relationship between rapid growth and DOD has been suggested in dogs (Hedhammar, 1974; Dämmrich, 1991), poultry, and pigs (Olsson and Reiland, 1978), however that same link between rapid growth and DOD in horses is unclear. Great Dane puppies grew rapidly and showed signs of physitis, increased bone density of the proximal radius, earlier closure of physes, broadened metaphyseal growth plates, increased incidence of hip dysplasia and OCD, and larger compressed cervical vertebrae when fed ad libitum as compared to puppies fed a restricted diet (Hedhammar, 1974). Another study reported that Great Dane puppies grew rapidly and had a higher incidence of OCD when fed ad libitum (Dämmrich, 1991). Dämmrich proposed that overnutrition due to ad libitum feeding shortened the time to skeletal maturity, caused an increase in size and volume of bone organs and increased bone remodeling, leading to enlarged bones formed of cancellous bone and compact low density bone, ultimately leading to lower mechanical
loading strength. Puppies fed an ad libitum diet had weight gains that exceeded skeletal growth rates (expressed as a ratio of bone length to body weight), which led to a mismatch of body weight increase and skeletal development as compared to the restricted-fed puppies (Dämmrich, 1991).

A study using Dutch Warmblood foals genetically predisposed to osteochondrosis (OC) reported that a lack of decreasing ADG with age in growing horses may have contributed to an increase in severity of OC, although there was no evidence of a direct cause-effect relationship (Barneveld and van Weeren, 1999; Firth et al., 1999; van Weeren et al., 1999). Horses that had OC of the stifle joint grew more rapidly at three and five months of age as well as having a higher ADG throughout the 11-month experiment than horses without OC of the stifle (van Weeren et al., 1999). Pagan and Jackson (1996) reported that horses that had OC of the hock were 5 kg heavier at 25 days of age and 14 kg heavier at 240 days of age than the average for Kentucky Thoroughbreds as reported by Pagan et al. (1996), however, no statistical analyses were reported. A study involving eight Standardbred foals with radiographic signs of OC in the hock had significantly higher ADG from birth to twelve months of age than the remaining 69 Standardbred foals in the study that did not have signs of OC (Sandgren et al., 1993). Weight gain rate in Dutch Warmblood weanlings affected the incidence of OC lesions in the stifle joint but not in the hock joint (van Weeren et al., 1999). Bone mineral density (BMD) was significantly lower in horses with more severe OC although there was no effect of weight gain on the variations seen in BMD measures (Firth et al., 1999). Bone mineral density has been shown to be positively correlated with bone strength (Muir and Markel, 1996). However, another study reported that weanlings with higher ADG had higher BMD and bone mineral content (BMC) in several regions of the appendicular skeleton as compared to weanlings with lower ADG (Petersen et al., 2001). Petersen et al. (2001) also reported that 3 of the 6 weanlings with higher growth rates showed transient signs of physitis during the middle of the 120-day study, while none of the weanlings with slow growth rates showed similar signs. The researchers proposed that the physitis was more likely caused by heavy body weights causing excess strain on the joints rather than the actual growth rates since it was seen only in the heaviest of the three horses (Petersen et al., 2001).

Considerations for Developing a Feeding Program:

1. What stage of development is your horse in (i.e., weanling, yearling, 2-year old)?
2. Determine your young horse’s current weight and body condition; also, what do you expect his mature body weight to be?
3. What do you plan to use him for? If you are looking to have a large attractive horse to race as a two-year old, then his early nutrition is especially important!
4. What average daily gain (ADG) do you expect to achieve? This is, in part, a function of his intended use but is limited by genetics.
5. What type of housing and exercise do you provide? A young horse getting lots of exercise, whether in the form of turn-out or forced exercise, needs more digestible energy than if he’s kept confined to a stall.
6. Start with the forage and build on to it! Have your primary forage source, be it pasture or hay, analyzed for nutrient content. Young horses need much higher quality forage than adult horses at maintenance requirements.
Table 2. Nutrient Recommendations for Growing Horses. NRC (1989) minimum requirements (100% DM basis) for growing horses.

<table>
<thead>
<tr>
<th></th>
<th>Digestible Energy, Mcal/lb</th>
<th>Crude Protein, %</th>
<th>Ca, %₁</th>
<th>P, %₁</th>
<th>Cu, ppm²</th>
<th>Zn, ppm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weanling</td>
<td>1.4</td>
<td>14.5</td>
<td>0.56-0.61</td>
<td>0.31-0.34</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Yearling</td>
<td>1.30</td>
<td>12.6</td>
<td>0.43-0.45</td>
<td>0.24-0.25</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Long-Yearling, in training</td>
<td>1.20</td>
<td>12.0</td>
<td>0.36</td>
<td>0.20</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Long-Yearling, not in training</td>
<td>1.15</td>
<td>11.3</td>
<td>0.34</td>
<td>0.19</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>2-year old, in training</td>
<td>1.20</td>
<td>11.3</td>
<td>0.34</td>
<td>0.20</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>2-year old, not in training</td>
<td>1.15</td>
<td>10.4</td>
<td>0.31</td>
<td>0.17</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>

₁ Higher level in the ranges reflect requirement for a more rapid growth rate at the specified age.
² Current NRC (1989) recommendations may not be high enough. Some studies suggest a Cu level as high as 30 ppm and a Zn level of 75 ppm in the diet.

Practical Considerations

Choosing the right forage

Young horses are like most young mammals – they are energetic during turnout and sleep more than their adult counter-parts. Add this to the smaller size of their digestive tracts and they not only don’t have as much time to eat as an adult, but they can’t physically eat as much. The NRC (1989) reports that young horses are able to eat anywhere from 2.5-3.5% (DM) of their bodyweight on a daily basis. However, it is more realistic to assume a more moderate intake of about 2-2.5% (DM) of body weight. This doesn’t leave much room for high fiber forages! As such, most forages are not energy-dense enough to meet the requirements of the growing horse (See Figure 2). For that reason, an energy concentrate should be added to the ration to make up for the deficiencies of the forage. It is always in the best interest of the horse to try to keep the level of concentrates at no more than 50% of the diet on a dry matter basis. Higher levels of concentrate can lead to digestive upset as well as making it easier for the horse to unbalance his own ration by overeating concentrate and not eating enough forage! Forages should be tested for nutrient content in order to determine the correct energy concentrate. The Forage Testing Association provides a list of all certified labs on their website www.foragetesting.org/certified_labs.html.
Legume forages usually contain more than enough CP for the horse at all stages of growth. Lysine is often also in sufficient quantity. However, as grass hays mature, CP levels can drop rapidly, leading to an increase in the need for protein supplementation. Full bloom grass hays can be as low as 5% CP! Weanlings and yearlings being fed primarily grass hay will usually need protein supplementation in order to meet minimum requirements.

Calcium requirements are generally easily met with legume hays, while grass hays are often Ca-deficient. Phosphorus is not in adequate supply in either type of hay for a growing horse. Taking all these factors into consideration, when selecting a forage for young growing horses, high quality early-bloom hays will be your best bet. Look for a hay that is leafy and green with few seedheads or flowers. Crude protein content should be between 13 – 18%.

Forage trace mineral contents are highly dependent upon concentrations of minerals in the soil, so using NRC (1989) reported values for trace minerals will not be accurate for all forages.

Choosing the right concentrate

After getting an analysis of the hay to be fed, a concentrate can be chosen that best meets the deficits of the forage. Fortunately, feed companies have begun to formulate mixed rations that are well-fortified in vitamins and minerals and high in protein. If you are feeding primarily legume hay, look for a lower-protein concentrate and consider adding a vitamin/mineral supplement if necessary. For example, an early-bloom alfalfa hay has approximately 19.9% (or higher) CP. The total dietary CP requirements of a weanling, as mentioned above, is only 14.5%. It is costly both to you and the horse to feed a “growth” concentrate with 16+% CP! Searching for a lower protein concentrate will often mean that the vitamin/mineral fortification is not high enough for a young horse, so you may need to top-dress the concentrate with a vitamin/mineral supplement or a “forage balancer” mixing pellet.

A lot of interest has been generated recently on the use of high fat concentrates as a replacement for the traditional high carbohydrate energy concentrate. Even young horses can benefit from this high density and highly digestible form of energy, and it has not been shown to have any detrimental effects on bone quality (Hoffman et al., 2001).

Other considerations

Make sure horses of all shapes, sizes and ages have free choice access to clean water and salt at all times. If free-choice access to water is not possible, offer water several times a day. Lack of water in the digestive tract can lead to intestinal impactions!
Exercise, as mentioned above, leads to increased requirements by the young horse. The NRC (1989) provides software that will allow you to customize requirement recommendations based on whether or not the horse is in training (forced exercise). Exercise has also been shown to have positive effects on bone strength. This phenomenon is known as Wolff’s Law of the Skeleton (Wasserman et al., 1993). Providing turn-out or forced exercise to horses whose skeletons are undergoing rapid modeling and remodeling will help increase bone strength that can persist throughout their adult lives (Raub et al., 1989). Conversely, studies have shown that stall-rest can actually lead to decreased bone content (Porre et al., 1998; Bell et al., 1999) and bone density (Barneveld and van Weeren, 1999; Firth et al., 1999). In addition, stall-rest can lead to weaker bones, but as little as 12 hours of turn-out per day was sufficient for increasing bone mineral content in weanling horses (Bell et al., 1999).

Conclusion

The future of research on nutrition of the growing horse is an ever-expanding area. The young horses of today represent the race, performance and pleasure horses of tomorrow, and their early nutrition is of extreme importance in order to promote healthy bone growth. Young horses have very high nutrient requirements, most of which cannot be met with forage (hay or pasture) alone. Energy and protein concentrates are usually necessary in amounts as high as 50% of the diet in order to make up for deficient forages. Copper and zinc, two trace minerals that are important in bone growth metabolism, are often at low concentrations in both forages and concentrates not designed for the growing horse. So be careful to consider them when selecting a concentrate for young horses – balancing the concentrate to meet the shortfalls of the forage is the key. High fat, high fiber diets seem to be of great interest as they are “safer” for young growing horses in terms of minimizing digestive upset. Unfortunately, there is still no clear cause-effect relationship between rapid growth and DOD in horses. Several research papers have indicated a possible relationship, however, other factors are most likely involved. It is probably safer to feed young horses a balanced diet aiming for a more moderate growth rate in order to eliminate rapid growth as a cause of DOD in those horses.

References


DIETARY CONSIDERATIONS FOR ATHLETIC HORSES:
SOURCES AND EFFECTS OF DIETARY ENERGY

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Summary

Horses perform at a wide range of exercise intensities; at one extreme the racing Quarter horse and Thoroughbred galloping at speeds up to 45 miles/hour for 20 s to 2 min and, at the other, endurance horses racing at much lower speeds (10-12 miles/hour) for 100 miles or more. Exercise requires the skeletal muscles of the body to convert large amounts of chemical energy into mechanical energy. Carbohydrate and fat are the major sources of chemical energy used by working muscles during exercise. These substrates are metabolized to produce adenosine triphosphate (ATP), the currency required to produce mechanical energy. Ultimately, this chemical energy is provided through the diet. Thus, it is apparent that diet is of primary importance for maximizing athletic potential. In particular, the source of energy supplied in the diet and how feeding of forage and grain is timed relative to exercise are important considerations in the nutritional management of athletic horses. This paper reviews aspects of energy metabolism in horses during exercise, the energy requirements of athletic horses, and sources of dietary energy to meet the demands of athletic training, with an emphasis on recent data regarding the effects of fat and sources of fermentable fiber (e.g. beet pulp) on metabolism and performance. The effects of pre-exercise feeding on exercise metabolism and dietary management of horses with chronic exertional rhabdomyolysis are also considered.

Substrate Utilization During Exercise

Glucose and fatty acids are the primary energy substrates during exercise. Although there is limited data in horses, research in other species indicates that amino acids (protein) are not a major contributor to ATP re-synthesis during exercise. Fatty acids (long-chain, non-esterified fatty acids or NEFA) are released from triglyceride stores in adipose tissue and in muscle, while glucose is derived from the breakdown of liver and muscle glycogen and de novo synthesis of glucose in the liver. A 1000-lb horse (450-kg) has approximately 3000-4000 g muscle glycogen (1-2% of skeletal muscle weight), 100-200 g liver glycogen (6-8% of liver weight), 1400-2800 g muscle triglyceride, and 35,000-45,000 g as adipose tissue triglyceride (Harris 1997).

In the context of exercise, the body’s store of triglycerides is virtually inexhaustible. In contrast, endogenous carbohydrate reserves are more limited and under some circumstances carbohydrate availability is a performance-limiting factor. Indeed, in human athletes there is unequivocal evidence that endurance capacity is correlated with glucose supply; during prolonged moderate to heavy exercise (workloads up to 75% of maximum oxygen uptake [VO$_{2\text{max}}$]), fatigue is often associated with depletion of muscle glycogen and/or development of hypoglycemia. Furthermore, dietary interventions that result in an increase in resting muscle glycogen concentrations (so-called “glycogen supercompensation”) are associated with improved endurance capacity. Studies in horses also indicate a relationship between glucose supply and performance. Run time to fatigue was increased by 20-25% when horses received an intravenous infusion of glucose during moderate intensity exercise (55% of VO$_{2\text{max}}$) (Farris et al., 1995). As well, a decrease in muscle glycogen concentration (~50% of normal) is associated with a reduction in sprint exercise performance (Lacombe et al., 1999; Lacombe et al., 2001).

Muscle Fiber Types
Muscle fiber recruitment patterns have a marked effect on energy metabolism. Type 1 fibers contract slowly, are able to “fire” (twitch) for long duration without fatigue – and are therefore ideally suited for endurance exercise (Table 1). The fatigue resistance of type 1 fibers is in part related to a high density of mitochondria that confers a high oxidative or aerobic capacity. Compared to the other fiber types, type 1 fibers also have the highest triglyceride stores, the lowest glycogen stores and glycolytic capacity, and the highest density of capillaries. The type 2, or fast-twitch, fibers are sub-classified into types 2A and 2B. Type 2B fibers have the fastest contractile speed, the largest cross-sectional area, the highest glycogen stores and glycolytic capacity, and the lowest oxidative capacity. The contractile and metabolic properties of Type 2A fibers lie between those of type 1 and 2A fibers.

**Table 1: Selected Properties of Muscle Fiber Types in Horses.**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Type I</th>
<th>Type IIA</th>
<th>Type IIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of contraction</td>
<td>slow</td>
<td>fast</td>
<td>very fast</td>
</tr>
<tr>
<td>Fatigue resistance</td>
<td>high</td>
<td>intermediate</td>
<td>low</td>
</tr>
<tr>
<td>Oxidative capacity</td>
<td>high</td>
<td>intermediate</td>
<td>low</td>
</tr>
<tr>
<td>Glycolytic (anaerobic) capacity</td>
<td>low</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>low</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>Fat content</td>
<td>high</td>
<td>intermediate</td>
<td>low</td>
</tr>
</tbody>
</table>

Muscle fiber composition varies between different breeds, individual horses and muscle groups. Fast-twitch, type 2 fibers predominate in the propulsive muscles (e.g. gluteus medius); in Quarter horses and Thoroughbreds, this muscle is 80-90% type 2 fibers, with Standardbreds somewhat lower (~75%). With growth and training, there is a change in the length and cross-sectional area of a fiber, but no change in the number of muscle fibers. There is also a change in the proportion of fiber types with intensive training in young horses, specifically an increase in the percentage of type 2A fibers and a like decrease in type 2B fibers.

The pattern of muscle fiber recruitment is primarily dependent on exercise intensity. At slow exercise intensities, type 1 fibers and a small number of type 2A fibers are activated. As the speed and duration of exercise increases, more muscle fibers will be recruited; first, the type 2A fibers, then 2B fibers. Type 2B fibers are recruited only at near maximal running speeds or after several hours of submaximal running (when recruitment is dictated by glycogen depletion and fatigue in the other fiber types).

**Energy Metabolism**

The relative contribution of different substrates to fuel metabolism during exercise is determined by a number of factors, including the intensity and duration of exercise, muscle fiber composition, the fitness of the horse, and the availability of substrates in plasma and in muscle. Habitual diet and the composition of a pre-exercise meal can also influence the mix of substrates oxidized during exercise. Within muscle fibers, there are three “energy systems” for the re-synthesis of ATP:

1. Intramuscular stores of ATP and creatine phosphate (CP); cleavage of the phosphate from ATP or the CP molecule supplies energy until ATP production from stored substrates (e.g. glucose) begins. This system provides enough energy for only a few seconds of muscle contraction.
2. Anaerobic glycolysis – metabolism of glycogen and glucose to lactic acid; and
3. Oxidative phosphorylation – complete oxidation of fatty acids and glucose to CO$_2$ and water in mitochondria; requires oxygen and therefore classified as aerobic metabolism.

Anaerobic glycolysis provides 2 molecules of ATP for each molecule of glucose metabolized, whereas complete oxidation of one molecule of glucose (oxidative phosphorylation) yields 38 ATP and the oxidation of one NEFA (16 carbon chain length) produces 146 molecules of ATP. Clearly, aerobic metabolism is much more efficient than anaerobic metabolism. In addition, oxidative metabolism does not alter intracellular pH because there is little or no accumulation of lactic acid.

The amount of ATP used by a muscle and the energy pathways and substrates used for ATP re-synthesis are primarily dependent on the speed and force of contraction. During the walk, mostly type 1 fibers are recruited and nearly all of the ATP is generated by aerobic metabolism. Fatty acids, including the volatile fatty acid (VFA) acetate, will supply much of the energy. As speed increases (walk $\rightarrow$ trot $\rightarrow$ canter $\rightarrow$ gallop), type 1 fibers alone cannot sustain the speed of contraction required to propel the horse and the other muscle fiber types are recruited. Type 2A fibers can function aerobically and anaerobically, utilizing both glucose and fatty acids. At trot and canter speeds, most of the ATP will be generated by aerobic metabolism. Note, however, that glucose can be metabolized aerobically twice as fast as fat for ATP re-synthesis (fat is regarded as a “slow fuel”). Therefore, as running speed/intensity increases ATP re-synthesis becomes increasingly reliant on the metabolism of glycogen and glucose, with a proportional decrease in the use of fat.

As maximum running speed or effort is approached, more muscle fibers including type 2B fibers are recruited and the horse consumes more oxygen until it reaches a speed where the ability to utilize oxygen becomes a limiting factor (the horse has reached its maximum aerobic capacity or VO$_{2\text{max}}$). At speeds above VO$_{2\text{max}}$ additional ATP requirements must be met by anaerobic glycolysis of muscle glycogen, principally in type 2B fibers. Therefore, at and beyond VO$_{2\text{max}}$ there will be a sharp increase in glycogen utilization (Figure 1) and lactate production; the latter will be reflected in an exponential rise in blood lactate concentrations (Figure 2). The main advantage of anaerobic glycolysis is a very rapid rate of ATP re-synthesis. The main disadvantage is the rapid decline in intracellular pH that occurs secondary to lactate and hydrogen ion accumulation – muscle pH can fall as low as 6.4 during maximal exercise. This decrease in muscle pH contributes to fatigue development because both glycolysis and excitation-contraction coupling are impaired in an acid environment.
Figure 1: Schematic Representation of Muscle Glycogen Utilization (solid line) as a Function of Running Speed in Horses. The dashed line represents relative fat utilization at the same running speeds to illustrate the inverse relationship between glycogen and fat use across this range of work intensities. Figure 2: Oxygen consumption (VO$_2$) and blood lactate concentrations in horses during incremental treadmill exercise.

Conditioning (physical training) does alter the metabolic profile of skeletal muscles, in particular, the volume density of mitochondria and thus the oxidative capacity of muscle increases with training. Over a 6-month period, the ratio of type 2A:2B fibers increases and the capillarization of all fiber types are also enhanced. These adaptations favor the delivery and utilization of oxygen and blood-borne substrates, increased utilization of NEFA by muscle fibers, and a muscle glycogen sparing effect during low-to-moderate intensity exercise.

Practical Considerations

The form of substrate used, and the speed of substrate utilization can be predicted based on the type of exercise performed. This information is useful when designing diets for different classes of athletic horse. The endurance horse travels at speeds which can be maintained almost entirely by aerobic ATP re-synthesis. However, a common misconception is that fat is the only fuel utilized during endurance racing. In fact, a mix of carbohydrate (blood glucose, muscle glycogen) and fat is oxidized and fatigue during this form of exercise can occur as a result of decreased glucose supply, particularly in high level endurance racing where the average running speed for 50 to 100 mile races can approach 13-14 miles/hour. Adaptation to a fat supplemented diet can result in a modest increase in the capacity oxidize fat which, together with physical training, can help to conserve precious carbohydrate stores during prolonged exercise. However, even in these circumstances the adequacy of muscle glycogen stores is of critical importance.

Racehorses, western performance horses and eventers (steeplechase and cross-country phases) perform at higher exercise intensities. During racing, it has been estimated that 70% of ATP re-synthesis is met by aerobic metabolism, with the balance provided by anaerobic ATP re-synthesis (i.e. use of stored ATP and CP, and metabolism of glycogen to lactate). The anaerobic component will be somewhat higher for some western disciplines. However, regardless of the relative proportions of energy supplied by
aerobic vs. anaerobic means, muscle glycogen is the predominant fuel utilized during these forms of exercise and there is negligible use of fat. Thus, as for endurance horse, the diet must supply some glucose for muscle glycogen synthesis.

Dietary Energy Requirements

The most basic nutritional concern for athletic performance is provision of adequate energy for training and competitive activities. The 1989 National Research Council recommendations state that daily energy intake be increased by 25, 50, and 100% above maintenance requirements for horses engaged in light, medium, and intense exercise, respectively. These recommendations are based on data from experimental studies, feeding surveys and practical experience. Light work might include equitation and other forms of pleasure riding, while horses engaged in racing, hunting, 3-day events, and endurance activities would fall into the intense category (Table 2). Clearly, if the diet does not provide sufficient energy to meet the increased demands associated with these athletic activities, loss of body condition and performance will ensue. This situation most commonly arises in racehorses, where the adequacy of feed intake can be a problem. On the other hand, pleasure horse owners have a tendency to over estimate the amount of work (physical activity) performed by their horses – the end result being weight gain due to energy intake in excess of requirements.

Table 2: Digestible Energy (DE) Requirements (Megacalories per Day) for a 500 kg Horse at Four Different Activity Levels.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Examples</th>
<th>DE Requirement (Mcal/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance</td>
<td>Horse at pasture</td>
<td>16</td>
</tr>
<tr>
<td>Light</td>
<td>Pleasure riding, equitation</td>
<td>20</td>
</tr>
<tr>
<td>Moderate</td>
<td>Reining, cutting</td>
<td>24</td>
</tr>
<tr>
<td>Intense</td>
<td>Racehorses, endurance horses</td>
<td>32</td>
</tr>
</tbody>
</table>

For Standardbred and Thoroughbred racehorses, field studies and observations have indicated that the NRC (1989) recommendations are appropriate. Gallagher and colleagues (1992a and 1992b) estimated the DE intake by Thoroughbred (mean body weight 505 kg) and Standardbred (mean body weight 449 kg) horses, respectively, at 31 to 36 Mcal per day and 28 to 31 Mcal per day. Practically, body condition scoring is the most reliable means for assessment of the adequacy of DE intake. A condition score between 4 and 5 is ideal for most athletic horses. Two recent studies investigated the relationship between body condition score and completion rate during the Tevis Cup (100 mile) endurance ride; the standard body condition scale of 1 to 9 was used (Garlinghouse and Burrill, 1999). The mean body condition score of horses that successfully completed the rides was 4.5, whereas horses that were eliminated for metabolic failure (colic, heat exhaustion, synchronous diaphragmatic flutter or “thumps”, or tying up) had a mean condition score of 2.9. Horses that were eliminated for non-metabolic reasons such as lameness and overtime had a mean condition score of 4.3. The researchers were careful to point out that their results may not apply to endurance competition as a whole given the difficult nature of the Tevis Cup course. Nonetheless, the take-home message from these studies is that there is an optimal level of “fatness” for horses competing in endurance events, and that training and feeding programs need to be adjusted accordingly. Thin horses (condition score <3) may be at a disadvantage because of low energy reserves, while over-conditioned horses could experience detrimental effects due to the insulating effect of a thicker fat cover.
Dietary Energy Sources and Their Effects on Metabolism During Exercise

Digestible energy (DE) requirements are calculated based on the horse’s maintenance DE needs plus the additional energy expended during exercise. A general recommendation is that the diet contain a minimum of 1 kg forage per 100 kg body weight (i.e. 1% of body weight; 11 lb of hay for an 1100-lb horse) to maintain gastrointestinal health. The amount of grain or grain-concentrate fed will depend on the amount of physical activity. For example, for an 1100-lb racehorse requiring 34 Mcal DE per day, energy needs may be met by the consumption of 14 lb of good quality hay (0.9 Mcal/lb = 12.6 Mcal) and 14 lb of a commercial concentrate (1.5 Mcal per lb x 15 lb = 22.5 Mcal). In contrast, a horse of similar body size performing only light exercise (DE requirement of 20 Mcal DE per day) and consuming a larger quantity of hay (16-17 lb) may require only 2-3 lb of a grain concentrate.

Several different energy sources are available for performance rations including starch and sugars (hydrolysable carbohydrates), fermentable carbohydrates, fat and protein. Traditionally, the high energy requirements of athletic horses have been met by diets high in hydrolysable carbohydrates (i.e. grains). In the past decade, however, there has been increased emphasis on use of alternative energy sources, such as fat and beet pulp, in equine rations and some data are available regarding the effects of such diets on the metabolic response to exercise.

Hydrolysable carbohydrates

These carbohydrate forms (starch and sugars) can be digested by mammalian enzymes with absorption of hexoses in the small intestine. Starch, a carbohydrate, is the primary component of cereal grains, comprising 50 to 70% of the grain’s dry matter; oats is approximately 50% starch while the starch content of corn approaches 70%. Molasses is the best-recognized source of sugar for the horse. Traditionally, cereal grains and molasses have been the major components of concentrates for athletic horses. On the one hand, provision of some starch and sugar in the diet is needed for the replenishment of muscle glycogen. However, a concern with hydrolysable carbohydrates (hCHO) is the potential to “overload” the system, wherein undigested starch and sugar enters the cecum and undergoes fermentation, resulting in a decrease in cecal/colonic pH and an increased risk for colic and laminitis. Recent epidemiological studies have clearly identified the level of grain concentrate feeding as a risk factor for colic. In one study of 364 horses in Texas, feeding >2.7 kg (6 lb) of oats per day significantly increased the risk of colic (Hudson et al., 2001).

Recognition of the adverse health effects associated with diets high in hCHO has lead to the use non-sugar and starch based energy sources (see below). Another approach to reducing the risk of grain-associated intestinal dysfunction is to limit the size of grain meals. There is some evidence that the upper limit of hCHO digestion in the small intestine is between 2 and 4 g hCHO per kg bwt. Currently, it is recommended that not more than 400 g straight cereal grain per 100 kg bwt or 500 g grain-concentrate per 100 kg be fed per meal (e.g. for a 500-kg [1100-lb] horse, not more than 2 kg of oats or 2.5 kg of grain concentrate per meal)(Harris and Kronfeld, 2003). The form of hCHO is another important consideration. Oat starch appears to be well digested, whereas a substantial portion of barley and corn starch by-passes the small intestine unless these grains are processed. Cooking or micronizing cereals improves starch digestibility and is recommended if corn or barley is to be included in equine rations.

Studies in human athletes have unequivocally demonstrated that a high CHO-low fat diet will result in higher muscle glycogen concentration when compared to a more conventional, lower CHO diet. Furthermore, there is a direct relationship between initial muscle glycogen stores and performance during moderate intensity (50-80% of VO2max) endurance exercise (Bergstrom and Hultman, 1967). Thus, for human athletes, dietary strategies that boost muscle glycogen stores enhance exercise performance. In the 1980’s, several researchers sought to determine whether similar benefits could be realized by “carbohydrate loading” in horses. However, only a modest increase in the glycogen content of muscle has been observed in horses fed diet rich in hCHO (Pagan et al., 1987; Essen-Gustavsson et al., 1991; Topliff
et al., 1983). For example, Essén-Gustavsson et al. (1991) reported a 12% increase in the resting muscle glycogen content of Standardbred horses fed a diet containing approximately 2 kg of starch and sugar when compared to an isocaloric diet that provided about 1.3 kg soluble CHO per day. However, no beneficial effect on performance was after such hCHO-rich diets (Pagan et al., 1987; Essén-Gustavsson et al., 1991; Topliff et al., 1983). On the contrary, higher heart rate (HR) and blood lactate accumulation is observed during intense exercise in horses fed diets high in hCHO (Pagan et al., 1987; Topliff et al., 1983). The underlying mechanisms of these responses are unknown but may be related to alterations in sympathetic outflow and circulating catecholamine concentrations. Interestingly, Jansson et al. (2002) reported higher HR during submaximal exercise in horses fed 1.5 kg of barley sugar per day when compared to an oat-based diet, suggesting that the form of CHO also may influence the HR response during exercise. There is also accumulating evidence that diets comparatively high in starch and sugar increase HR and excitability of horses at rest (MacLeay et al., 1999a).

Although athletic horses do require a dietary source of glucose, it is now clear that exclusive use of hCHO to meet energy needs is problematic. Accordingly, a more balanced approach that makes use of fat and other energy sources is recommended. This is especially true for horses suffering from chronic exertional rhabdomyolysis (see below).

Fermentable carbohydrates

Non-starch carbohydrate feeds, such as sugar beet pulp (SBP) and soya hulls, are now commonly added to diets for athletic horses. The carbohydrate fraction of these feeds is devoid of starch, but rich in non-starch polysaccharides (fiber). SBP contains major fractions of pectins, arabinans and galactans that are extensively fermented in the hindgut (Sunvold et al., 1995). Beet pulp is available in two forms, molassed (i.e. molasses is added to the shreds, generally at the 5% level) or non-molassed. Both SBP and soya hulls provide more energy than typical forages. Studies have demonstrated that up to 3.0 g SBP per kg bwt may be fed adult horses without any adverse effects on overall nutrient utilisation or performance (Lindberg and Palmgren Karlsson 2001). Replacing oats with plain SBP will reduce the glycemic and insuliniemic responses to a meal (Lindberg and Palmgren Karlsson 2001). However, when oats are replaced by molassed SBP there is no appreciable change in glycemic response although the post-prandial increase in insulin was mitigated (Palmgren Karlsson et al., 2002).

Replacing oats with SBP also mitigates the rate of muscle glycogenolysis and the increases in muscle and plasma lactate in Standardbred trotters performing a treadmill exercise test that simulated a race (Palmgren Karlsson et al., 2002). Similarly, replacing oats with barley sugar resulted in a significant reduction in muscle glycogen utilization during intense exercise (Jansson et al., 2002). Therefore, a reduction in dietary starch, with replacement by SBP or barley sugar, modifies the muscle glycogenolytic response to high-intensity exercise. The mechanism of this apparent glycogen-sparing effect when oat starch is replaced by barley sugar or SBP is not known. Potentially, an increase in sugar intake results in enhanced use of plasma glucose for energy with a concomitant decrease in energy transduction from muscle glycogen.

Dietary Fat

The inclusion of fat (as vegetable oil) in diets for athletic horses is now widespread, and it is common for horses to receive up to 20% of total daily digestible energy (DE) from fat. Vegetable oils contain approximately 2.5-3.0 times as much DE as the cereal grains, are well tolerated by horses and have high digestibility (>90%). Sources include soy or corn oil, rice bran (18-22% fat), flaxseed (40% fat) and copra meal (8-9% fat).

The ideal amount of dietary fat for horses has not been determined. Common equine feedstuffs contain 2 to 4% fat, but horses have safely consumed diets containing up to 12% oil by weight (i.e. total diet). However, the typical level of fat supplementation is usually lower with a suggested upper limit of
100 g oil per 100 kg bwt per day. This level of fat supplementation is much higher when compared to that provided by commercially-available performance horse concentrates that contain between 5 to 12% fat (as fed), or between 10 and 25% of the DE. Horses readily accept fat-supplemented diets and there are usually no adverse effects providing the level of fat supplementation is gradually increased over a 2-3 week period.

Fat supplementation in horses is characterized by an increase in plasma phospholipids and cholesterol and a decrease in plasma TGs (Orme et al., 1997). Changes in the activities of lipoprotein lipase and the enzymes of β-oxidation also suggest that horses adapted to a fat-supplemented diet have increased capacity for the uptake and oxidation of fatty acids in muscle. Indeed, some recent studies have shown lower respiratory exchange ratio in fat-adapted horses during low and moderate intensity exercise (Dunnett et al., 2002; Pagan et al., 2002). Pagan et al., (2002) also reported a decrease in glucose flux during low intensity in horses adapted to a diet providing 25% of DE from corn oil. Thus, fat-supplementation enhances lipid oxidation and spares the use of endogenous CHO (plasma glucose, muscle glycogen) during moderate exercise. Theoretically, such a glycogen-sparing effect could improve exercise performance. However, the actual effects of fat-supplementation on endurance exercise performance have not been reported. The minimum (or optimum) level of dietary fat necessary for expression of these metabolic adaptations has not been established. Most studies have fed diets containing 3% to 12% fat (total diet on a dry matter basis) and in one study a dose-response relationship was detected between fat intake (3.0 to 10.8% fat) and heparin-released plasma lipoprotein lipase activity (Geelen et al., 2001).

The length of time required for metabolic adaptation to dietary fat is a somewhat contentious issue. Some nutritionists believe that a minimum of 10-12 weeks is required for adaptation (Harris and Kronfeld, 2003). However, metabolic adaptations to fat supplementation have been observed as early as 3-5 weeks after the start of supplementation (Orme et al., 1997; Pagan et al., 2002). Thus, whereas a 2-3 month period may be required for complete adaptation to a fat-supplemented diet, some of the metabolic responses are evident much earlier. In one study, the metabolic responses to fat-supplementation were abolished within 5 weeks of withdrawal of the oil-supplemented diet (Orme et al., 1997). Thus, the putative benefits of fat-supplemented diets in horses are dependent on continued use of such rations.

There is some evidence that fat-supplementation also improves the performance of horses undertaking higher intensity exercise (e.g. racehorses). Harkins et al. (1992) reported improved racetrack performance in Thoroughbreds fed a fat-supplemented diet, and attributed this improvement to increased muscle glycogen content and glycogen utilization rate during exercise. Furthermore, Eaton et al., (1995) reported that a fat-supplemented diet resulted in a small but statistically significant increase in run time to fatigue and MAOD in horses undertaking treadmill exercise at an intensity equivalent to 120% of VO_{2max}. However, in this study there was no corresponding change in resting muscle glycogen content or glycogen utilisation rate during exercise. The mechanism for enhancement of high-intensity exercise performance with fat supplementation is unclear, although increased activation of glycolysis and glycogenolysis is a possibility, the data of Eaton and colleagues notwithstanding.

Fat-supplemented diets for horses have lower hCHO content when compared to grain-based rations. These diets, therefore, provide less substrate (glucose) for muscle glycogen synthesis. However, although a drastic reduction in the soluble CHO content of the diet (<10% of DE from starch and sugar) is associated with marked reductions in muscle glycogen content (Toppliff et al., 1983), it appears that muscle glycogen content is largely unchanged when horses are fed diets containing moderate amounts of fat (up to 7.5% of total dry matter or 20% of DE) (Hyyppa et al., 1999, MacLeay et al., 1999a). However, in horses not adapted to a fat-supplemented diet, consumption of meals containing 7.5% fat (rapeseed oil) slows the rate of muscle glycogen replenishment (Hyyppa et al., 1999).

**Protein**

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Horse owners often view protein as an important source of energy. In reality, protein is not a major energy source for athletic horses although excess dietary amino acids can be used for ATP re- 
synthesis (alternatively, the carbon skeletons can be used for gluconeogenesis or lipogenesis). Unlike fat and carbohydrate, protein is not stored in the body; most dietary amino acids are used for synthesis of functional proteins (e.g. structural proteins, albumin, enzymes), while excess dietary amino acids are deaminated and used as an energy source. However, this process of conversion is inefficient with higher 
higher amounts of waste in the form of heat, acid and nitrogen when compared to the catabolism of 
carbohydrate and fat. The nitrogen is removed as urea with a resultant increase in water requirements. 
Increased urinary nitrogen also may increase stall ammonia concentrations, thereby predisposing to respiratory ailments. Therefore, excess dietary protein is undesirable in athletic horses.

More recent research has focused on the effects of lower protein diets in horses. Graham-Thiers 
and colleagues (2001) have evaluated the effects of a restricted protein diet (7.5% crude protein with 
added lysine and threonine) on acid-base responses in horses subjected to repeated bouts of high-intensity 
exercise. When compared to a 14.5% crude protein diet, the restricted protein diet mitigated exercise-
associated acidemia. However, quantitatively the effects were very small and further studies are needed 
to determine their physiological significance.

**Effects of Preexercise Feeding**

The timing and composition of a meal consumed before exercise can influence metabolic 
response. Most notably, the hyperglycaemic and insulinemia associated with the digestion and absorption 
of grain meals affects the mix of substrates utilized during a bout of exercise. Insulin is a potent inhibitor 
of lipolysis and fatty acid oxidation in skeletal muscle, and also promotes glucose uptake into muscle via 
recruitment of the transporter protein GLUT4 to the sarcolemma. Thus, hyperinsulinemia at exercise 
onset will suppress FFA availability and lipid oxidation and increase reliance on carbohydrate stores 
(including plasma glucose) for energy transduction.

Accordingly, several equine studies have demonstrated that a grain meal (1-3 kg of oats, corn or a 
mixture of the two) consumed 3 hours or less before exercise results in hyperglycaemia, hyperinsulinemia 
and decreased plasma FFA concentration at the start of exercise, and a subsequent marked decrease in 
plasma glucose concentration during the initial period of exercise (Lawrence et al., 1993; Stull and 
Rodiek, 1995; Pagan and Harris, 1999). This decrease in plasma glucose concentration tends to be short-
lived such that during prolonged moderate-intensity exercise (e.g. 60 min at 50% of VO2max), the plasma 
glucose concentrations of grain fed horses is not substantially different from horses fasted before exercise. 
On the other hand, plasma FFA remains lower when compared to the fasted state throughout exercise. 
Jose-Cunilleras and colleagues (2002) utilized isotopic tracer methods and indirect calorimetry to 
determine the effects of hay (alfalfa cubes; ~3 kg) and starch (cracked corn; 1.7 kg) feeding on glucose 
flux and substrate oxidation during exercise. The alfalfa cubes were consumed between 2 and 3 hours 
before exercise, while the cracked corn was ingested 90 min pre-exercise. Feeding corn before exercise 
resulted in increased utilization of blood-borne glucose and whole-body carbohydrate oxidation when 
compared to a meal of alfalfa or not feeding.

The effects of pre-exercise grain feeding on endurance exercise performance in horses have not 
been reported. In humans, carbohydrate ingestion during exercise unequivocally improves performance 
during prolonged (more than 2 hours) moderate-intensity (>50-60% of VO2max) exercise, presumably by 
maintaining glucose supply in skeletal muscle at a time when glycogen stores are depleted. On the other 
hand, the performance affects of pre-exercise glucose feedings in human athletes are more equivocal. For 
horses performing endurance exercise, the acceleration in carbohydrate oxidation (and suppressed fat 
oxidation) associated with grain feeding may result in premature fatigue as a result of carbohydrate 
depletion.
As demonstrated in the study by Jose-Cunilleras et al. (2002), together with the results of earlier studies by Pagan and Harris (1999), forage meals (<2 kg) consumed 2 to 3 hours before exercise have minimal effect on substrate availability and oxidation during sustained exertion. However, free choice consumption of hay in the 12-24 hour period before exercise may adversely affect performance because of an increase in body weight (gut fill). Large meals (hay or grain or a combination) consumed near the start of exercise will also result in a decrease in plasma volume as a result of fluid shifts into the gastrointestinal tract (Pagan and Harris, 1999). Such reductions in plasma volume could compromise cardiovascular function during exercise.

There is some evidence that a short-term reduction in forage intake is beneficial in horses undertaking high-intensity exercise. When compared to ad libitum hay consumption, restricting hay intake to ~1% of bodyweight for a 3-day period before a treadmill exercise test (2 min at 115% VO₂max) resulted in a 2% decrease in body weight and a reduction in anaerobic energy expenditure during exercise, as evidenced by reduced oxygen deficit and plasma lactate concentrations. The reduction in body weight was attributed to a reduction in gut fill (Rice et al., 2001).

In summary, small forage meals consumed 2-3 hours before exercise have minimal affect on substrate metabolism during exertion. However, meals containing hCHO (starch and sugar) consumed within 3 hours of the start of exercise accelerate carbohydrate utilization and decrease lipid oxidation. Although the performance affects of these feeding practices are not known, this suppression in lipid oxidation may be detrimental during endurance exercise (e.g. endurance races; speed and endurance test of a 3-day event).

**Dietary Energy for Horses with Chronic Muscle Disorders**

Over the last 10 years, tremendous advances have been made in our understanding of the etiology of exertional rhabdomyolysis (ER) or “tying-up” in horses. Clinically, tying-up can be divided into two syndromes: 1) Sporadic exertional rhabdomyolysis – the occasional episode of tying-up that occurs in horses with a history of satisfactory performance; and 2) Chronic ER in horses with repeated episodes of tying-up from a young age. Two forms of chronic ER have been identified:

1. Polysaccharide storage myopathy (PSSM), also termed equine polysaccharide myopathy (EPWM), a form of tying-up in Quarter Horse-related breeds, draft horses and warmbloods (Valberg et al., 1997; Valentine et al., 1998); and
2. Recurrent exertional rhabdomyolysis (RER), a disorder of Thoroughbreds (although a similar condition probably also affects Standardbred and Arabian horses)(MacLeay et al., 1999b; Valberg et al., 1999).

PSSM is characterized by the accumulation of glycogen and abnormal polysaccharide in skeletal muscle. Biopsies of PSSM horses reveal abnormal complex polysaccharide accumulations in muscle fibers. As well, muscle glycogen concentrations are 1.5 to 4 times higher compared to normal horses and other breeds of horses with chronic ER. The underlying metabolic defect has not been identified, but a problem with the regulation of glycogen synthesis is suspected. Affected horses have high insulin sensitivity and after an IV or oral glucose load clear glucose from the bloodstream at a much higher rate compared to normal horses. Similarly, the increase in blood glucose concentrations after a grain meal is lower in PSSM horses than in healthy control horses, perhaps reflecting a more rapid clearance and uptake of glucose by skeletal muscle.

RER is a disorder with highest prevalence in young racehorses. In horses less than 4 years of age it is most common in fillies, with a more equal sex distribution in older horses (perhaps because many of the fillies have been retired). Many affected horses have a nervous disposition. In Thoroughbreds, RER has been identified as a heritable defect in intracellular calcium regulation leading to excessive muscular contraction and, eventually, muscle necrosis. Clinical expression of RER is often stress-induced. In this

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regard, diets high in hCHO may increase expression of the disease because predisposed animals are more excitable when fed such diets.

Despite the apparent differences in the etiology and pathogenesis of PSSM and RER, there is experimental and clinical evidence that a reduction in dietary hCHO with substitution by fat (and fermentable carbohydrates) results in clinical improvement in both conditions. For horses with PSSM, complete elimination of grain from the diet (hence, a drastic reduction in hCHO intake) is recommended. In addition, a modest amount of fat supplementation provides a favorable effect. However, the extent of dietary alterations required for clinical improvement is somewhat contentious. Some authors have recommended that horses with PSSM receive at least 25% of DE as fat (Valentine et al., 1998), while others have indicated that improvement occurs with lower levels of fat supplementation (McKenzie and Valberg, 2002). Practically, however, the appropriate dietary alteration will depend on the level of physical activity. “Easy keeper” Quarter horses and warmbloods in only light work will maintain condition on a diet that is predominantly forage, with perhaps a small quantity of fat (e.g. 1-2 lb rice bran per day). On the other hand, a PSSM horse in intense work will require a substantial quantity of concentrate to meet energy needs and much of this DE must come from fat to keep hCHO intake at a minimum (less than 10% of daily DE). It is also important to realize that dietary alterations alone will not prevent further episodes of tying up in horses with PSSM. Rather, a combination of regular physical activity and dietary control is necessary for successful management.

The diet of a typical racehorse with RER often includes 10-18 lb of a sweet feed or similar grain-concentrate i.e. very high hCHO intake. Interestingly, when RER prone horses are fed a moderate caloric intake, the incidence of sub-clinical rhabdomyolysis is low regardless of diet. However, when calorie intake is increased to levels commensurate with racehorses in training, diets high in hCHO are associated with higher incidence of sub-clinical and clinical rhabdomyolysis. Recent research has demonstrated that replacing some of the grain in the diet with fat and highly fermentable fiber is beneficial in reducing the muscle damage associated with exercise (MacKenzie and Valberg, 2002). Clinically, RER-affected horses are calmer when fed a higher fat, lower hCHO diet. Thus, one mechanism by which such diets decrease the severity or incidence of exertional rhabdomyolysis is a reduction in excitability. In general, the diet should provide no more than 20% of DE as hCHO, thus requiring substantial fat and fermentable fiber (e.g., beet pulp) to meet the horse’s energy needs. Commercial feeds that contain 10-13% fat (by weight) and less than 10% hCHO are now appearing on the market and have been used with success in horses with RER (McKenzie and Valberg, 2002).

Conclusion

Traditionally, cereal grains have been a dietary mainstay for athletic horses. However, research over the past 20 years has indicated that diets high in hydrolysable carbohydrate increase the risk of intestinal dysfunction and increase the expression of certain medical conditions e.g. chronic exertional rhabdomyolysis. Therefore, modern rations for athletic horses emphasize of alternative energy sources such as fat and fermentable carbohydrates, with lower starch and sugar.

References


FAT SUPPLEMENTATION IN THE EQUINE DIET

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Summary

Fats increase energy density, improve performance, and avoid grain-associated disorders. Dietetic difficulties are poor acceptance and poor digestibility if introduced too rapidly. Adding fats (‘empty calories’) to already complete and balanced feeds risks multiple deficiencies of essential nutrients. Thus it is safer to incorporate fats into feeds and balance the formula accordingly.

Acceptance (palatability) in published comparison tests is highest for corn oil, lowest for tallows. It is also high for rice bran according to common experience.

Ether extract is nearly 100% triglyceride in extracted vegetable oils and refined animal fats, which have lecithins and other polar lipids removed. It is about 30 to 40% unavailable waxes, pigments and sterols in most forages and common ingredients of horse feeds. True digestibility of triglycerides approaches 100%. Apparent digestibility is about 95% for corn oil, 85-95% for many fat sources, 75-85% for fast-fortified concentrates, 50-60% for unfortified concentrates, and 40-50% for most forages. Corresponding digestible energy contents are 9.0, 8.0-9.0, 6.2-8.0, 4.7-5.7, and 3.8-4.7 Mcal/kg dry matter. Excessive calcium may bind fatty acids, hence decrease absorption. Fat fortification has depressed protein digestibility in a few but not most experiments. Soybean oil depresses fiber digestibility.

Fats are used to increase energy density for performance and to avoid adverse effects of excessive starch and sugars. Equine grain-associated disorders (EGAD) include a fermentative set (gastric ulcers, osmotic diarrhea, distention colic, acidic colitis, laminitis, founder) and a metabolic set (laminitis, tying-up, osteochondrosis)—equine syndrome X.

Fat-fortification reduces the bowel ballast of horses by up to 30 lbs. The increase in power-weight ratio should occur in 10 to 20 days and improve most kinds of athletic performance. Further improvement develops over 12 weeks as a set of metabolic and regulatory adaptations to the combination of physical conditioning and feeding a fat-fortified diet—fat adaptation. Other advantages for fat-fortification during growth, gestation and lactation have been demonstrated at the Middleburg Agricultural Research and Extension Center, mainly in collaborative studies with the WALTHAM Centre for Pet Nutrition.
Introduction

Maestro Bart Cardon of Arizona Feeds pioneered fat fortified horse feeds in the 1930s. He encountered the same difficulties all of us see today (personal communication to DSK). The way has not been easy for incorporating fats into horse feeds. The old clash is between cost and palatability, the new one between cost and depressed fiber digestibility. The common culprit is soybean oil, so its continued dominance in fat-fortified horse feeds is probably attributable to effective marketing.

The second difficulty is interference with digestion when fats are introduced too rapidly. Stools become shiny, then loose, then grayish, watery and foul smelling—steatorrhea. These undesired effects can be avoided by introducing fats or fat-fortified feeds in progressive steps (one-quarter, half, three-quarters, full) of 1 to 4 days per step.

Fats are the most concentrated chemical form of dietary energy and consist mainly of empty calories. Thus adding fats to an already complete and balanced feed risks insidious development of multiple deficiencies. These are most likely to manifest simply as poorer performance. Consequently it is safer to avoid supplementing fat sources post facto and to incorporate fats into feeds and balance the formula accordingly.

Dietary fats are needed for the absorption of fat-soluble vitamins, which may become inadequate from low fat feeds. Certain fatty acids are essential, especially during growth and later for sustaining tissues that continue to turn over quickly, such as skin and coat. Omega-6 fatty acids (most vegetable oils) favor inflammation and repair, in contrast to omega-3 fatty acids (flax oil, fish oil). A high omega-3:omega-6 ratio is present in certain specialty horse feeds, supported by claims of reducing inflammation. If effective, it should also delay healing. In medicine in general, physical and chemical attempts to regulate repair and inflammation (the first steps in repair) are usually kept under careful temporal control ("timing is everything"). Fine temporal control is impossible with the chronic feeding of a high omega-3 diet.

Following the above four knocks, you may wonder why fats are used in horse feeds. First intentions were to increase energy density and avoid adverse effects of excessive starch and sugars. In the last three decades, fat fortification has been found to improve actual athletic performance in a few studies, and to improve metabolic proxies of performance in many.

Palatability

Our consumption preference tests used a cafeteria presentation of 3 to 5 fats (Holland et al., 1998). Corn oil was so overwhelming in these trials that sometimes we had to remove it to obtain useful numbers for other test fats. Looking back at the horse’s evolution on diets low in vegetable oils and negligible in animal fats, poor acceptance, especially of tallows, is not surprising.

Many studies of Dr Gary Potter and associates (Texas A&M University) used animal tallow without interference from poor acceptance (Potter et al., 1992a). This conflict with the VT experiments moved us to seek an explanation. The TAMU tallow is supplied by a single renderer, who is advised 4 days in advance to avoid 4 D (down, disabled, diseased, dead), hence unusual but obtainable with special effort.

Rice bran is a popular fat source. It is consumed readily by horses, according to common experience, and perhaps slowly if it does not blow away.

A second difficulty with acceptance of fat fortified feeds may emerge after a month or two. It seems to be analogous to a problem with dry dog foods. In that context, efforts to ensure prevention of
oxidation and rancidity have not provided a clear answer. This problem is still in the art (or perhaps proprietary science) of feed manufacture.

**Digestibility**

Ether extract (EE) is nearly 100% triglyceride in extracted vegetable oils and refined animal fats, which have lecithins and other polar lipids removed. It is about 30 to 40% unavailable waxes, pigments and sterols in most forages and common ingredients of horse feeds. True digestibility of triglycerides approaches 100%. Apparent digestibility is about 95% for corn oil, 85-95% for many fat sources, 75-85% for fast-fortified concentrates, 50-60% for unfortified concentrates, and 40-50% for most forages (NRC, 1989, Kronfeld *et al.*, 2001a). Corresponding digestible energy (DE) contents are 9.0, 8.0-9.0, 6.2-8.0, 4.7-5.7, and 3.8-4.7 Mcal/kg dry matter.

Calcium soaps of fatty acids are not absorbed, so must be split by acidic conditions in the stomach. The apparent digestibility of EE in a commercial product containing calcium soaps of palm oil was 48% (Hintz and Schryver, 1989), about 4.5 Mcal/kg of fat in the product.

Soybean oil (158 g/kg DM) depressed crude fiber digestibility from 71 to 57% (Jansen *et al.*, 2002), reinforcing several previous experiments in the same laboratory. In our hands, the combination of added soybean oil (50 g/kg) and soy lecithins (50 g/kg) had no deleterious effects (Kronfeld *et al.*, 2001a). Moreover, following smooth introduction of the fat-fortified feeds, we observed no shiny stools, the first sign of poor fat digestion, or increase in abundance, the first sign of poor fiber digestion. Also, our soybean oil and lecithin data are consistent with the rest, showing no quantitative sign of reduced digestibility up to 180 or 230 g/kg, depending on the model. The discrepancy between Utrecht (Jansen *et al.*, 2002) and Middleburg (Kronfeld *et al.*, 2001a) begs for any explanation except the amount—5% of soybean oil being tolerated well, 15% not.

Protein digestibility has been depressed in a few but not most studies of associative effects of added fats (Kronfeld *et al.*, 2001a). The low desired protein content in feeds for equine athletes is helped by adding fats (Graham-Thiers *et al.*, 2001). Attention should be given to protein quality for several reasons, including the possibility, however small, that added fat may impair protein digestibility.

Twenty-three complete feeds, containing from 3 to 23% EE, were tested in digestibility balance experiments at Virginia Tech (Kronfeld *et al.*, 2001a). Mean estimates of apparent digestibility are about 55% for basal feeds and 80% for fat-fortified feeds, regardless of type of fat. True digestibility of added fats approached 100%, and endogenous fecal fat was about 60 g/day. A common assumption is that endogenous fecal fat accounts for the difference between true and apparent digestibilities. It is consistent with our data for EE contents of 16% or more, but not lower EE contents. A common explanation for the low apparent digestibility of EE in forages and feeds is the presence of waxes and other indigestible extractives, but this indigestible component was not sufficient to account for the data. A third explanation—a new hypothesis—is that enzyme kinetics are rate-limiting at low substrate concentrations, given the brief residence time of ingesta in the small intestine.

A first-order reaction typical of enzymes was found for digestibility versus dietary fat contents, with asymptotic or saturation values of about 95% (Kronfeld *et al.*, 2001a). This physiological model showed no diminution in fat digestibility of 95% up to 23% fat (230 g/kg DM). In contrast, an empirical quadratic model fit to the same data suggests a peak of fat digestibility at 18.5% (Figure 1).
Amount of Fat in Horse Feeds

Fat fortification should lead to a final content of 12 to 19 % (120 to 190 g/kg) according to the quadratic model of digestibility (Figure 1). Another quadratic model, muscle glycogen concentration versus dietary fat content, indicates a peak at 12% (Kronfeld et al., 1994). Many authors describe the effects of dietary fat on muscle glycogen concentration (a major factor in exercise) as inconsistent or erratic, probably upon finding no linear relationship.

Avoiding Equine Grain-Associated Disorders

In contention in a recent court case was the possible adverse effects of feeding two 10-lb meals of grain-and-molasses pellets a day, a lapse of 2 days, then abrupt resumption of 10-lb meals. One of us (DSK) attributed the distension colic in 3 valuable horses to feeding mismanagement. Two junior surgeons from a nearby veterinary college testified that any adverse consequences of grain ingestion require abrupt intakes of the order of 50 lb, in effect grain overload. They won, because the jury was more inclined to blame the manufacturer’s feed than the owner’s feeding management.

The two brazen young expert witnesses in that trial were apparently unaware that their mentor was the principal investigator of a cogent epidemiologic study, which identified a 6-fold relative risk of colic associated with feeding meals of 11 lb or more of grain a day (White, 1997). Also they were unaware that physiological studies suggest that meals of grain-and-molasses should not be more than about 5 lb in order to prevent rapid and abnormal fermentation in the cecum (Potter et al., 1992b, Meyer et al., 1995). If higher intakes are desired, then we should feed more meals or fortify the feed with fat.

The fat-splitting hydrolytic (lipolytic) capacity of the equine small intestine appears to adapt over a few days, perhaps up to 3 weeks, to higher intakes of fat (Kronfeld et al., 2001a). In contrast, starch- and sugar-splitting hydrolysis appear non-adaptive to higher intakes and to be limited to 2 or 4 g/kg body weight per meal (Potter et al., 1992, Meyer et al., 1995). This upper limit is only approximate and dependent on many factors, including the type of grain and other ingredients in the meal, especially those that affect rate of passage through the small intestine.
Rapid fermentation leads acutely to excessive production of gas and lactic acid, which is poorly absorbed and accumulates. Lactic acid attracts water, so may cause osmotic diarrhea. On the other hand, unfavorable condition in the colon will close the cecal-colic valve, and the cecum will distend with accumulating fluid and gas, causing pain—colic. Lactic acid also lowers the pH, causing acidic colitis and enabling entry of bacteria into the intestinal wall and blood. Acid lysis of bacteria releases endotoxins, which contribute to some (but not all) forms of laminitis.

Excessive glucose absorption following large meals rich in starch and sugar leads acutely to an exaggerated feeding-fasting cycle of metabolites and hormones and chronically to insulin resistance. In humans, the popularity of low-fat diets over the last 40 years has led to an epidemic of disorders associated with high starch-sugar diets and insulin resistance, a set sometimes called syndrome X. The low-fat, high-carbohydrate diets were promoted initially by the American Heart Association and Ancel Keys, who over-generalized his studies on blood cholesterol and heart disease under constrained conditions to apply to free-living populations. Ironically, syndrome X not only includes obesity and type 2 diabetes mellitus but also high blood pressure and atheromatous heart disease.

In horses, others and we have demonstrated exaggerated responses of (blood) plasma glucose and insulin to 5 lb meals of grain and molasses (sweet feeds or pellets) compared to fat-fortified, low starch feeds or to forages. We have proposed an equine syndrome X, which may include some but not all forms of gastric ulcers, exertional rhabdomyolysis, and developmental orthopedic disease (Kronfeld et al., 2001b).

**Fat Enhanced Performance**

Feeding fat-fortified feeds will lower bowel ballast in 3 weeks, perhaps less. Compared to hay only, a ration of hay:oats (50:50) lowers bowel ballast of a 500 kg 3-day event horse by about 60 lb (Kronfeld, 1996). Compared to hay:oats (50:50), a ration of hay-oats-oil (45:45:10) lowers bowel ballast of our horse by about 30 lb. This improvement in power-weight ratio should improve every kind of exercise performance.

Further improvement in performance depends on fat adaptation, the set of metabolic and regulatory adjustments to the combination of physical conditioning and high fat feeding for 12 weeks or more (Graham-Thiers et al., 2001,Kronfeld, 1998). Here are high points:

- Less spontaneous activity and reactivity, that is, more calmness and tractability.
- Improved work, anaerobic (sprinting) as well as aerobic (stamina).
- Less production during exercise of acid, a major fatigue factor.
- Improved energetic efficiency, demonstrated by a bioenergetic model based on typical published data.
- Improved metabolic regulation, revealed by responses of (blood) plasma lactate to aerobic versus anaerobic exercise.
- Alleviation of growth slumps and spurts in yearlings during winter and spring.
- Reduction of insulin resistance and changes in the somatotropic axis that probably contribute to developmental orthopedic disease in growing horses.
- Reduction of insulin resistance in mares during pregnancy, that is, diminishing the diabetogenic effect of pregnancy.
- Increasing the contents of immunoglobulins in colostrum (initial milk), hence foal blood, which should help prevent infections and sepsis that are common diseases of neonatal foals.
- Maintaining the contents of fat, protein and other nutrients in mare’s milk until 6 months, compared to the usual drop from about 2 months onwards.
Conclusion

The main disadvantages of fat-fortification of horse feeds have been recognized for about 6
decades, although a few details continue to emerge. One such is the adverse effect of 15% soybean oil on
digestibility of fiber, although 5% soybean oil has done no harm in our hands. The main advantages of
fat-fortification have been emerging for about 3 decades, and especially the last decade. Empirical models
suggest the final fat content of the ration (total daily intake) should be 12-18%, but most current ‘high-fat’
feeds have only 8-12% fat and are fed with forages containing 1-2% triglycerides. From where we sit in
the ivory tower, we are left with two inter-related questions: Are feed manufacturers being too
conservative nutritionally? Or are they justified economically in balancing cost against effectiveness? Our
conclusion is that fat-fortification of horse feeds now needs only one thing—a marketing genius.

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VITAMIN AND MINERAL REQUIREMENTS IN THE HORSE

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Vitamins

The nature of vitamins is as varied as any group of nutrients. In general, vitamins are organic compounds required in minute amounts that cannot be produced in adequate quantities in the body and must be obtained from food or the environment. More specifically, vitamins serve the similar purpose of maintaining normal body function. Vitamins are necessary for optimal growth, health, feed conversion and reproduction. To further complicate this, vitamins are also necessary for proper physical performance in horses. Vitamins are either fat-soluble (vitamins A, D, E & K) or water-soluble (vitamin C & B vitamins). Function, signs of deficiency, and the current recommended minimum requirement for the fat- and water-soluble vitamins can be found in Table 1.

Minerals

Unlike vitamins that are organic, minerals are inorganic compounds. Like vitamins, minerals also play an important role in a wide range of biochemical systems that affect virtually every metabolic function in the horse. The type and quantity of minerals required by the horse are very diverse. Essential minerals include the major or ‘macro minerals’, which are needed in larger amounts (calcium, phosphorus, magnesium, potassium, sodium, chloride, sulfur) and the trace or ‘micro minerals’, which are needed in minute amounts (iron, copper, zinc, manganese, selenium, iodine, molybdenum, fluoride, cobalt). Function, signs of deficiency, and the current recommended minimum requirement for the macro- and micro-minerals can be found in Table 2.

Requirements

Vitamin and mineral requirements are affected by age, reproductive status, environment, amount of exercise and a variety of stresses like gastrointestinal infections and intense muscular exercise. The need for vitamin and mineral supplementation also depends on the type and quality of the diet, the length of exposure to sunlight, the amount of microbial vitamin synthesis in the digestive tract, and the extent of vitamin absorption from the site of synthesis. Non-working horses grazing high quality pastures are likely to need little or no vitamin and mineral supplementation because forages are a rich source of most fat- and water-soluble vitamins and have a fairly decent mineral balance. Because many horses do not have the advantage of lush green pasture year-round, supplementation of vitamins and minerals becomes necessary. Today, nearly all commercial horse feeds are fortified with vitamins (primarily fat-soluble vitamins) and minerals to supplement the low natural vitamin and mineral content of the grains as well as balance mineral content to complement forage.

Several methods can be used to estimate mineral requirements (Hintz, 2001). In the balance study, intakes above and below the estimated requirement of a specific mineral are fed to mature animals in metabolism stalls. Intake, fecal and urinary mineral losses are determined. Intake is plotted against retention. The intake of the mineral needed for zero retention (maintenance) can then be calculated. Another approach is to measure obligatory (endogenous) losses in urine and feces. Endogenous losses are those that the body loses regardless of intake and can be measured with isotope studies or with balance studies in which losses are extrapolated at zero nutrient intake. Knowledge of endogenous losses is useful
not only in the estimation of maintenance requirements, but also for the factorial method to measure requirements above maintenance.

Table 1: Vitamin function, signs of deficiency and NRC recommendations (Lewis, 1995).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Major Function</th>
<th>Signs of Major Deficiency</th>
<th>NRC Requirement (Concentration in total diet, DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Vision, mucous tissue, tissue keratinization, immunity, reproductive integrity</td>
<td>Decreased feed intake and growth, anemia, poor hair coat, increase in respiratory disease and diarrhea, tearing, night blindness, cornea keratin, decreased conception, weakness and convulsions</td>
<td>2000 – 3000 IU/ kg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Calcium and phosphorus balance metabolism, bone calcification, immunity</td>
<td>Decreased feed intake, growth and bone density, enlarged metaphysis, emaciation and recumbency, Rickets, osteomalacia</td>
<td>300 – 800 IU/ kg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Intracellular respiration, antioxidant, membrane integrity</td>
<td>Encephalomalacia, depressed immune status, skin edema, steatitis, jaundice, liver necrosis, anemia, erythrocyte hemolysis, muscular dystrophy, fetal death, reduced fertility</td>
<td>50 – 80 mg/kg</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>Blood coagulation</td>
<td>Prolonged blood clotting, low prothrombin, intramuscular bleeding, anemia, hemorrhage</td>
<td>No requirement established</td>
</tr>
<tr>
<td>Thiamine (B1)</td>
<td>Metabolism of carbohydrates, nerve transmission</td>
<td>Decreased growth, loss of appetite, ataxia, muscle tremors, stiff, cold extremities, generalized congestion and hemorrhage, and pulmonary edema</td>
<td>3 – 5 mg/kg</td>
</tr>
<tr>
<td>Riboflavin (B2)</td>
<td>Function in coenzymes FMN and FAD in energy metabolism, antioxidant, ligament integrity, H-transfer</td>
<td>Never reported in the horse. Rough hair coat, dermatitis, photophobia, decreased growth, fatty liver, anemia.</td>
<td>2 mg/kg</td>
</tr>
<tr>
<td>Pantothenic Acid</td>
<td>Conversion of amino acid groups as coenzyme A, skin integrity</td>
<td>Never reported in the horse. Dermatitis rough hair coat, graying of hair, goose stepping, demyelination of spinal cord, depressed immune system, gastrointestinal inflammation</td>
<td>No requirement established.</td>
</tr>
<tr>
<td>Niacin</td>
<td>Metabolism of carbohydrates, protein and fats, basis of enzymes NAD and NADP</td>
<td>Never reported in the horse. Loss of appetite, reduced growth, muscular weakness, rough hair coat, diarrhea</td>
<td>No requirement established.</td>
</tr>
<tr>
<td>Pyroxdine (B6)</td>
<td>Metabolism of proteins</td>
<td>Never reported in the horse. Dermatitis around the eyes, alopecia, anemia, muscular weakness, impaired immune function</td>
<td>No requirement established.</td>
</tr>
<tr>
<td>Choline</td>
<td>Component of phospholipids in cell membranes.</td>
<td>Never reported in the horse. Fatty liver, duodenal ulcers, anemia.</td>
<td>No requirement established.</td>
</tr>
<tr>
<td>Cobalamin (B12)</td>
<td>Protein metabolism, methionine synthesis and folate entry into cells, required for utilization of propionate</td>
<td>Never reported in the horse. Anemia, poor growth, poor hair coat, fatty kidney, kidney damage, ataxia, uncoordinated hind legs, impaired thyroid, dermatitis.</td>
<td>No requirement established.</td>
</tr>
<tr>
<td>Folacin</td>
<td>Red blood cell synthesis, movement of single carbon units in metabolic pathways along with methionine and choline.</td>
<td>Never reported in the horse. Poor growth, anemia, poor skin condition, reduced fertility immunity.</td>
<td>No requirement established.</td>
</tr>
<tr>
<td>Biotin</td>
<td>Metabolism of fats, carbohydrates and proteins as coenzyme for CO 2 fixation and walled transcarboxylatio.</td>
<td>Hoof wall cracks, crumbling shelly hooves, tender soles.</td>
<td>No requirement established.</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Antioxidant, hormone synthesis, conversion of vitamin D to calcitriol, essential for bone calcification.</td>
<td>Never reported in the horse. Poor hair coat, depressed immune system, delayed wound healing, capillary fragility and hemorrhage.</td>
<td>No requirement established.</td>
</tr>
</tbody>
</table>
### Table 2: Mineral function, signs of deficiency and NRC recommendations (Lewis, 1995).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Major Function</th>
<th>Signs of Major Deficiency</th>
<th>NRC Requirement (Concentration in total diet, DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Bone structure, neural muscular function, blood coagulation, cell membrane function, temperature regulation, glandular secretion, enzymes.</td>
<td>Developmental Orthopedic Disease (DOD), lameness, tendonitis, Hyperparathyroidism, spontaneous fractures</td>
<td>0.24 – 0.68 %</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Buffer, energy metabolism, numerous cellular functions.</td>
<td>Same as calcium.</td>
<td>0.17 – 0.38 %</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Bone structure, muscle contraction.</td>
<td>Muscle tremors, ataxia, collapse, sweating, convulsions, tetany.</td>
<td>0.08 – 0.13 %</td>
</tr>
<tr>
<td>Sodium &amp; Chloride</td>
<td>Regulation of body fluids, maintenance of acid-base balance, generation of membrane potential, conduction of nerve impulses.</td>
<td>Decreased sweating and performance, decrease food and water intake, weight loss, weak dehydration, excess licking, pica, constipation.</td>
<td>Sodium 0.1 – 0.3 %</td>
</tr>
<tr>
<td>Potassium</td>
<td>Regulation of body fluids Never reported</td>
<td>Fatigue, weak, lethargy, decrease feed and water intake, weight loss.</td>
<td>0.30 – 0.43 %</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Constituent of amino acids, vitamins, hormones, enzymes and other body constituents, important in cartilage, hoof and hair.</td>
<td>Never reported in horse, decreased appetite, growth, hair or wool production and milk production.</td>
<td>0.15 %</td>
</tr>
<tr>
<td>Iron</td>
<td>Component of molecules and enzymes involved in oxygen transport.</td>
<td>Anemia.</td>
<td>40 – 50 mg/kg</td>
</tr>
<tr>
<td>Copper</td>
<td>Collagen stabilization, elastin syntheses, mobilization of iron stores, melanin syntheses.</td>
<td>DOD, anemia, change in hair color.</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Zinc</td>
<td>Component of metalloenzymes involved in protein and carbohydrate metabolism.</td>
<td>DOD, decreased feed intake and growth, parakeratosis, hair loss.</td>
<td>40 mg/kg</td>
</tr>
<tr>
<td>Manganese</td>
<td>Essential for carbohydrate and lipid metabolism, synthesis of chondroitin sulfate, superoxide scavenger.</td>
<td>Possibly bone abnormalities in newborn.</td>
<td>40 mg/kg</td>
</tr>
<tr>
<td>Iodine</td>
<td>Production of thyroid hormone.</td>
<td>Hypothyroidism, goiter, rough hair coat and loss, DOD</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>Selenium</td>
<td>Protect cell membranes, enzymes from oxidative damage.</td>
<td>Juvenile: decreased immunity and growth, stiff, listless, difficult nursing, dypnea, lung edema, white muscle disease. Adult: stiffness.</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Component of enzymes involved in purine metabolism.</td>
<td>Never reported.</td>
<td>No requirement established.</td>
</tr>
<tr>
<td>Flouride</td>
<td>Incorporated into teeth and bone.</td>
<td>Never reported.</td>
<td>No requirement established.</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Integral part of B12.</td>
<td>B12 deficiency.</td>
<td>0.1 mg/kg</td>
</tr>
</tbody>
</table>

Unlike research conducted on humans and food animals, actual vitamin requirements for horses have not undergone intensive scrutiny. Most of the attention has been focused on fat-soluble vitamins because of their problem with possible toxicity. Research into requirements is time consuming due to the lengthy period it takes to initiate obvious outward signs of vitamin depletion. Further, research may involve considerable suffering of the animal before death occurs, and many researchers are reluctant to put the animals through this suffering. Currently, function tests are used most frequently in vitamin studies in an attempt to detect the onset of vitamin depletion before it causes irreversible damage and suffering. With the use of function tests, it is becoming apparent that there is a clear distinction between the minimal requirement of a vitamin and an optimal requirement (Greive-Crandell et al., 1995; Craig et al., 1991).
Minimum versus Optimum Requirements

The minimum vitamin or mineral requirement is that quantity which has to be supplied daily to the animal to prevent or correct deficiency symptoms. Many of these values were determined earlier in the twentieth century under experimental laboratory conditions; however, these values have only a theoretical value as far as practical animal feeding is concerned. Many times the research animals were maintained in a rigidly controlled environment, not in a natural situation. Animals were also not subjected to any form of physical exercise or reproductive effort, physiological states which may alter minimal requirements.

The optimum requirement is the quantity that promotes maximal growth rate, performance, health, feed utilization and body reserves. Experience indicates that the optimum supply is probably several times higher than the minimum vitamin requirement. Researchers are attempting to define the optimum requirement for many vitamins and minerals in the horse but can only be determined by using sensitive biological and physiological criteria. Symptoms of a minor vitamin or mineral deficiency are not always immediately visible. Suboptimal intake can occur frequently in practice and result in a nonspecific depression of performance, increased susceptibility to disease, reduced fertility, and shorter productive life. For these reasons, the objective in designing the ideal diet is to meet optimum requirements through vitamin and mineral supplementation.

The National Research Council (NRC) last published Nutrient Requirements of Horses in 1989. The recommendations presented in this edition are restricted to the research that had been done for each nutrient before 1989. In compiling the requirements, many different recommendations had to be considered and a consensus was decided upon to establish requirements. Since then, a great deal of research has been directed towards microminerals, particularly as they affect skeletal development in growing horses. Still, many questions remain unanswered about specific requirements for other minerals and vitamins in many classes of horses. The NRC vitamin and mineral requirements should be considered the minimum vitamin requirement under ideal circumstances to prevent clinical symptoms of deficiency. Since these requirements often differ drastically from what may be the optimal vitamin or mineral requirement under true field conditions, the NRC recommendations should be considered only a part of the total requirement. Figure 1 illustrates the concept of optimal nutrition.

The most recent Nutrient Requirements of Horses (NRC, 1989) provides estimated requirements for vitamins A, D, E, thiamin and riboflavin, but not for the other vitamins. Requirements of other vitamins in the horse were not established prior to 1989 because outright deficiencies were not experienced. However, in designing diets for optimum health and well-being of horses, the gray zone between minimal and optimum requirement needs to be taken into consideration. For example, McMeniman et al. (1995) reported that exercising horses had lower peak plasma lactate concentrations and lower maximum heart rates when they received a diet containing a multi-vitamin supplement (A, D, E, K, thiamin, riboflavin, nicotinamide, pyridoxine, pantothenic acid, biotin, choline, folic acid and cyanocobalamin), but the indices of performance were not improved. The fact that performance was not improved may be justification to not change the minimal requirement, but the changes in heart rate and lactate are sufficient to suggest that there can still be improvement with optimal levels.
Figure 1: Effects of amount of nutrient consumed on animal health and performance. Suboptimal intake of a nutrient impairs health and performance, cannot be detected clinically, but for some nutrients can be detected by laboratory tests. Clinical signs occur only at an intake below or above that which is deficient or toxic, respectively. The intake range between requirement and toxic levels differs for different nutrients (adapted from Lewis, 1995).

Beyond Requirements

Aside from the basic and optimal vitamin and mineral requirements in the horse, there is increasing interest in using nutrients as healing foods. This is drastically changing the manner in which vitamin and mineral supplementation of horses is being viewed. Vitamin and mineral supplementation has evolved from meeting the basic physiological requirements of the animal to providing a food that will improve the quality of life. This is essentially the same development that occurred in human nutrition over the last ten years. Human supplementation with antioxidants (vitamins E and C, beta-carotene, selenium) and bone builders (calcium, magnesium, zinc) has become commonplace. Many times the amounts taken are well in excess of the minimal requirement and are considered therapeutic amounts. The main justification for this supplementation is to improve the quality of life and to reduce risks associated with aging such as cancer, bone loss, low immune competence, cardiovascular diseases, cataracts and renal failure. The consumer may conclude that such supplementation may improve the quality of life for horses as well. Supplement products for horses found on the market frequently contain vitamins or minerals in therapeutic amounts.

Influencing Factors on Vitamin and Mineral Supplementation

There are many factors that can influence the requirement of a vitamin or mineral. Anti-factors can affect nutrient availability in the feed. The requirement may vary depending on the make-up or level of other nutrients in the feed, such as protein, energy, other minerals, or other vitamins. For example, high fat feeds may increase the requirement for vitamin E (antioxidant) because metabolism of fat in the body increases the production of free radicals and reactive oxygen species (McMeniman and Hintz, 1992; Hargreaves et al., 2001b). Bioavailability of vitamins or minerals existing in the natural feedstuffs may influence the additional amount needed to meet the requirement. For example, oxalates found in tropical forages tie up calcium and make it unavailable for absorption in the horse so that the amount of calcium needed in the rest of the diet needs to be significantly higher (McKenzie et al., 1981). Poisonous plants
can interfere with absorption of a nutrient; such as, Horsetail (Equisetum arvense) which has a thiaminase enzyme that denatures thiamin before the horse has the opportunity to absorb it (Lewis, 1995). A possible problem with the manufacture of commercial concentrates is the destruction of vitamins during heat processing (extruding or pelleting) or oxidation and catalytic effects from trace minerals present or peroxidation from rancidifying of polyunsaturated fats (McDowell, 1989). The form the mineral is in may influence availability or digestibility, whether it is bound to sulfate or oxide or chelated (Siciliano et al., 2001). The form of vitamin, whether it is palmitate or acetate, natural or synthetic, or different isomers will affect absorption (Ganzen et al., 1995; Greiwe-Crandell et al., 1997; Hargreaves et al., 2001a). Also vitamins can bind with something in the feed to make digestion less efficient. Further, the growth of fungi, bacteria and yeast in a feed will be detrimental to the stability of the added vitamins to that feed (McDowell, 1989).

Within the horse there can be factors that influence the requirement of a vitamin or mineral. There may be considerable variability in the requirements of different types of equine (mule, pony, horse) or breeds (light horse, heavy horse, crossbred). Within individuals there may be genetic variation for requirements just as there is variation in body metabolism. In a study on biotin supplementation to Lippizaner horses, improvement in hoof quality was only seen in horses that had a problem with poor quality hoof wall and not horses with good hooves (Josseck et al., 1995). There can be variation in the ability of an individual to absorb the nutrients in the gut, some of which may be the result of scarring and damage from parasites. If there is not enough fat in the diet, it will make absorption of fat-soluble vitamins difficult (McDowell, 1989). Low or lack of the appropriate mechanism involved with absorption through the gut wall will certainly have an adverse affect on availability of a nutrient. This can be from a diminution of gut enzymes, like lipase or thiaminase, or competition between nutrients that use similar absorption mechanisms (such as calcium and magnesium or vitamins A and E). Other factors can affect absorption of minerals in the intestinal tract. In particular, the age of an animal (young animals are more efficient in absorbing essential elements than older animals) and the pH of the intestinal tract can affect the rate of absorption (Hall et al., 1991, Neilsen et al., 1997; Greiwe-Crandell et al., 1992). Once in the blood stream, there may be inadequate levels of transport proteins in the liver for delivery of the nutrients to their target sites.

Further, the health of the animal can influence its vitamin and mineral requirements. Disease, particularly chronic states, will affect the need for certain vitamins or minerals, depending on the etiology of the disease. Malabsorption may occur due to destruction of microvilli in the small intestine. Parasites not only can cause irreparable damage but also can compete for nutrients in the body (Reed and Bayly 1998). Mycotoxins and peroxides from the feedstuffs are often responsible for malabsorption of vitamins (BASF, 2000). Stress can play a major role in the need for certain vitamins and minerals, and increased supplementation may support the immune system during or after periods of major stress. For example, foals that had undergone transportation stress responded to vitamin E and C supplementation by being able to fight off viral infection (Ralston, 2001). Amount of work being required of a horse may influence its requirement. For example, serum ascorbate (vitamin C) has been found to be depressed in heavily working polo ponies and responded to supplementation (Hoffman et al., 2001). Environment can effect vitamin requirements, as seen in horses housed in hot humid climates that had higher sodium and chloride needs than horses in cooler climates (McCutchion and Geor, 1996). Season can also influence nutrient status, as was seen with vitamin status in horses in Finland. Vitamin A and E were depressed in the winter and were less responsive to supplementation than during the summer (Maenpaa et al., 1988a, 1988b).

Conclusion

Numerous factors influence the requirements of vitamins and minerals in the horse. Continued investigation into vitamin and mineral requirements in horses will broaden the understanding of the vital
importance of these delicate organic and inorganic compounds, and further define the significance of optimal, not just minimal, nutrition in the equine.

References


Summary

Horse owners feed a large variety of substances classified as nutraceuticals. Nutraceuticals are dietary supplements sold for the treatment or prevention of disease. The term "nutraceutical" combines the word "nutrient" (a nourishing food or food component) and the word "pharmaceutical" (a medical drug). This dual identity has prevented their regulation by the American Association of Feed Control Officials (AAFCO) and the Food and Drug Administration (FDA). Equine feeds are required to have nutritive value which is accountable by labeling (guaranteed analysis), and contain ingredients identified by AAFCO to be "generally recognized as safe" (GRAS). Nutraceuticals contain ingredients with nutritive value, but some of these ingredients may not have GRAS status, which prevents their inclusion into an equine feed. Conversely, when a claim for a nutraceutical for the treatment or prevention of disease is made, it is subject to regulation as a drug. This requires demonstration of safety and efficacy for its intended use in order to meet FDA approval, which is lacking for these products. The regulation of nutraceuticals as dietary supplements is limited to human products, preventing their regulation for animal use. There are no federal guidelines for enforcement of safety or efficacy of nutraceuticals sold as dietary supplements for equine use. The following nutraceuticals are discussed in relation to anecdotal claims, mode of action, and research results and effectiveness: oral joint supplements, MSM, gamma oryzanol, creatine, DMG, HMB, coenzyme Q₁₀, carnitine, and echinacea. Although anecdotal evidence and a clinical trial are promising, there is no published data demonstrating sodium hyaluronate to be an effective oral joint supplement. Glucosamine hydrochloride and glucosamine sulfate have not been demonstrated to be effective oral joint supplements for lameness in horses. Chondroitin sulfate is an effective oral joint supplement, but chondroitin sulfate used in combination with glucosamine hydrochloride or glucosamine sulfate is more effective for treatment of arthritic conditions of horses. MSM is a bioavailable source of sulfur for the horse, but its effectiveness for any claims or benefits is anecdotal. Rice bran and rice bran oil contains gamma oryzanol. Ferulic acid is a component of gamma oryzanol and it is an effective antioxidant in body tissues. High levels of ferulic acid, achieved through supplementation of gamma oryzanol, rice bran, or rice bran oil, could be responsible for the beneficial effects of gamma oryzanol, rice bran and rice bran oil supplementation observed in working horses. These benefits are consistent with the antioxidant property of ferulic acid in reducing the effects of prolonged anaerobic exercise and free radical oxidation, with improvement in muscle function and endurance, and the lowering of blood lactate levels in performance horses. There is no evidence for creatine supplementation increasing muscle performance in the horse, but frequency of daily supplementation may be critical to effectiveness. There is evidence for beneficial results of oral DMG supplementation, but results are contradictory. HMB supplementation appears promising in reducing muscle damage and increasing performance in working horses, but no improvements in performance are apparent. There were no advantages observed for supplementation of Coenzyme Q₁₀ for improving athletic performance of horses. Carnitine supplementation altered cardiac and hematological changes consistent with increasing fitness in exercising horses, but no increases in muscular performance were evident. Echinacea increased immune function and overall blood quality in the horse.
Introduction

Every one who owns a horse wants it to look and perform at its best, and part of this is good nutrition. Horse owners are often looking for new feed supplements that will improve the horse's health or provide a competitive edge. Unfortunately, the subjective nature of most evaluations of equine performance lends itself to the promotion and sale of many dubious products. The term "nutraceutical" was coined to describe the increasing number of products offered for the prevention or treatment of disease or performance enhancement, but marketed as dietary supplements. The term "nutraceutical" combines the word "nutrient" (a nourishing food or food component) and the word "pharmaceutical" (a medical drug). Many of these substances are present in commercial equine feeds and common feedstuffs provided to horses. These include products that contain levels of essential nutrients beyond what is considered required for a particular species, as well as products that contain nutrients that are not recognized as being required. A nutraceutical has been defined as "Any nontoxic food component that has scientifically proven health benefits, including disease treatment and prevention." The theory behind the mode of action of nutraceuticals is to increase the supply of natural building blocks in the body to provide a beneficial effect. Providing these building blocks can work to prevent or reduce signs of disease, or to improve athletic performance. There are a limited number of nutraceuticals whose effects have been researched in the horse. Regulation of nutraceuticals has not been resolved, there are no laws or regulations that define what a nutraceutical is, consequently, federal guidelines for enforcement of safety or efficacy of nutraceuticals sold for equine use don't exist. Besides anecdotal evidence from horse owners of the effectiveness of these products, there is a lack of scientific data to support the claims for many nutraceuticals. The following is discussion on the current state of regulation for nutraceuticals, and a review on a limited number of nutraceuticals regarding mode of action, summary of research results, and effectiveness.

Regulation of Nutraceuticals

The Federal, Food, Drug, and Cosmetic Act governs the use of food products. A food is defined by the Food, Drug and Cosmetic Act as "An article that provides taste, aroma, or nutritive value." This act requires that animal feeds, like human foods, be pure and wholesome, contain no harmful substances, and be truthfully labeled. Failure to meet these requirements can result in a product judged as adulterated or misbranded and not allowed for sale. Adulteration includes, food packaged or held under unsanitary conditions, food or ingredients that are filthy or decomposed, food that contains any poisonous or deleterious substance, and food that contains unapproved food additives.

The Food and Drug Administration carries out the responsibility of regulating animal feed products in cooperation with the each state feed control organization. For example, the FDA cooperates with the Association of American Feed Control Officials (AAFCO), which coordinates implementation of uniform policies that regulate animal feed products with each state. AAFCO helps to coordinate feed laws and regulations in the U.S. by establishment of model feed laws and regulations, uniform feed ingredient definitions, and proper labeling rules to assure the safe use of animal feed products. A nutrient is defined by AAFCO (2000) as "A feed constituent in a form and at a level that will help support the life of an animal." The definition of a feed by AAFCO (2000) is "Edible materials which are consumed by animals and contribute energy and/or nutrients to the animal's diet". AAFCO requires that animal feeds have nutritive value and are accountable, with labeling and a guaranteed analysis, specific for each species. Animal feed ingredients such as corn, oats, soybean meal, minerals, vitamins, flavorings, preservatives, etc. must be included on AAFCO's approved list to be generally recognized as safe (GRAS). Feed ingredients must have GRAS status for legal inclusion into an animal feed.

For decades, the FDA (Food and Drug Administration) regulated dietary supplements for humans and animals as foods, to ensure that they were safe, and that their labeling was truthful and not
misleading. However, with passage of the Dietary Supplement Health and Education Act (DSHEA) of 1994, the regulations were expanded to include ingredients beyond essential nutrients. A dietary supplement is defined by DSHEA as "A product that contains one or more of the following dietary ingredients: vitamin, mineral, herb, or other botanical, and amino acid (protein). Includes any possible component of the diet as well as concentrates, constituents, extracts or metabolites of these compounds." This act allowed substances such as ginseng, garlic, fish oils, and psyllium to be sold that were not subject to prior pre-market safety evaluations. The DSHEA also permitted certain limited claims to be made about dietary supplements without classifying the dietary supplement as a drug. This act "opened the door" for the manufacture and sale of human nutraceuticals because the FDA now has to prove that a substance is unsafe rather than the manufacturer having to document product safety (Dzanis, 1998). Many dietary supplements for human consumption have a long history of usage that can be used to establish reasonably safe levels. However, when compared to the human use of supplements, there is less information on the safe use of dietary supplements in animals. Each animal species requires different nutrients, absorbs and metabolizes nutrients differently, and can exhibit different toxic reactions to these food ingredients.

FDA's Center for Veterinary Medicine (CVM) is responsible for the regulation of animal drugs, medicated feeds, animal food additives, and devices for use in animals. Claims on animal feed products that have an intended use to cure, treat, prevent or mitigate disease are classified as drugs. Unless the product has been shown to be safe and effective for its intended use by controlled clinical studies it will not be approved for sale. In addition, any claims for the product to affect the structure or function of the animal's body may cause CVM to classify the supplement as a drug. Feed ingredients, food additives, and drugs used in horse feeds are subject to the same laws as feed ingredients, etc. for other animal species. In 1996, the CVM published a revision of the DSHEA for it to exclude regulation of products for use in animals. The lack of information on the safe use of dietary supplements in animals, the danger of harmful residues from consumption of these supplements in food-producing animals, and the fact that the animal population is not as uniform as the human population, are some of the reasons why FDA determined that DSHEA should not apply to dietary supplements for animals. This act does not permit FDA to consider a new product a "drug" or "food additive" if it falls under the definition of a "dietary supplement". In addition, the DSHEA does not address how the effect of supplements on food-producing animals and human food safety is to be assessed. Currently, there are no federal guidelines for enforcement of safety or efficacy of nutraceuticals sold as dietary supplements for animals, including horses.

The FDA and AAFCO established a Novel Ingredient Task Force in 1999 that has the responsibility to propose a regulatory framework for feed supplements, including nutraceuticals. There have been several committees formed and surveys undertaken to determine which ingredients are marketed as dietary supplements and nutraceuticals for animals before any regulations are issued. The National Animal Supplement Council (NASC) has been formed by manufacturers of nutritional supplements for companion and other animals not used for human consumption. Equine supplement manufacturers have formed the National Association of Equine Supplement Manufacturers (NAESM). The goal of these two organizations is to work with FDA and AAFCO regarding the definition and approval of nutraceuticals and other dietary supplements for animals.

Oral Joint Supplements

Joint lameness resulting from athletic performance in horses is a common problem. The horse's joint has two means of protection from the mechanical stresses from physical activity, synovial fluid and articular cartilage. Oral joint supplements contain ingredients to replace or manufacture these two substances. Obviously, for joint supplements to be effective, the substance must be absorbed into the horse's bloodstream, transported into the joint capsule, and utilized for repair or replacement functions. The main ingredients used in oral joint supplements are sodium hyaluronate, glucosamine sulfate,
glucosamine hydrochloride, and chondroitin sulfate. Oral joint supplements are available in powdered, pelleted, liquid, and gel forms. Other ingredients that are commonly included in oral joint supplements include MSM (methylsulfonylmethane), copper, zinc, manganese, and vitamin C. MSM is a bioavailable form of sulfur, which is a component of collagen. The trace minerals copper, zinc and manganese are required cofactors for enzymes involved in cartilage metabolism. Vitamin C or ascorbic acid is a component of hydroxyproline, an amino acid that is a major component of collagen.

Synovial fluid, which provides lubrication for ease of movement in the horse’s joints, contains proteins, enzymes, water, leukocytes, and sodium hyaluronate. Sodium hyaluronate or hyaluronic acid is a low molecular weight glycosaminoglycan (GAG) composed of glucuronic acid and N-acetylglucosamine. Sodium hyaluronate functions as a lubricant and shock absorber in the joint. Intra-articular, intravenous and intra-muscular injections of sodium hyaluronate have been used to effectively treat joint disease in the horse. Addition of sodium hyaluronate by these methods increases viscosity of synovial fluid, inhibits cartilage-damaging enzymes, and promotes production of sodium hyaluronate. Oral sodium hyaluronate is poorly absorbed in humans, and there is no data on intestinal absorption of sodium hyaluronate in horses. Oral sodium hyaluronate products for horses are commercially available, and anecdotal evidence and a clinical trial (Pierce, 2001) suggest similar effects for treatment of lameness and joint disease as injectable products after two to three weeks of daily administration.

Glucosamine is included in oral joint supplements either as glucosamine sulfate or glucosamine hydrochloride. Cartilage is composed of collagen fibers containing chondrocytes. Chondrocytes synthesize glucosamine, which is a precursor of sodium hyaluronate and the disaccharide unit of GAG, which comprises the proteoglycan found in articular cartilage. Inflammation of a joint from physical stress results in excess fluids, destructive enzymes and prostaglandins in the joint capsule. This results in loss of lubricating GAG molecules and lowered viscosity of synovial fluid. Proteoglycan synthesis by chondrocytes decreases, which lowers cartilage repair rate. Absorption of glucosamine hydrochloride following oral administration has been demonstrated in the horse (Eddington and White, 2001). There is no available literature documenting absorption of glucosamine sulfate in horses after oral dosing, but there is a large amount of evidence documenting effective absorption in man and dogs. In vitro data with equine cartilage supports the concept that glucosamine hydrochloride is effective at stimulating cartilage synthesis and inhibiting prostaglandin release (DeChant et al., 2001; Fenton et al., 1999; Mello et al., 2001). However, there are no published equine studies showing the effectiveness of glucosamine hydrochloride or glucosamine sulfate for treatment of lameness or arthritis.

Lameness can result from damage to any of the tissues associated with the joint, which results in a change in the normal range of motion. Damage to the articular cartilage such as breakdown of collagen or loss of proteoglycan can result in weakened cartilage. This weakened cartilage can develop cracks that disrupt its smooth articulating surface, resulting in lameness. Chondroitin sulfate is the most abundant GAG present in articular cartilage. Since it is known that joint injury leading to inflammation causes a reduction in the amount of proteoglycan, replacement of chondroitin sulfate could replace proteoglycan and restore articular cartilage to a normal state. Chondroitin sulfate has also been proposed to have anti-inflammatory properties and inhibit destructive enzymes associated with cartilage breakdown. Data to support these proposed actions of chondroitin sulfate from an equine in vitro study has shown positive results (Orth et al., 2002). Although molecular weight of chondroitin sulfate is greater than glucosamine products, bioavailability of low molecular weight chondroitin sulfate has been documented in horses after oral administration (Eddington and White, 2001). Chondroitin sulfate is an effective oral joint supplement. Oral administration of chondroitin sulfate significantly reduced lameness scores over controls for horses induced with aseptic arthritis (Dorna and Guerrero, 1997).

There is evidence that an oral joint supplement with a combination of chondroitin sulfate and glucosamine hydrochloride is more effective than a product with chondroitin sulfate alone. In vitro
studies using equine cartilage have shown that a combination of glucosamine hydrochloride and chondroitin sulfate had a synergistic effect on stimulation of cartilage synthesis and protection from degradation (DeChant, et al., 2001; Orth et al., 2002). Significant improvement in lameness scores were observed for horses provided with a powdered oral joint supplement containing chondroitin sulfate and glucosamine hydrochloride (Hanson et al., 2001). Combination products with chondroitin sulfate and glucosamine sulfate are also effective in reducing lameness. Clayton (2002) found that a liquid oral joint supplement containing chondroitin sulfate and glucosamine sulfate produced a more symmetrical gait pattern in horses diagnosed with degenerative joint disease.

MSM

Methylsulfonylmethane (MSM) is a derivative of dimethylsulfoxide (DMSO). With a sulfur content of 34%, MSM serves as a bioavailable source of sulfur for methionine, cysteine, thiamin, biotin and GAG. Since sulfur levels are reduced in damaged articular cartilage, MSM could play an important role as a readily available source of sulfur for cross-linking of collagen fibers during cartilage repair. There are claims for numerous beneficial effects of MSM for horses including: relieving pain and inflammation from arthritis and muscular disorders, moderating allergic reactions and gastrointestinal tract upset, antioxidant, antiparasitism, and antimicrobial functions, and correcting nutrient malabsorption for minerals involved in developmental orthopedic disease (DOD). MSM is widely prescribed for joint injury in horses, its probable mode of action is to reduce inflammation by stabilizing cell membranes and preventing the release of lipids that are a source of prostaglandins. The net result of this process would be reduced pain, swelling and joint effusion.

However, the effectiveness of MSM for treatment of the above conditions is anecdotal. Although MSM is included in many oral joint supplements, no studies have investigated the sole use of MSM for treatment of lameness or DOD in horses. Only one study with oral supplementation in horses has been reported that demonstrated absorption and utilization of MSM (Pratt et al., 1983). Using radioactive labeling, MSM was present in collected urine, feces, blood, and was incorporated into proteoglycan complexes collected from synovial fluid.

Gamma Oryzanol

Gamma oryzanol is a dietary supplement for humans and horses, with claims of increased muscle mass and improved athletic performance. Rice bran and rice bran oil are used as dietary supplements for horses. Gamma oryzanol is a two-part molecule composed of a plant sterol with a ferulic acid ester. It was first isolated from rice bran oil, thus gamma oryzanol is present in rice bran oil and rice bran. The oryzanol fraction of rice bran oil amounts to 1.3% to 2.6% of the total oil content (Seetharamaiah and Prabhakar, 1986). Gamma oryzanol is metabolized by the liver into a ferulic acid portion and a sterol portion. The sterol portion is excreted in the feces, but ferulic acid is absorbed in animals with the highest concentrations of ferulic acid found in intestinal, liver, adrenal and brain tissues (Child, 1987). Ferulic acid is an antioxidant, and has been shown to have greater antioxidant properties than ascorbic acid (Vitamin C) in enhancing the resistance of low-density lipoprotein to oxidation in an in vitro study (Castelluccio, 1986). The net result of free radical production due to prolonged anaerobic exercise would be muscle fatigue, loss of endurance performance and delayed muscle soreness. High levels of antioxidants could reduce the effects of prolonged anaerobic exercise and free radical oxidation, with improvement in muscle function, greater endurance, and lower blood lactate levels.

No significant differences were observed between human males on a nine-week exercise training program when one group was supplemented with 500 mg daily of gamma oryzanol (Fry et al., 1997). The parameters tested were maximum muscular strength, vertical jump power, resting heart, blood pressure, body weight, body composition, and circulating blood concentrations of testosterone, cortisol, estradiol,
growth hormone, insulin, ß-endorphin, calcium, magnesium, total cholesterol, triglycerides, HDL-cholesterol, and binding protein (albumin).

However, feeding rice bran resulted in lower blood lactate accumulation and lower heart rates compared to corn oil in exercised Thoroughbred horses (Kennedy et al., 1999). These lower values for rice bran-fed horses may be due to the antioxidant effect of gamma oryzanol present in rice bran consumed during this study, where daily intake was 2.5 kg per horse. Lower lactate levels and lower heart rates shows a faster metabolic response to the effects of exercise and could result in a performance advantage for horses fed rice bran or rice bran oil.

Using an estimate of 2% gamma oryzanol content for rice bran oil and a 20% crude fat guarantee for rice bran used in this study (EquiJewel™ High Fat Stabilized Rice Bran, Producers Rice Mill, Inc., Stuttgart, AR 72160), daily intake of gamma oryzanol for each horse in the treatment group was 10,000 mg (2.5 kg rice bran x 20% fat = 0.5 kg rice bran oil x 2% gamma oryzanol = 10,000 mg). Daily amount of gamma oryzanol ingestion would be approximately 3 times greater on a per body weight basis for horses on this trial than for the human study, and may explain why differences due to the possible antioxidant effects of ferulic acid were observed for horses but not in humans. This may explain the beneficial effects that have been observed with supplementation of gamma oryzanol, rice bran, and rice bran oil in working horses.

Creatine

The availability of creatine phosphate has been proposed as one of the most likely limitations to muscle performance during intense exercise because it is present in limited amounts in the muscle cell and has a fairly rapid turnover rate. A chemical reaction involving creatine phosphate results in generation of adenosine triphosphate (ATP), which is the fastest source of energy generation in the muscle cell for contraction.

Research studies in humans indicated that creatine phosphate content of muscle was increased with creatine supplementation, and performance was improved by ingestion of creatine over a period of days prior to the exercise test (Greenhaff et al., 1993; Harris et al., 1993). A major disadvantage with creatine supplementation is that the supplement must be taken 4 to 6 times daily, making it impractical for proper supplementation in the horse. A study in racing Thoroughbreds showed no significant increase in muscle creatine or improvement in performance with creatine supplementation (Sewell and Harris, 1995).

DMG

Dimethylglycine (DMG) is a derivative of the amino acid glycine. DMG is converted in the body to N, N-dimethylglycine, an ester of DMG that is a normal intermediate of choline metabolism. DMG has been proposed to enhance creatine phosphate stores in the muscle cell, increasing the amount of ATP or energy available for muscle contraction, although the exact mechanism is not known. Claims of the benefits of DMG supplementation include increased oxygen utilization, reduction of lactic acid accumulation in muscle, enhancement of immune system function, and prevention of tying up (exertional rhabdomyolysis), and overall increased muscular performance.

DMG, also called vitamin B₁₅ and pangamic acid, first received attention when it was found that Russian athletes were using it to increase muscular performance. Studies in the horse have provided mixed results, but there could be some benefits proven with additional research. Supplementation of DMG in Standardbreds showed reduced blood lactate and greater speed at maximal heart rate (Levine et al., 1982), and research with exercised Quarter horses found lower blood lactate levels for the DMG-
supplemented group (Moffit et al., 1985). However, a study with Thoroughbreds undergoing treadmill exercise found no differences due to supplementation with DMG (Rose et al., 1989).

**HMB**

β-hydroxy-β-methylbutyrate (HMB) is a metabolite of the amino acid leucine. Leucine is a branched-chain amino acid believed to be used by the body during stress and exercise. HMB functions as a building block for intramuscular cholesterol synthesis, which may be limited for maximal muscle function during periods of heavy training or stress. Research in humans has indicated that aerobic exercise performance and muscular strength can be improved with supplementation of HMB. Increased performance with HMB supplementation in horses also appears promising, but more research is needed. A treadmill study with HMB-supplemented horses observed higher blood glucose levels during exercise than controls (Nissen et al., 1997). Another study conducted with horses under actual race training and racing conditions found lower levels of muscle enzymes that are indicative of muscle damage in horses supplemented with HMB after a race (Miller and Fuller, 1998).

**Coenzyme Q<sub>10</sub>**

Coenzyme Q<sub>10</sub> or ubiquinone is found in the body as a component of the mitochondrial respiratory chain. It works in combination with other substances to regenerate ATP in the cell. Coenzyme Q<sub>10</sub> also functions as an antioxidant and free radical scavenger. In humans, coenzyme Q<sub>10</sub> supplementation has been reported to have been used successfully in the treatment of heart problems, muscular dystrophy, cardiac myopathy, and periodontal disease (Greenburg and Fishman, 1990; Hishikawa et al., 1989). However, there is no evidence that coenzyme Q<sub>10</sub> improves performance in the horse. One study in horses found that coenzyme Q<sub>10</sub> may have an indirect effect on tissue oxygen utilization, but had no effect on lactate metabolism or heart rates (Rathgeber-Lawrence et al., 1991).

**Carnitine**

Carnitine is an amino acid present in high levels in cardiac and skeletal muscle. Carnitine is involved with the utilization of fatty acids for energy in the muscle cell. It has been hypothesized that supplementation of carnitine could be glycogen sparing and reduce lactic acid production, which would result in improved muscle function and endurance. Cardiac supplementation improved cardiac and skeletal muscle function in humans with impaired oxygen supply (Cerrerelli and Marconi, 1990). However, human studies have not shown an increase in muscle carnitine or improved athletic performance with healthy individuals. These results would suggest that endogenous synthesis of carnitine is adequate in the normal healthy adult but may be deficient in states of disease.

Carnitine is present in high amounts in muscle tissue, thus a carnivorous human diet is high in carnitine, while the herbivorous diet of a horse would have a very low carnitine level. This would require the horse to produce most, if not all of its carnitine supply endogenously. Researchers had investigated oral carnitine supplementation in adult and yearling horses. Results have shown that supplementation increased plasma carnitine levels in adult and yearling horses, but long-term feeding did not increase muscle carnitine concentrations. Since carnitine must be inside the muscle cell to provide a benefit, there is no evidence that supplementation would improve performance in the horse. Harmeyer et al. (2001) observed cardiac and hematological changes consistent with increasing fitness in standardbreds performing aerobic and anaerobic with carnitine supplementation, but no increases in performance were evident.
Echinacea

Echinacea (*Echinacea augustifolia*) is a perennial herb whose root extracts have been used in human holistic medicine for supportive therapy for influenza-like infections because of its known immuno-stimulating properties. Supplementation of echinacea to horses improved immune function and overall blood quality in the horse (Pearson-O'Neill *et al.*, 2001). An aqueous extract of echinacea *augustifola* from powdered root standardized to 4% echinacoside was fed to horses for 42 days. Echinacea significantly increased the number and size of red blood cells and blood hemoglobin levels of treated horses versus controls. Significant increases were also observed for blood lymphocytes and phagocytic ability of neutrophils for echinacea-fed horses.

Conclusions

Horse owners feed a variety of substances classified as nutraceuticals. Nutraceuticals are dietary supplements sold for disease treatment or prevention or improvement in performance. There are no federal guidelines for enforcement of safety or efficacy of nutraceuticals sold as dietary supplements for equine use. Although anecdotal evidence and a clinical trial are promising, there is no published data demonstrating sodium hyaluronate to be an effective oral joint supplement. Glucosamine hydrochloride and glucosamine sulfate have not been demonstrated to be effective oral joint supplements for horses. Chondroitin sulfate is an effective oral joint supplement, but chondroitin sulfate used in combination with glucosamine hydrochloride or glucosamine sulfate is more effective for treatment of lameness in horses. MSM is a bioavailable source of sulfur for the horse, but its effectiveness for any claims or benefits is anecdotal. Rice bran and rice bran oil contain gamma oryzanol. Ferulic acid is a component of gamma oryzanol, which has antioxidant properties and may be effective at reducing the effects of prolonged anaerobic exercise in performance horses. High levels of ferulic acid achieved through supplementation of gamma oryzanol, rice bran, or rice bran oil could be responsible for the beneficial effects of gamma oryzanol, rice bran and rice bran oil supplementation observed in horses. There is no evidence for creatine supplementation increasing muscle performance in the horse, but frequency of daily supplementation may be critical to effectiveness. There is evidence for beneficial results of oral DMG supplementation, but results are contradictory. HMB supplementation appears promising in reducing muscle damage and increasing performance in working horses, but no improvements in performance are apparent. There was no advantage for supplementation of Coenzyme Q10 for improving athletic performance of horses. Carnitine supplementation altered cardiac and hematological changes consistent with increasing fitness in exercising horses but no increases in muscular performance were evident. Echinacea increased immune function and overall blood quality in the horse.

References


This conference, the Golden Anniversary of The Maryland Nutrition Conference for Feed Manufacturers, is dedicated to Dr. Gerald Combs, Sr. Dr. Combs was instrumental in initiating the first conference and has maintained interest and input throughout these 50 years.

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