Antimicrobial Resistance in Livestock

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Take Home Message

Antimicrobial resistance is a threat to public health and there are concerns that a post-antibiotic era may occur if action is not taken to control the development of resistance. In agriculture, antimicrobials have been used for the last 70 years as growth promoters and to treat and mitigate bacterial infection in animals. Bacterial resistance is universal and complex but the abundance of resistance has increased as a result of antimicrobial use in humans and animals. Subtherapeutic use has been the most controversial application of antimicrobials in animal production systems and has led some countries to ban growth promoters. However, this may lead to an increase in the use of antimicrobials of greater importance. It is estimated that global consumption of antimicrobials by food animals will increase 67% by 2030. Thus alternatives to antimicrobials are greatly needed. As alternatives are derived or policies are made to change antimicrobial use, it is important to have surveillance systems in place to measure the effects of altering production practices.

Introduction

The discovery of penicillin in 1928 initiated the antibiotic era that revolutionized human medicine (Aarestrup, 2015). Over the last 80 years, additional antimicrobial agents have been developed and are the foundation to treating infectious diseases in both human and veterinary medicine. Antibiotics are critical to public health and have reduced mortalities and morbidities not only through treatment but also prevention of infections. Shortly after large scale production of antibiotics for use in clinical medicine in the 1940's, they started to be used in agriculture therapeutically and for growth promotion (You and Silbergeld, 2014). Antimicrobials have become integrated into modern agriculture and in 2010, global usage of antimicrobials for food animal production was estimated to be 63, 151 tons (Van Boeckel et al., 2015). It is estimated that consumption of antimicrobials by food animals will increase 67% by 2030.

However, soon after their clinical use, bacteria resistant to antibiotics were detected. Penicillin-resistant *Staphylococcus aureus* (Barber and Rozwadowska-Dowzenko, 1948) was isolated from patients in hospitals and resistance to streptomycin was observed in community isolates of *Mycobaterium tuberculosis* in 1948 (Crofton and Mitchison, 1948). This was followed by the detection of multidrug resistant bacteria in enteric pathogens in the 1960's (Cantas et al., 2013). Recently, the World Health Organization has listed resistance to antimicrobials as an increasingly serious threat to global public health (WHO, 2014), which is highlighted by the medical impact of resistance. In the United States, it has been estimated that at least two million people are infected with resistant bacteria, directly causing 23,000 deaths annually (CDC, 2013).

Antimicrobial-resistant bacteria are not a new phenomenon and it is now becoming clear that resistance is ancient and has always been part of the bacterial pangenome. This is evidenced by recent studies showing that bacteria never before exposed to modern antimicrobials were resistant

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to those commercially available (D'Costa eta l., 2011; Bhullar et al., 2012). It is also clear that the improper use of antimicrobials can lead to an increase in the prevalence of resistant bacteria. To this end, for decades, the most controversial use of antimicrobials in animal husbandry has been subtherapeutic application for growth promotion. This led to the Swan report in 1969 that recommended in-feed antimicrobials for animals be limited to those that have no application for therapeutic use in humans (Hao, 2014). The European Union in 2006 banned the use of growth promoters in food-producing animals, though they are still currently used in Canada, the United States, and other countries around the world. Resistance is complex and as industry and policy makers address concerns, multiple factors need to be considered. This paper will provide a brief overview of antimicrobial use in agriculture, the development and dissemination of resistance, and alternatives to antibiotics.

Types of antimicrobial use in farm animals

Antimicrobials are readily used for animal production to treat and prevent disease, and to promote growth. For treating disease, they are administered at therapeutic doses to animals displaying symptoms of bacterial infection. Disease prevention can be separated into two categories. The first is prophylactic use, which is the treatment of healthy animals in order to prevent disease at times of high risk. Examples of this type of use include dry cow treatment between lactations and administering antimicrobials to piglets at early weaning (Aarestrup, 2015) or after surgical procedures. The second type of disease prevention is metaphylactic use, in which antimicrobials are administered at therapeutic concentrations to healthy animals that are part of a group displaying disease. This is done to prevent an outbreak and mitigate infection. In the North American beef industry, metaphylactic antimicrobials are often used for high-risk cattle entering feedlots to control respiratory disease. For example, in western Canada, 20 to 50% of newly arrived feedlot placements receive injectable metaphylactic antimicrobials for respiratory disease prevention (Checkley et al., 2010) while 75% of U.S. feedlots with capacities of >8,000 head use injectable antimicrobials at arrival (USDA, 2012).

Antimicrobials have also been used for growth promotion. Used in this way, they are generally given at subtherapeutic concentrations in the diet, either at specific times of high disease incidence, or on a continuous basis throughout production to improve performance. This type of use began in the 1940's after it was discovered that the addition of waste by-product from tetracycline to animal diets improved production (Aarestrup, 2015) and early research showed enhanced growth in poultry fed tetracycline (Whitehill et al., 1950) and swine fed penicillin (Speer et al., 1951). While studies have shown increased performance from antimicrobial growth promoters ranging from 2-40% (Hao et al., 2014), the mode of action is not entirely known and long-term studies on their efficacy for promoting growth under modern agricultural practices are few. One hypothesis is that subtherapeutic antimicrobials alter the gastrointestinal microbiota to improve feed utilization and this is true for ionophores which have been shown to reduce methane emission (Guan et al., 2006). However, there is less evidence for other classes of antimicrobials. It is more likely that they act by limiting infection, which can reduce performance, either directly or indirectly through immunomodulation (Costa et al., 2011) or altering commensal bacteria (Collier et al., 2003). Indeed, certain antimicrobials are marketed for subtherapeutic application to control disease. For example, both infeed tetracycline and tylosin are indicated to reduce liver disease in cattle, which is caused by the pathogens *Fusobacterium necrophorum* and *Actinomyces pyogenes*. However, their effectiveness appears to be limited to high-risk cattle (Stanford et al., 2015). Similarly, the advantages of in-feed antimicrobials in healthy chickens may have limited efficacy (Diarra and Malouin, 2014). This suggests that management practices are a key component to reducing the use of subtherapeutic antimicrobials.

Development of resistance

Antimicrobial resistance is common in bacterial communities and serves as a method of competition. Certain bacteria will produce antimicrobials to inhibit other microorganisms and will also encode

resistance genes to be immune to their effects. This type of community interaction is ancient and has existed before antibiotic use by humans. Evidence to support this include the detection of resistant genes in 30,000 year-old permafrost sediment (D'Costa et al., 2011) and more recently, in the microbiota of Yanomami subjects in the Amazon, who have been isolated for more than 11,000 years (Clemente et al., 2015). The latter case highlighted that bacteria resistant to natural and even synthetic antimicrobials were prevalent in humans prior to the antibiotic era. However, what differs from now and the past is the abundance of selection pressure from anthropogenic antimicrobial use. This has caused a widespread increase in the number of resistant bacteria around the world.

In 1976, one of the first empirical studies was published that showed in-feed subtherapeutic tetracycline led to an increase in antimicrobial-resistant bacteria in both poultry and animal handlers (Levy et al., 1976). Numerous studies have been published since then that link antimicrobial use to the development of resistance in livestock (Katsunuma et al., 2007; Akwar et al., 2008). Recently, Chantziaras (2013) and colleagues compared antimicrobial use in seven European countries and resistance in *Escherichia coli* isolated from pigs, swine and poultry. They found that on-farm antimicrobial use highly correlated to resistance in *E. coli*, with coefficients of determination of 0.99 for fluoroquinolones and amphenicols, 0.94 for third-generation cephalosporins and sulphonamides, 0.80 for gentamycin and tetracycline.

Most resistance studies have focussed on isolated bacteria within the genera *Escherichia*, *Enterococcus*, *Campylobacter*, and *Salmonella* (Marshall and Levy, 2011). While viable indicator bacteria provide useful baseline resistance data, the capacity for bacteria to transfer or acquire antibiotic resistance genes stresses the importance of considering the total level of encoded resistance in a bacterial community. Increasingly, research utilizing molecular tools to analyze resistance in uncultured microbiota are being used. Metagenomic analysis of the swine gut microbiota showed that after in-feed administration of chlortetracycline, sulfamethazine, and penicillin, the bacterial community shifted after 14 days of antimicrobial use (Looft et al., 2012). Compared to control animals that received no antimicrobials, there was an increase in both abundance and diversity of resistance genes in treated animals. An increase in resistance genes that were unrelated to the classes of antimicrobials administered was also observed, highlighting that indirect selection occurred. Similarly, Kanwar et al. (2015) reported that chlortetracycline can co-select for genes encoding cephalosporin resistance in cattle. They also noticed that using a PCR-based metagenomics approach to analyze resistance can lead to differences compared to culture-based data. This implies that monitoring resistance in indicator bacteria may bias results and that the total resistance in microbial communities is important to understanding the impact of antimicrobial use in animals.

In-feed antimicrobials may also select for resistance in extra-intestinal bacterial. Administering tylosin to pigs resulted in a rapid increase in erythromycin-resistant fecal enterococci and also a gradual increase in resistant *S. hyicus* from skin swabs (Aarestrup and Carstensen, 1998). In contrast to this, tylosin did not affect resistance in nasopharyngeal *Staphylococcus* when fed to cattle (Zaheer et al., 2013), although it did lead to increased number of erythromycin-resistant enterococci in feces. In the study by Zaheer and colleagues (2013), they also investigated the effects of the therapeutic antimicrobials tulathromycin and tilmicosin. Subcutaneous injection of these antimicrobials had no effect on resistance in nasopharyngeal bacteria but like tylosin, caused and increase in the number of fecal erythromycin-resistant enterococci. The differences in these studies highlight the complexity of resistance and show how animal species may affect resistance. Similarly, rates of avilamycin resistance were shown to be higher in *Enterococcus* sp. from poultry than those in swine despite use in both species (Marshall and Levy, 2011).

In addition to species and antimicrobial use, other factors can affect resistance in food animals. For example, in evaluating the effects of subtherapeutic antimicrobials on resistance in *E. coli* from beef cattle, it was observed that more than 40% of steers shed tetracycline-resistant *E. coli*, before ever being exposed to antimicrobials (Alexander et al., 2008). In that same study, ampicillin-resistant isolates were obtained from approximately 10 to 20% of the steers within each treatment before

feeding antimicrobials. Presumably, the majority of these resistant *E. coli* would have been acquired from the range environment in which the calves were raised, either from their dams or other environmental sources. Diarra and Malouin (2014) also observed multi-drug resistant enterococci in day-old chicks and feed rations, emphasizing that the environment can be a source of contamination.

Apart from being a source of resistant bacteria, dietary factors have been implicated in the development of antimicrobial-resistant *E. coli* populations in ruminants. A non-medicated dietary supplement fed to dairy calves increased the prevalence of *E. coli* resistant to streptomycin, sulfadiazine, and tetracycline (Khachatryan et al., 2006) and changing from a forage- to concentrate-based diet increased prevalence of tetracycline-resistant *E. coli* in feedlot cattle (Alexander et al., 2008). In addition, indirect effects of the environment, such as cold stress, have been related to the increased prevalence of tetracycline- and ampicillin-resistant *E. coli* strains in swine, an effect that may have been attributable to changes in the level of feed consumption (Moro et al., 1998). These effects need to be taken into account when investigating resistance in farm animals.

Transfer of resistance

The main concern regarding antimicrobial use in food animals is the development and spread of resistance to humans. This remains a point of controversy still today, as there is a lack of studies measuring the risk associated with antimicrobial-resistant bacteria from agriculture. There is however evidence that bacteria from food animals colonized humans. Whole genome sequencing has shown that transfer of *S. aureus* and *Salmonella* occurred from livestock to humans in the past (Woolhouse et al., 2015). The transfer of resistant pathogens or commensals from food animals to humans can occur through direct animal contact and consumption of contaminated food, as well as indirectly from environmental sources contaminated with agricultural waste.

Foodborne outbreaks with bacteria conferring multi-drug resistance genes have been described (Djordjevic et al., 2013) and confirm the potential for transmission of resistant bacteria to humans. In addition to pathogens, transfer of antimicrobial-resistant commensals is a concern. Bacteria have multiple ways to transfer resistance genes and therefore commensals may act as a reservoir of resistance and have been shown to transfer resistance elements in the intestinal tract and food products (Economou and Gousia, 2015). The detection of antimicrobial-resistant bacteria in abattoirs (Aslam et al., 2009) and commercial meat products (Schroeder et al., 2003) has been reported. One study genetically profiled *E. coli* in a beef packing plant and found multiple sources of resistant *E. coli*, with few genotypes being shared across contaminating sources (Aslam and Service, 2006). There are fewer studies however that have reported direct links between resistant bacteria harboured by livestock and those entering the food supply. Alexander et al. (2010) investigated *E. coli* in beef cattle that were administered subtherapeutic antimicrobials from the farm through processing at an abattoir. They observed that compared to control animals given no antimicrobials, the prevalence of ampicillin- and tetracycline-resistant *E. coli* was greater in feces of cattle administered tetracycline. Interestingly, no other samples, including the hide, carcass, and ground beef were different between the two groups of animals. The study showed that resistant *E. coli* can contaminate meat at the abattoir and enter the food chain regardless of whether cattle were administered antimicrobials.

A critical factor in the dissemination of antimicrobial-resistant bacteria is persistence in agricultural-related matrices. As described above, fecal material from food animals is a diverse source of resistant bacteria and genes and remains a source of resistance for months after defecation (Alexander et al., 2011). Additionally, up to 75% of ingested antimicrobials have been estimated to be excreted in fecal and urine waste of livestock, presenting a continued source of selection pressure on bacteria (Chee-Sanford et al. 2009). Applying manure to land is an economically viable method to manage animal waste and minimize the need to purchase mineral fertilizers for crops. While strict rules apply to manure management in order to safeguard water supplies, bacteria from fecal material can be transferred in runoff water (Schuster et al., 2005).

Numerous studies have shown that application of manure to land increases the level of resistant genes and bacteria in soil (Jechalke et al., 2014). Recently, it was reported that application of dairy cow manure enhanced the proliferation of resident antibiotic-resistant bacteria and genes encoding beta-lactamases in soil (Udikovic-Kolic, et al., 2014). This effect was observed whether the manure originated from cows administered antimicrobials or not. Thus manure can alter resident soil bacteria and increase background levels of resistance through mechanisms that are not related to agricultural antimicrobial use. Marti and colleagues (2013) have shown that resident soil bacteria can also be a source of antimicrobial resistance contaminating vegetables. They tested the effect of manure fertilization on the abundance of resistant bacteria and frequency of resistance genes in soil and vegetables. While manured soil showed an enrichment of antimicrobial-resistant bacteria and resistant determinants, there was no effect on the level of resistance in vegetables. Regardless of manuring, genes encoding resistance and transferrable elements were in soil and vegetables.

Mobile genetic elements are increasingly recognized as important factors in the emergence and horizontal spread of resistance genes. It is especially concerning when mobile elements encode multiple resistance genes because it can allow for co-selection of resistance elements. Integrons are natural genetic elements that have specific structures and abilities to capture or excise one or more resistance gene cassettes by site-specific recombination. They have been detected in food-producing animals and there is some evidence to show that subtherapeutic antimicrobials can cause an increase in their prevalence in *E. coli* (Wu et al., 2011). One of the more important families of mobile genetic elements in bacteria is integrative conjugative elements (ICEs). These elements are unique in that they are self-transmissible, encoding all of the mechanics required for integration, excision and transfer (Wozniak and Waldor, 2010). Acquisition of ICEs allows bacteria to rapidly adapt to changing environmental conditions and colonize new niches (Burrus and Waldor, 2004) by mediating the transfer of accessory genes that alter phenotype, including antimicrobial resistance and virulence (Wozniak and Waldor, 2010). As a result, they are key contributors to bacterial pathogenicity and the shaping of bacterial genomes (Burrus and Waldor, 2004). They have also been shown to acquire and transfer multi-drug resistance in bacteria from livestock. In beef cattle mortalities, isolates of the respiratory pathogens *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* were observed to possess ICEs that conferred resistance for up to seven different antimicrobial classes and all the major antibiotics used to treat respiratory disease (Klima et al., 2014). ICEs were also shown to be transferred via conjugation from *P. multocida* to *E. coli* and from *M. haemolytica* and *H. somni* to *P. multocida*. The ICEs encoded resistance genes to oxytetracycline, tulathromycin, and tilmicosin, all which are used for treatment and metaphylactic control of respiratory disease in feedlots. The impact of respiratory bacteria carrying these ICE elements is not fully known but they could be a major detriment to current antimicrobial strategies used to control respiratory illnesses in cattle. Thus resistance is a concern not only for human, but animal health as well.

Limiting antimicrobial resistance

Methods to limit the amount of antimicrobial resistance from food-producing animals include: i) banning the use of subtherapeutic antimicrobials, ii) implementing proper management strategies, and iii) utilizing alternatives to antimicrobials. Realistically, multiple approaches are needed to reduce antimicrobial use without impacting animal production.

As mentioned before, few studies have investigated the effects of subtherapeutic antimicrobial growth promotion on modern agricultural practices. In 1999, poultry and pig producers in Denmark voluntarily banned use of antibiotics for growth promotion and in 2006, this type of use was banned in Europe. In Denmark, mortality in swine initially increased and production decreased, but this trend reversed five years after the band. This was attributed to altering pig management strategies (Aarestrup, 2015). In a study investigating prophylactic antimicrobial use in dairy calves, it was found that implementing daily health evaluations and limiting therapeutic treatment to only calves displaying disease was as effective as complete herd treatment (Berge et al., 2009). It not only led to reduced antimicrobial use but also a $10 per head savings. Stanford et al. (2015) also showed that

subtherapeutic antimicrobials in low-risk beef cattle did not improve health and production parameters, suggesting that management practices reducing animal stress could be an alternative to in-feed antimicrobials. Combined, there is merit in how farmers can adopt methods to reduce subtherapeutic antimicrobial use. However, banning subtherapeutic use may lead to an increase in other types of antimicrobials. In Europe, the ban on growth promoters did not lead to a consistent decrease in total antimicrobial consumption, as an increase in metaphylactic and prophylactic use occurred (Woolhouse et al., 2015). This has also led to an increase in higher-risk antimicrobials being used in animal production. Therefore, it is critical to have surveillance that properly accounts for both antimicrobial use and levels of resistance in agriculture. This is necessary when evaluating any changes to antimicrobial practices, in order to evaluate the impact of the modifications.

Managing waste is also important to limiting the spread of antimicrobial resistance elements from agricultural. While manure is an important source of resistant bacteria and can lead to greater levels of resistance after being applied to soil, it has been shown that the effects are temporary and baseline resistance returns to normal levels less than one year after application (Marti et al., 2014). Thus properly scheduling the application of manure to land can help limit the burden of resistance. In addition, processing of manure can significantly reduce the level of resistance genes prior to land application. For example, composting is an effective method to reduce pathogen load in manure and also resistant bacteria. We have seen a 100-fold reduction in certain resistance genes throughout the 90-day composting of cattle manure (unpublished).

Most research on alternatives to antimicrobials has been on novel vaccines, plant secondary chemicals, bacteriophages, prebiotics and probiotics. Without changing management practices, viable alternatives to metaphylactic antimicrobials have yet to be developed (Ribble et al., 2010). However, advances in DNA sequencing technology may help overcome previous limitations. For example, genome sequencing is being used for reverse vaccinology to identify novel antigen candidates and metagenomics studies are helping to elucidate the mechanism of antimicrobials on gastrointestinal microbiota in an effort to find alternatives with similar effects. This information can be used for the targeted development of alternatives, which has been a challenge in the past. We have recently described the bacterial microbiota in calves that develop respiratory disease and those that are healthy (Hollman et al., 2015). From that study, we identified bacteria common to healthy calves and have since isolated them and found that they can inhibit respiratory pathogens and immuno-modulate bovine epithelial cells (unpublished). It is hypothesized that these bacteria may be useful as probiotics to reduce metaphylactic antimicrobial use in cattle. Thus the sequencing technologies, which are becoming faster and cheaper, are advancing the field of targeted therapies to reduce antimicrobial use in food-producing animals.

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Prevalence and Impacts of Genetically Engineered Feedstuffs on Livestock Populations

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Take-Home Messages

- Soy and corn, two major components in commercial animal feed, make up two-thirds of global grain trade. The main countries that grow and export soybeans and corn are the U.S., Brazil and Argentina, all of which grow significant quantities of genetically engineered (GE) varieties of both crops.
- In the US alone, more than 95% of sugar beet, 93% of soy and 90% of cotton and corn acreage are planted with GE varieties. The largest consumers of these crops are livestock populations, namely chickens, pigs and cattle.
- Hundreds of controlled, peer-reviewed animal feeding studies, including long-term and multigenerational studies, have shown that that the performance and health of GE-fed animals are comparable to animals that are fed near-isogenic, non-GE lines.
- As a result of the rapid increase in GE adoption rates since 2000, it can be predicted that the vast majority of food animals in the U.S. consumed GE-derived feed in the past decade. Collectively, this totals more than 100 billion food animals (mostly broiler chickens) that have consumed some level of GE feed between 2000 and 2011.
- Commercial livestock productivity and health data from publicly-available databases for a period before the introduction of GE crops in 1996 and through 2011, a period with high levels of GE crops, do not reveal unfavorable or perturbed trends in the performance or health of livestock that have been raised on diets containing predominately GE feed.
- There is no method to detect whether an animal has been fed using GE feed based on testing their milk, meat, and/or eggs which presents source verification challenges for proposals to require mandatory labeling of such products.
- “Zero-tolerance” policies for crops approved for cultivation in exporting countries and unapproved for food and feed import in export markets are causing trade disruptions.
- Numerous GE crops that are enhanced for animal nutrition are far advanced in the regulatory pipeline. The approval of these so-called “second generation” GE crops will further complicate the sourcing of non-GE feedstuffs as well as present regulatory and commercialization challenges.
- There is an urgent need for international harmonization of both regulatory frameworks for GE crops and governance of advanced breeding techniques to prevent recurrent disruptions in international trade of livestock feedstuffs in the future.

The media has given extensive coverage to a handful of highly controversial studies that claim deleterious health effects in a small number of animals that have consumed “genetically modified (GM) feed. These studies, despite being widely criticized for experimental design and methodological flaws, in combination with anecdotal stories on the internet, have been used to suggest that there is a need to label milk, meat and eggs from animals that are fed GM crops.

Since their introduction in 1996, GM or more accurately “genetically engineered (GE)” feed crops have been widely adopted. Genetic engineering is a breeding method that enables the manipulation of an organism’s genes by introducing, eliminating or rearranging specific genes using the methods of modern molecular biology, particularly those techniques referred to as recombinant DNA (rDNA) techniques. In the US alone, more than 95% of sugar beet, 93% of soy and 90% of cotton and corn acreage are planted with GE varieties. The largest consumers of these crops are livestock populations, namely chickens, pigs and cattle.

Globally, multiple generations of food-producing animals have been consuming 70-90% of GE crop biomass for almost 20 years. Numerous carefully-conducted studies have shown that GE crops are compositionally equivalent to non-GE crops and no significant differences have been detected in animals that are fed GE feed in terms of performance or health. A recent review by Van Eenennaam and Young (2014)[1] summarizes published animal feeding studies and commercial livestock productivity and health using data from publicly-available databases for a period before the introduction of GE crops and through 2011, a period with high levels of GE crops. Overall, the results did not reveal unfavorable or perturbed trends in the performance or health of livestock that consume GE feed.

GE feeding studies in livestock

Before a GE crop event is approved, it undergoes risk analysis governed by the Codex Alimentarius Commission’s international guidelines. New GE varieties are tested for substantial equivalence; that is, they are compared to an equivalent, conventionally-bred variety that has a known history of safe use. To date, all 148 GE transformation events evaluated by the USDA have been found to be substantially equivalent to their conventional counterparts[2]. Despite the fact that many toxicologists agree that animal feeding trials of whole GE food do not have much power with respect to the safety assessment of whole foods, the EU now mandates 90-d subchronic toxicity rodent feeding studies for each GE transformation event.

In contrast, studies in which GE crops are fed to food-producing animals have focused more on evaluating nutrition, performance and health. The International Life Sciences Institute has developed clear guidelines for experimental design of these types of studies which to date have included sheep, goats, pigs, chickens, quail, cattle, water buffalo, rabbits and fish that have been fed different GE crop varieties. Recent studies conforming to these international guidelines support conclusions made a decade ago that the performance and health of GE-fed animals are comparable to animals that are fed near-isogenic, non-GE lines and commercial varieties. The Federation of Animal Science Societies (FASS) maintains an extensive bibliography of food-producing animal GE feeding studies including poultry (http://www.fass.org/page.asp?pageID=52).

Additionally, long-term and multigenerational studies have been conducted with pigs, cows, quail and fish. Two thorough, publicly-funded, multigenerational studies, one in dairy cattle and one in pigs, using a specific variety of GE corn, are particularly notable since they included appropriate controls that consumed isogenic non-GE lines of corn, comprehensively examined phenotypes and used state-of-the-art techniques to determine the presence or absence of recombinant DNA and protein. Results revealed no differences in composition or nutrition of the GE corn variety compared to the control and did not show any long-term adverse effects on the animals due to consumption of GE corn. In addition, test results indicated no recombinant DNA and protein were present in the blood, organs and products of GE corn-fed animals[3-9].

Although it may seem informative to conduct more long-term and multigenerational target animal GE feeding studies, the data observed in such studies to date have not revealed enhanced sensitivity as compared to endpoints examined in 90-day rodent feeding studies. The absence of novel, useful data comes at the cost of time, expense and animal experimentation. Long-term studies have not uncovered new effects that were not seen in short-term rodent studies. Therefore, long-term and

multigenerational feeding studies would only appear justified when a potential compositional hazard exists in the GE crop and some reasonable doubt remains following a 90-d rodent feeding trial.

GE-fed livestock populations: Field datasets

In addition to the aforementioned long-term food animal GE feeding studies, livestock worldwide have been consuming GE feed for more than 15 years, resulting in a large and powerful publicly-available dataset. In 2011, less than 5% of the animals in each of the major U.S. livestock sectors were raised under National Organic Program (NOP) certification (which prohibits use of GE feed). As a result of the rapid increase in GE adoption rates since 2000, it can be predicted that the vast majority of food animals in the U.S. consumed GE-derived feed in the past decade. Collectively, this totals more than 100 billion food animals that have consumed some level of GE feed between 2000 and 2011.

Although the duration and level of GE feed exposure is presumed to vary depending on the animal industry, affected by factors including the animal species, production system, management practices, feeding stage and relative feed prices, it is reasonable to hypothesize that any deleterious effects resulting from consuming GE feed on this scale would have resulted in discernible negative impacts on animal performance and health attributes. Productivity metrics of the different livestock industries are routinely assessed by the USDA, and all animals that arrive at USDA-inspected slaughter facilities are subject to antemortem and postmortem inspections by veterinarians. Visibly ill animals and carcasses with lesions or tumors present are documented, condemned and excluded from the food supply. These publicly-available data sets (USDA Economics, Statistics, and Market Information System, USDA Food Safety and Inspection Service, National Chicken Council, and the National Non-Fed Beef Quality Audit) on broiler, dairy, hog and beef health in the U.S. were compared for the period 1983 through 1994, which represented a period of time with no GE feed, and 2000 through 2011, a period with very high levels of GE feed.

The results of these analyses showed no unfavorable or unexpected trends in production and health parameters, for any livestock industry over time. In fact, improvements were seen for the available health parameters of somatic cell count (an indicator of mastitis and udder inflammation in dairy cows), postmortem condemnation rates in cattle and poultry, and mortality rates in poultry (**Figures 1 and 2**).

These data are in agreement with the many peer-reviewed, controlled animal feeding studies that have reported no biologically-relevant differences between the nutritional attributes and safety of feed from GE plants as compared to feed derived from conventional crop varieties. The publicly-available livestock data show no indication of worsening animal health subsequent to the introduction of GE feed and improvements in productivity continued in the same direction and at similar rates during both time periods.

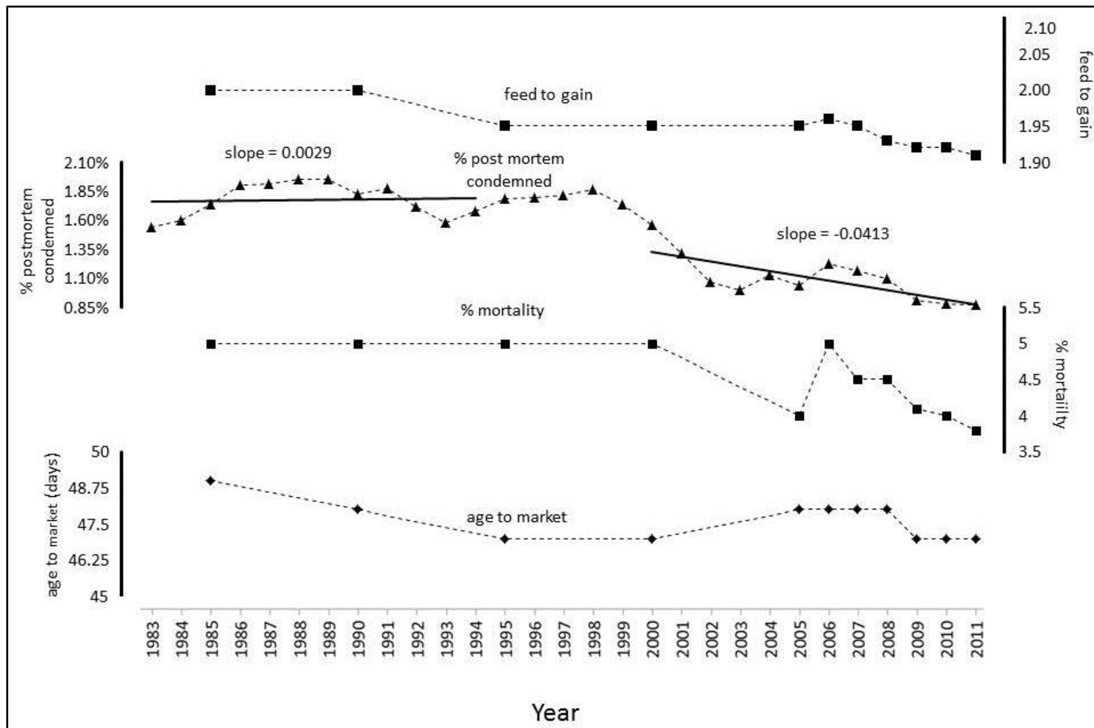

Figure 1. US broiler statistics prior to and subsequent to the introduction of GE crops in 1996[1]. Sources: USDA National Agricultural Statistics Service, 2013; National Chicken Council, 2011. Slope differs between time periods 1983-1994 and 2000-2011 (*P < 0.05).

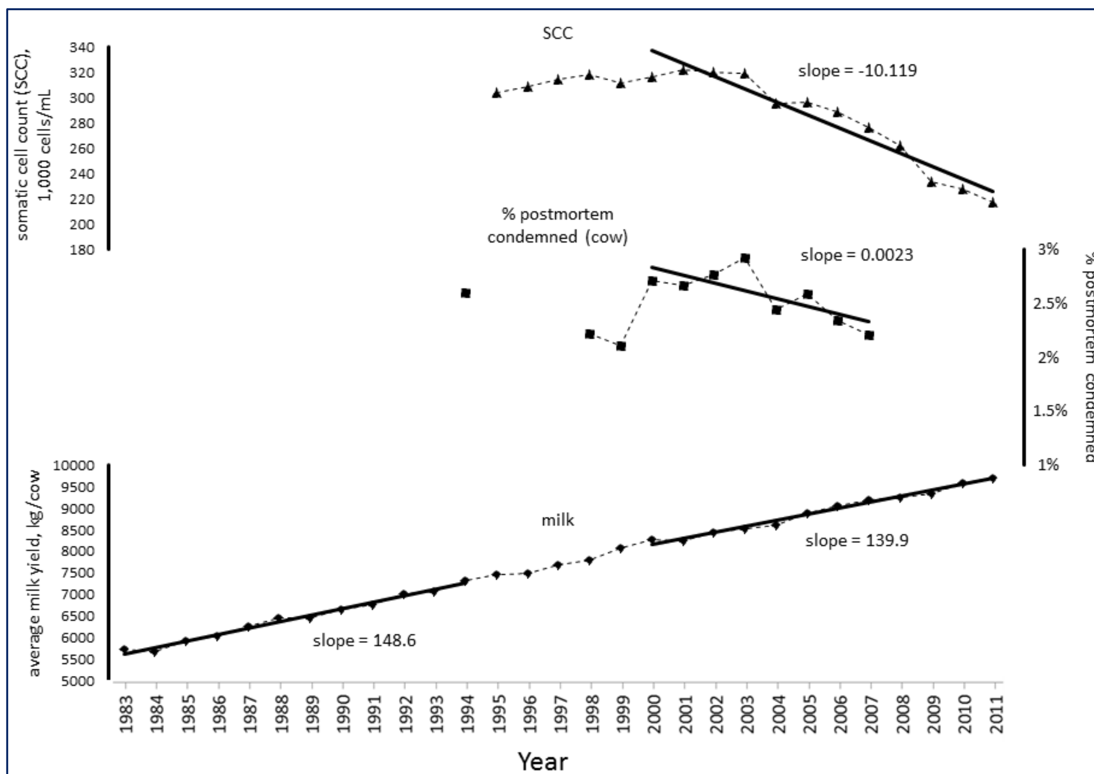

Figure 2. Milk production, percent post-mortem condemned and somatic cell counts for the US prior and subsequent to the introduction of GE crops in 1996. Sources: USDA National Agricultural Statistics Service, 2013; USDA Food Safety and Inspection Service, 2013; Slope does not differ significantly between time periods 1983-1994 and 2000-2011.

Data on recombinant DNA/protein in animal products

Although it is not possible to detect differences in nutritional profiles in products derived from GE-feed-consuming animals and no reliable traces of GE components have been detected in meat, milk, or eggs from those animals, some proposed state GE labeling laws would require mandatory labeling of these products. Currently, the major global livestock feed exporters are the countries that cultivate GE corn and soy; sourcing non-GE feed crops is problematic due to low supply and expense. As a result, there are a relatively small number of livestock producers that feed non-GE diets. Consequently, more than 95% of milk, meat and eggs currently on the market in the United States would require labeling.

Animals and humans regularly digest DNA, RNA and protein without any adverse consequences. DNA from GE crops is chemically equivalent to DNA from non-GE crops and studies have shown that it is broken down in the same way during digestion. Consequently, neither recombinant DNA nor protein from GE feed crops is detectable in milk, meat, or eggs from animals that have consumed GE feed. The absence of detectable traces of recombinant DNA and protein is especially important when it comes to the prospect of labeling animal products such as milk, meat and eggs.

In the absence of any way to analytical test products to see if they came from animals that had consumed GE-containing feed, labeling of animal products derived from GE-fed livestock would have to be based on documenting the absence of GE crops in the production chain. This would require identity preservation and supply chain segregation constraints for producers and importers. Finished products in this tracking system, which would effectively be segregating indistinguishable foodstuffs, could not be tested to guarantee the absence of milk, meat, and eggs from animals that might have eaten GE feed[10].

With respect to mandatory labeling, some countries, such as Australia, New Zealand and Japan, target the presence of detectable traces of GE components in the finished product. In other countries, namely the EU, Brazil and China, regulations target foods that use GE technology as part of the production process. It is interesting that even in GMO-adverse Europe, where animal agriculture is heavily dependent on imported GE soybeans as a feed protein source, no tracking or labeling of food products from animals that have consumed GE feed is required. Brazil is the only country that currently requires labeling of products from animals that consume GE feed, but the law governing this labeling has yet to be fully implemented.

There are additional costs associated with sourcing product from non-GE fed animals, and that choice is currently available through the organic supply chain. Additionally, a voluntary, process-based label that allows companies to label products that meet the Non-GMO Project's standard (<0.9% tolerance for GE presence) for the avoidance of GE feed in the diet of animals producing meat and liquid egg products was approved in 2012 by the USDA's FSIS. Such voluntary, process-based label claims are allowable provided that they are truthful, accurate, and not misleading. The requirements of these programs are supported by documented quality management systems.

GE and non-GE feedstuffs: Global production and trade

Soy and corn, two major components in commercial animal feed, make up two-thirds of global grain trade. The main countries that grow and export soybeans and corn are the U.S., Brazil and Argentina, all of which grow significant quantities of GE varieties of both crops. The EU does not grow GE soybean varieties and is heavily reliant on soybean imports for feed, a significant proportion of which is GE. Corn is the second largest category of GE products imported into the EU after soy, although EU corn production is generally sufficient to meet consumption needs, with imports accounting for only 10% of the total supply.

World grain markets fall into one of four categories: conventional (non-GE grain that is not certified), mixed (GE and conventional undifferentiated), identity-preserved (certified non-GE) and organic. It is estimated that 4% of global soybean trade and 7% of global corn trade is required to be identity-preserved, certified, non-GE. Reliance on imported feed, as in the EU, is becoming increasingly complicated for countries looking to source non-GE products due to the high GE adoption rate in the major feed exporting counties (**Table 1**). The EU's stringent tolerance levels for low level presence (LLP) are complicating the maintenance of non-GE supply chains and some countries that previously committed to source only non-GE feed for certain sectors have had to abandon those plans.

An additional complication in worldwide grain commerce is trade disruptions that arise due to asynchronous approvals. This occurs when approvals for the cultivation of GE varieties in an exporting country occur in advance of import approvals in another. Thirty-three countries currently have regulatory systems that handle approval for the cultivation or importation of new GE crops. There are considerable discrepancies in the amount of time required to review and approve new GE crops in different countries. Notably, this issue has resulted in the removal of the U.S. as the main supplier of corn to the EU and has caused trade disruptions between the EU and other countries, such as Argentina. Demand for EU corn imports has consequently been concentrated on Brazil and feed prices have increased in turn for EU feed producers.

Table 1. Share of global crop trade accounted for by genetically engineered (GE) crop production. 2012/13 (Million Metric Tonne)[1].

Variable	Soybeans	Corn	Cotton	Canola
Global production	266	862.9	26.8	62.6
Global trade (exports)	97.2	100.1	10.0	12.0
Share of global trade from GE producers	94.6 (97.3%)	71.3 (71.2%)	6.9 (69%)	10.2 (85%)
Estimated size of market requiring identity preserved (certified non-GE) market (in countries that have import requirements)*	4.0-4.5	7.3	Neglig.	0.1
Estimated share of global trade that may contain GE (i.e. not required to be segregated)	90.1-93.2	64-92.8	6.9	10.1
Percentage of global trade that may be GE	92.75-95.9%	64-92.7%	69%	84.2- 85%

China, the sixth largest corn importer, has rejected corn shipments from the U.S. due to asynchronous approvals, despite regulatory requests for food and feed import approval of those crops. China has a zero-tolerance policy for adventitious presence (AP) of unapproved events, meaning that even minute traces of unapproved GE crops in exports are illegal and must be withdrawn from the market. Under a zero tolerance policy, trade of relevant commodities between countries with asynchronous regulatory approvals will likely cease as importing and exporting firms will act to avoid the risk associated with a positive test of an unapproved event in an export market[11]. Countries with zero tolerance policies will be perceived as risky export markets, and importers will pay higher prices and insurance premiums to offset risks taken by the supplier. The realities of agricultural production systems, in which harvesting and storage facilities are shared, means that trade disruptions due to "zero-tolerance" policies for crops approved for cultivation in exporting

countries and unapproved for food and feed import in one or more export markets are virtually unavoidable.

In the U.S., producers who want to purchase non-GE feed for their livestock generally contract with growers or source identity-preserved (certified non-GE) or organic feed. However, the availability and cost of certified organic feeds is a major challenge for U.S. organic livestock producers. Organic producers can source organic feed from other countries, and recently China and India have expanded their organic soybean exports to the U.S., although improved data collection is necessary to better understand whether these organic soybean imports are being used for food or animal feed.

In the U.S., less than 5% of the market is made up of products derived from animals raised in organic production systems (**Table 2**).

Mandatory labeling of products derived from animals that have consumed GE feed would currently require labeling greater than 95% of all animal products on the supermarket shelf. If the market response is to increase the proportion of products from animals fed non-GE feed, an increase in the non-GE feed supply would be required. Shifting to non-GE feed would be associated with higher feed costs, which typically make up 70% of animal production costs, and these costs would be reflected in higher priced milk, meat and eggs from animals fed non-GE feed.

Table 2. Organic livestock production statistics in U.S. (2011)[1].

Industry	Number of organic farms in U.S.	Number of animals on organic farms	Total number of livestock animals in U.S.	Organic livestock numbers as % of U.S. Total
Broiler	153	28,644,354	8,607,600,000	0.33%
Layers	413	6,663,278	338,428,000	1.97%
Turkeys	70	504,315	248,500,000	0.20%
Beef cows	488	106,181	30,850,000	0.34%
Dairy cows	1,848	254,711	9,150,000	2.78%
Hogs	97	12,373	110,860,000	0.01%

Sustainability, second generation crops and the future

Projected increases in global milk and meat consumption will require increases in feed production to support the additional animal numbers necessary to meet demand. GE crops, to date, have increased the global production of soybeans and corn since the 1990s, especially in resource-poor developing countries. A 2014 meta-analysis[12] of the impacts of GE crops concluded that on average, "GM technology adoption has reduced chemical pesticide use by 37%, increased crop yields by 22%, and increased farmer profits by 68%. Yield gains and pesticide reductions are larger for insect-resistant crops than for herbicide-tolerant crops. Yield and profit gains are higher in developing countries than in developed countries". Despite the fact that some weed resistance has arisen as the result of poor pest management practices and overreliance on a single herbicide, the adoption of GE technology has had a positive sustainability outcome in terms of increased global yield and reduced environmental impacts due to reduced insecticide use and the adoption of no-till agricultural practices. Reverting back to the use of non-GE crop technologies would have significant negative environmental and economic consequences.

Numerous GE crops that are enhanced for animal nutrition are in the research and development pipeline in many countries[13]. GE offers new possibilities for raising crop yield per hectare and improving the rate of conversion of vegetable calories into animal calories as well as improving the nutritional value of feed, lowering nitrogen and phosphorus pollution and reducing manure excretion. Some of these crops are far advanced in the regulatory pipeline. The approval of these so-called "second generation" GE crops will further complicate the sourcing of non-GE feedstuffs as well as

present regulatory and commercialization challenges. In addition, ongoing developments in precise gene-editing technologies that enable targeted editing of specific nucleotides will further complicate the situation in terms of regulation and testing since there would often be no way to differentiate a gene-edited DNA alteration from a naturally-occurring (i.e. spontaneous) mutation, and therefore no way to trace and track gene-edited crops, or differentiate them from genetic modifications resulting from spontaneous mutations.

Recent issues arising from asynchronous regulatory approvals have emphasized a critical need for productive dialog on a global scale to prevent widespread trade disruptions now and in the future. The development of "second generation" and precision-edited crops will further challenge global regulatory agencies as these technologies progress and become more widely utilized. Given these developments, there is an urgent need for international harmonization of both regulatory frameworks for GE crops and governance of advanced breeding techniques to prevent recurrent disruptions in international trade of livestock feedstuffs in the future.

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Antibodies for Healthy Gut Function

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TAKE HOME MESSAGE

The gastrointestinal tract is colonized by trillions of microorganisms termed the microbiome. These organisms have pivotal roles in the immunological, metabolic, nutritional, and physiological functions of the host and help keep the body in immune homeostasis, immune balance. The gastrointestinal tract is at the major intersection of the immune system and microorganisms. Antibodies, immunoglobulins, are large proteins produced by the gut-associated lymphoid tissues (GALT) found in the lining of the intestines. These antibodies help limit the growth of gut microorganisms, and pathogens that enter via the oral route. When the specificity or levels of immunoglobulins produced by the intestines is in insufficient quantities to protect the young, administration of commercially-available antibodies may be warranted.

MICROBIOME

The gastrointestinal tract (Bauer et al., 2006) and skin (Oh et al., 2014) of animals are colonized by trillions of dynamic ecosystems of microorganisms (Bauer et al., 2006). In totality it is estimated that these organisms express 10-fold more genes than does their host's genome. (Ley et al., 2006; Belkaid and Hand, 2014*).* As a community, the microorganisms in and on the body are termed the microbiome, and play key roles in the immunological, metabolic, nutritional, and physiological functions of the host (Bäckhed et al., 2005; Wu and Wu, 2012; Scott et al., 2013; Ji and Nielsen, 2015).

The regulatory functions of the microbiome are so vital that the microbiome is considered by some as a separate organ (O'Hara and Shanahan, 2006; Baquero and Nombela, 2012; Ji and Nielsen, 2015).

HOMEOSTASIS OF IMMUNE AND INTESTINAL FUNCTIONS

The intestine is constantly exposed to fluids, foodstuffs, microorganisms, and inorganic (Hooper et al., 2002) and organic materials. Among its many functions are: obtain nutrients, protect the host from infection, and modulate immunological homeostasis of the host (Hooper and Macpherson, 2010; Hooper et al., 2012; Wu and Wu, 2012; Brown et al., 2013; Levesque, 2014).

The lining of the intestine is made up of a single layer of epithelial cells which is highly permeable to permit fluids and nutrients to be transferred to the blood. The gastrointestinal tract has a complex system of immune cells immediately below the epithelium (Tomasello and Bedoui, 2013) that produces antibodies that inhibit the attachment of pathogens to the gut wall and help keep the intestines in microbial and immune homeostasis.

The importance of the gastrointestinal system for defense against orally-acquired infections is supported by the presence of high concentrations of immune cells in the intestines. About 70% of the immune system is represented within the gut-associated lymphoid tissues (GALT) (Jung et al., 2010; Vighi et al., 2010) with 80% of the body's immunoglobulin-producing plasma cells (Vighi et al., 2010) Peyer's patches, residing here as well.

As part of the digestive system's protective mechanisms, when the gut is exposed to antigenic pathogens, the plasma cells will produce immunoglobulins, antibodies, specific to the organisms that stimulated the response. Immunoglobulins, antibodies, are large Y-shaped proteins that bind to the

antigens that initially triggered their production. Immunoglobulins attach to and “mark” the pathogen for destruction by immune factors and cells. Antibodies also inhibit the binding of microbes to the intestinal walls, decreasing the likelihood of infection.

There is constant interaction between the members of the gastrointestinal microbiome, immune networks (Shirkey, et al., 2006; Nicholson et al., 2012; Ji and Nielsen, 2015), and the intestinal epithelium cells (Shulzhenko, et al., 2011; de Vrese and Schrezenmeir, 2007; Goto and Ivanov, 2013). Exposure to gut microflora is essential for the full development of the immune structures in the intestines (Tomasello and Bedoui, 2013), and the microbes also have major influences on the structure and function of Peyer’s patches (Barman et al.,1997) and other immune components of the gut.

Significant bi-directional influences between the microbiota and the immune system (Tomasello and Bedoui, 2013) play pivotal roles in defending the body from infection and mutating cells, and maintaining the host’s immune and physiological homeostasis (Mason et al., 2008; Wu and Wu, 2012; Goto and Ivanov, 2013; Belkaid and Hand, 2014).

Imbalances in concentrations and composition (Belkaid and Hand, 2014) of microbiota affect immunity and local and systemic inflammation in areas distal from the intestines (Ichinohe, et al., 2011; Hand et al., 2012; Belkaid and Naik, 2013; Iida et al., 2013; Belkaid and Hand, 2014). As an example, broad-spectrum oral antibiotic treatment results in changes of the gut flora and influences immunological responsiveness to infection with influenza (Ichinohe et al., 2011).

IMMUNE PROTECTION IN THE NEWBORN

Born agammaglobulinemic, without significant amounts of protective antibodies (Hurley and Theil, 2011), newly born mammals, such as pigs and cows, are vulnerable to infection (Bauer et al., 2006) and other environmental challenges until their own immune systems and microbiome mature.

Initial protection from infection is through the first milk, colostrum, which is passively transferred from the mother (Bauer et al., 2006; Langer, 2009) for the first few days after birth. As the newborn’s immune system matures and continues to nurse, the concentration of immuno-globulins and proteins provided in mother’s milk decreases (Langer, 2009).

Ingestion of colostrum by the young significantly affects development of the gastrointestinal tract (Blum, 2006) and its microbiome (Cabrera-Rubio, et al., 2012). In pigs and cows, for the first 12-24 hours of life, whole antibodies move easily from the intestine into the circulatory system without being digested by enzymes. However within 24-36 hours after birth, “closure” of the digestive system occurs, and intestinal cells become selective in what passes through the gut wall (Staley and Bush, 1985; Blum, 2006; Hurley and Theil, 2011).

Until the newborn’s immune and microbiota has developed sufficiently for it to protect itself, the newly born must have adequate amounts of protective antibodies to meet challenges from invading pathogens. If the antigenic challenge is too overwhelming, or the antibody titers are not sufficient or the specificity of the antibodies not appropriate for the challenge, there may not be enough passively acquired antibody to adequately protect the newborn. Insufficient amounts of antibodies require that the young be cross-fostered onto another mother, or given supplemental antibodies (ThePigSite.com).

HETEROLOGOUS TRANSFER OF PASSIVE IMMUNITY

Typically, immunoglobulin-rich colostrum, hyperimmune milk, or hyperimmune eggs are used in humans and livestock to help the newborn defend itself from pathogenic challenges until its own immune and microbiota systems are mature.

A useful extension of our knowledge of passive transfer of protective immunoglobulins is the opportunity to use immunoglobulins from one host for prevention or treatment of disease in a secondary host.

Decades ago, clinicians orally transferred anti-rotavirus human immunoglobulin to three children with primary immunodeficiency syndromes that were suffering from chronic excretion of gastrointestinal rotavirus. The antibody survived passage through the gut and retained its ability to attach to rotavirus. Antigen-antibody complexes were found in the stool for 4-6d post administration of a single dose. For several days after treatment no viral antigen was detected in stools (Losonsky, et al., 1985).

Significant immune protection can also be transferred orally by using antibodies from one species for another (Hammarström et al., 1994; Kovacs-Nolan and Mine, 2004; Hurley and Theil, 2011). Immunoglobulins are fairly resistant to digestion and maintain their immunological activity (Losonsky, et al., 1985; Vega, et al., 2011). Even when immunoglobulins are enzymatically digested, the Fab2 and Fab fragments are still able to bind to the antigen and continue to have neutralizing properties. (Akita and Nakai, 1998; Carlander, 2002).

Hyperimmune products provide a high level of targeted protection to animals. Such proteins are typically produced by stimulating chickens or mammals multiple times with inactivated bacteria and/or viruses. In response to this massive antigenic exposure, the host produces both specific and non-specific immunoglobulins, along with high concentrations of other biological and immune factors that help maintain host immune homeostasis.

Hyperimmune proteins have been studied extensively in humans and other animals and shown to enhance functioning of joints, digestive and other organ systems (Heckert, et al., 1999; Mine and Kovacs-Nolan, 2002; Larsson and Carlander, 2003; Kelly GS, 2003/4; Schade R, et al., 2005; Hurley and Theil, 2011; Xu et al., 2011; Kramski et al., 2012).

EGG PROTEINS AS A SOURCE OF IMMUNOGLOBULINS AND OTHER IMMUNE FACTORS

The hen passively transfers immunity, nutrients, and growth factors to her chicks by means of the egg.

Eggs have been used since antiquity to enhance animal and human health. One of the oldest works on the medical treatment of donkeys, mules, and horses P. Vegeti Renati Digestorum Artis Mulomedicinae libri (Fischer, 2011) mentions egg as a remedy for diseases.

In human applications, the Roman army traveled extensively and had purpose-built hospitals with physicians trained and influenced by the Greeks. Chronicles suggest that raw eggs from the local areas were used to prevent and treat diseases such as dysentery (Lommatzsch, 1903) as the Roman army moved from territory to territory.

In response to antigenic stimulation of hens, either via environmental exposure to pathogens, or injection of microorganisms, antibodies are concentrated in the yolk, along with a wide-spectrum of bioactive elements which are found both in the white and in the yolk of the egg.

Hyperimmune egg is not only rich in immunoglobulins and nutrients, but it contains a multiplicity of pro- and –anti-inflammatory molecules, interferons, chemokines, anti-viral and anti-bacterial biological factors (Wu et al., 2010). Investigators have calculated that there is a 30x greater concentration of small immune factors in a single egg from a “hyperimmunized” hen as compared to a regular egg.

The discovery that immunoglobulin was detectable in egg yolks of vaccinated hens, as well as in the blood, pre-dates the use of an antibiotics (Klemperer, 1893). Although functionally equivalent to

mammalian IgG (He et al., 2014), the type of immunoglobulin produced by birds was deemed different enough from mammalian antibodies and was thus designated as “IgY” (Leslie and Clem, 1969).

IgY not found in mammals, but is the dominant class of immunoglobulin found in avian egg yolk. It has multiple advantages over mammalian antibodies. For example, mammalian-derived antibodies can trigger inflammatory processes when they interact with rheumatoid factor or complement. IgY does not interact with either of these immunological factors (Gottstein and Hemmeler, 1985; Schade et al., 1991).

Another important advantage of IgY is that for microorganisms, its avidity, i.e., the binding capacity of IgY, is much tighter than the avidity of mammalian antibodies. Additionally, since fats and proteins in egg help protect IgY from enzymatic degradation, chicken immunoglobulins are less susceptible to digestion than bovine antibodies. Also a higher degree of protection is obtained with egg antibodies as compared with the same amount of mammalian immunoglobulins (Ikemori, et. al. 1997).

Despite the fact that chicken egg is an impressive source of antibodies (Tini et al., 2002), egg-derived immunoglobulins are under-utilized for animal and human applications (Kovacs-Nolan J, Mine Y 2004; Carlander et al., 2002; Schade R, et al., 2005; Xu et al., 2011).

Oral administration of egg antibodies may be a natural means to reduce or eliminate the use of antibiotics and control infections from bacteria and viruses (Kollberg et. al. 2003; Larsson and Carlander, 2003; Kovacs-Nolan and Mine, 2004).

A partial list of pathogens against which IgY antibodies have been produced are: *Helicobacter pylori* (Shin et al., 2002; Wang et al., 2014; Yang et al., 2012), influenza (Yang et al., 2014), *Pseudomonas aeruginosa* infections in cystic fibrosis patients (Kollberg et al., 2003), hemorrhagic and bovine and human rotavirus viruses (Yolken et al., 1988; Kuroki et al., 1997; Sarker et al., 2001; Kovacs-Nolan and Mine, 2004; Li et al., 2014),), canine parvovirus-2 (Van Nguyen et al., 2006), infectious bursal disease (Yousif et al., 2006), *Toxoplasma* (Ferreira Júnior et al., 2012) and *Trypanosoma* (Sampaio et al., 2014).

Egg-derived IgY have also been studied as a less arduous means of producing antibodies against bovine and human diarrheas (Yokoyama et al., 1993; Erhard et al., 1993; Ikemori et al., 1997; Kweon et al., 2000; Vega et al., 2011; Diraviyam et al., 2014), *H. pylori*-induced gastritis (Shin et al., 2002), *Escherichia* coli (Akita and Nakai, 1998), and botulism neurotoxins (You et al., 2014), and snake (Aguilaret al., 2014), spider and scorpion venoms (Schade et al., 2005).

Orally-administered IgY is transferred and absorbed into the circulatory system of a piglet as efficiently as are the IgG antibodies from the sow’s colostrum (Yokoyama et al., 1993). Immunoglobulins remain detectable in the neonatal circulation for 24-48h. Additionally, as seen above, specifically-induced egg yolk antibodies are protective against diarrhea in pigs (Yokoyama et al., 1993; Diraviyam et al., 2014) and other mammals.

As a side-benefit, administration of hyperimmune egg has been reported to contribute significantly in daily increases in weight gain (Heckert et al., 1999; Ikemori et al., 1997). Weight gains may be the result of the consumed immunoglobulins helping to control bioburdens in the lumen of the gut requiring less expenditure of energy to maintain gut homeostatic balance.

CONCLUSION

It is becoming increasingly evident that the gastrointestinal system is not merely an organ for digestion of feedstuffs, but has a myriad of other functions. Antibodies produced by the gut-associated lymphoid tissues, and immunoglobulins introduced exogenously, serve to protect the

host from pathogens and partner with the gut microbiota and the immune system to achieve homeostatic balance.

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Amino Acids and Dairy Rations… What is There to Know About Sources and Costs?

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Take-Home Message

The lactating dairy cow really relies upon two main sources of essential amino acids. The first main source is from rumen microbes and the second is referred to as RUP. The dairy NRC (2001) predicts the supply of amino acids from feed from estimates of RUP and the intestinal digestibility of RUP. To determine RUP samples of the feed are often placed in a nylon bag and placed in the rumen, with the amount remaining after a fixed time being considered RUP. Using the remaining residue in the bags the intestinal digestibility of RUP may be determined using the mobile bag or one of several in vitro techniques. Estimates of ruminal and intestinal digestion of both protein and amino acids may then be used as inputs into nutritional models or to determine the value of feed.

Introduction

The lactating dairy cow really relies upon two main sources of essential amino acids. The first main source is from the protein within rumen microbes. When a cow consumes feed, the rumen microbes have the first opportunity to degrade and metabolize the amino acids. These microbes ultimately use this protein to synthesize their own protein and when these microbes wash out of the rumen, and in turn, supply amino acids to the cow. The second main source is referred to as rumen undegradable protein (RUP). This is protein, which is contained in the feed and not degraded by rumen microbes, passes out of the rumen before reaching the small intestine where the RUP is available for digestion, absorption, and ultimately utilization by the cow. A dairy nutritionist balancing rations to support milk protein production is often focused on how to optimize the contribution of these two sources of amino acids. The synthesis of microbial protein in the rumen is dependent on several factors, most notably availability of carbohydrates and nitrogen. The success of rumen microbes is related to their capacity to degrade feeds that are not easily digested and produce end products of high value to the animal (i.e. volatile fatty acids and microbial protein). As more nutrients are degraded by rumen microbes, the greater the population and ultimately flow of microbial protein to the small intestine. In practice, this is one reason why the importance of forage quality is stressed. The objectives of this paper is 1) to outline the major sources of amino acids (microbial, RUP, commercial bypass), 2) to describe how the supply from these sources may be determined analytically, and 3) to discuss how feedstuffs and commercial protected amino acid products may be characterized for nutritional models.

Balancing for Amino Acids

Our growing understanding of protein nutrition has led nutritionists to consider the use and supply of individual amino acids during ration balancing procedures. Limiting amino acids are defined as those amino acids that are in shortest supply for what is needed for milk protein production. The National Research Council (NRC; 2001) for dairy publication has proven to be a useful tool because it has provided a simple ways of conceptualizing and balancing for AA. More specifically this publication indicates the proportions of methionine (Met) and lysine (Lys) that should be in metabolizable protein to maximize milk protein concentration. Biologically speaking the relationship between these amino acids on milk protein yield is generally positive. With high producing dairy cows, even when there are large amounts of amino acids coming from rumen microbes and feeds high in rumen undegradable protein, the supply of individual amino acids may still be limiting to milk protein production. To meet this need, a number of commercial technologies exist which in essence are aimed at protecting

specific amino acids from rumen degradation, but also ensuring that they are directly available to the cow. The need for such technologies generally increases with the level of milk production and also as the concentration of protein in the diet decreases. These technologies represent added costs and as a result when considering these technologies nutritionists should have a good understanding of the extent, amount, and quality of research available supporting the claims of rumen protection and also intestinal digestibility.

Determining the Rumen and Intestinal Digestibility

The concentration of rumen undegradable protein (RUP) in feed has been determined through in vivo (Vanzant et al. 1996), in situ (Ørskov and McDonald, 1979) and in vitro (Krishnamoorthy et al. 1983; Poos-Floyd et al. 1985) methods. To understand how these methods are used to characterize feed protein we can use the study of distillers grains and solubles as an example. Paz et al. (2013) summarized a number of studies in which the ruminal disappearance of CP from an array of distillers grains and solubles (DG) was estimated using either in situ or in vitro methods. These observations suggest that the degradability characteristics of protein contained in DG may be influenced (Aines et al. 1987) by a number of factors (Aines et al. 1987) including production plant (Spiehs et al. 2002), degree of heat used to dry the feed (Kleinschmit et al. 2007a), amount of solubles added back to DG (Corrigan et al. 2009), and particle size. It should also be noted that, variation in estimates may also be attributed to analytical procedures unique to each laboratory (NRC, 2001). Nonetheless Paz et al. (2013) also calculated the RUP using the parameters reported in each study and assumed a rate of passage of 5%/h. When studies employed the model of Ørskov and McDonald (1979) mean RUP for corn dried DG was reported to be 47.4 ± 12.6 (mean ± SD) while when studies employed the lag model of McDonald (1981) mean RUP for corn dried DG was reported to be 53.4 ± 8.2 %. Across models, RUP for corn dried DG was observed to be 50.4 ± 10.4%.

The Dairy NRC (2001) assumes that intestinal digestibility of RUP (dRUP) in corn dried DG is 80%. Since this was published, the assumption has been tested in a number of recent studies also summarized by Paz et al. (2013). Analytically speaking these studies can be grouped into three groups based on the adopted technique (several others exist but for simplicity are not included here).

1) The mobile bag technique (MB) in which a small sample of the feed is first incubated in the rumen and then directly inserted through a duodenal cannula into the small intestine and ultimately recovered in the manure (Hvelplund, 1985).
2) The three step in vitro procedure (TSP) which involves rumen incubation followed by pepsin and pancreatic digestion (Calsamiglia and Stern, 1995).
3) The modified three step in vitro procedure (MTSP) (Gargallo et al., 2006) which is similar to the TSP but does not include the use of trichloroacetic acid and includes the use of a batch incubator.

Overall in the dataset generated by Paz et al. (2013) the average digestibility of RUP (dRUP) in corn dried DG was observed to be 83.9 ± 10.5 %. This digestibility coefficient is similar to the NRC (2001) assumption of 80% but the reported estimates are also highly variable ranging from 59.2 - 95.0%. Analytically speaking the TSP and MTSP appear to be promising techniques to estimate dRUP because they do not require the use of cattle fitted with duodenal cannulas and may be used to analyze large numbers of samples rapidly and with precision. However the MB technique is the only technique that ensures that samples are exposed to all physiological digestive processes.

Commercial Products, Determining Animal Response

Providing single amino acids to lactating dairy cattle is an important consideration when formulating diets for optimal performance. Feeding free amino acids is typically not considered an acceptable

practice for ruminants due to the high extent of degradation within the rumen. Both Met and Lys are protected from rumen degradation and commercially available to provide higher concentrations of the amino acids to the site of absorption of the small intestine. Research using the rumen in situ procedure has been successfully used to evaluate rumen protection of feeds and supplements (Bach and Stern, 2000). This tool helps investigators evaluate if the product is stable in the rumen and thus available to be absorbed post- ruminally (Wu et al., 2012). Additionally the MB (Overton et al., 1996; Berthiaume et al., 2000) and the TSP/MTSP have been used to estimate intestinal digestibility (Bach and Stern, 2000). Although use of nylon bags yield valuable information their use does have several limitations including the fact that it should not be used with small-sized products as those particles may exit the bag despite not being degraded or absorbed, while remaining residues may also be contaminated by rumen bacteria. In addition, all the techniques described rely on passage rate assumptions, which in the case of the rumen in situ and TSP/MSTP techniques an assumed mean residence time or passage rate is used and in the case of the MB technique it is assumed the bag does not influence transit time through the intestine.

An alternative to this approach is to conduct a study that measures the appearance of the amino acid in blood, using this to determine bioavailability (Rulquin and Kowalczyk, 2003). In animal nutrition the term “bioavailability” may be defined as the, “… degree to which a consumed nutrient in a [feed] source is absorbed in a form that can be utilized in metabolism (Ammerman et al., 1995; Littell et al., 1997).” In the context of the ruminant, bioavailability of rumen-protected amino acids is the sum of what flows out of the rumen, therefore escaping rumen degradation, and what is absorbed at the small intestine. Experimentally, it can be challenging to test and determine bioavailability but in most cases investigators are really most interested in comparing the bioavailability of a test substance to a standard substance. For the case of amino acid nutrition the response usually measured in the appearance of the amino acid in the plasma (Bach and Stern, 2000) while the response in milk protein has also been used (Schwab et al., 2001). When testing the appearance in the blood investigators may take a dose-response approach where the differences in the slope of blood amino acid concentrations between graded doses of the amino acids are compared (Borucki Castro et al., 2008; Schwab and Ordway, 2003). An alternative approach is to pulse dose the different sources of amino acids into the rumen and compare treatment difference in the area under the plasma response curve (Graulet et al., 2005). Mathematically responses from a test substance to a standard substance can be denoted as x_s/x_t, where x_s and x_t are amounts of standard and test substances and the solution is commonly known as “relative bioavailability” (RBV) (Littell et al., 1997).

Example, Predicting the Cost of Increasing the Supply of Amino Acids

Practically, when a formulation change is made, such as the addition of a new feedstuff or commercial supplement, it may be useful to calculate the predicted bioavailability of individual amino acids and then to estimate the cost of this per unit of feed. To do so, known or expected rumen bypass and intestinal digestibility can be used. To return to our previous example of DG we can calculate the value of bypass Met in 1 ton of DG. To do so we assume the following (if different composition and digestibility is known these values may be modified):

- CP, 31.2
- RUP 50.4 CP
- dRUP 83.9 %
- Met in DM, 1.87 % DM
- Met in RUP, 1.93 % RUP
- Cost of DG $146/ton or $ 0.0730/lb (wet basis); $131/d or $0.0665/lb (dry basis, at 90% DM)

Amount of RUP in 1 ton (DM basis) of DG,

Amount of feed × CP × RUP =

2000 lb × 0.312 × 0.504 = 314.5 lb

Amount of RUP Met in 1 ton of DG,
314.5 lb × 0.0193 = 6.07 lb

Amount of digestible RUP in 1 ton of DG,
Amount of RUP × dRUP
314.5 lb × 0.839 = 263.9 lb

Amount of digestible Met in 1 ton of DG (assuming the digestibility of Met is equal to dRUP),
Amount RUP Met × dRUP,
6.07 lb × 0.839 = 5.09 lb

Value of RUP and Met in RUP,
$131/ton ÷ 314.5 lb/ton = $0.42/lb RUP
$131/ton ÷ 5.09 lb/ton = $25.74/lb digestible Met

The above calculations are simple and only serve as an example of how data from feed characterization may be used to further understand the value of a feed. The principles of this may be used in understanding other feeds and supplements. Additionally, the example illustrates what feed characterization inputs are important for the Dairy NRC (2001) model. Obviously, it is important to estimate the value of nutrients within similar groups such as feeds used for high bypass protein, or energy.

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Feedlot Water Quality and Quantity

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Availability of water for use in beef cattle production is a growing concern for both consumers and cattlemen due to a growing number of conflicts over water management. Water use by beef operations is often overlooked because quality and quantity has not been a challenge. Quality of water has a significant impact on performance and water sources should be tested during different times of the year. Feedlot cattle water consumption is directly related to climatic variables and prediction equations can be used to determine needs.

Introduction

Water is the most critical and most abundant nutrient we have access to in the U.S. Many processes such as growth, regulation of body temperature, reproduction, lactation, digestion, metabolism, excretion, etc. are dependent on adequate intake (NRC, 1996). Intake is primarily met through consumption followed by water contained in feedstuffs. Metabolic water is produced by oxidation of nutrients in feedstuffs. The amount of water produced from oxidation of nutrients does not result in an appreciable amount of water available for other processes (NRC, 1996). Water use for beef production was not a concern in the past because it was not considered to be limiting in terms of supply and price (Pluske and Schlink, 2007). Currently, there is an increase in demand for water in rural and urban sectors. Quality and quantity of water is often overlooked in our production systems but may become more important in the future.

Water Use Estimates

Several authors have reported an estimate for the amount of water needed for beef production. Many parameters in these models are estimated based on crop and livestock production in different regions. Variability exists among all models for estimates of water use per unit of product produced (**Table 1**). Most authors agree that water use in beef production systems is primarily influenced by feedstuff production. Within different beef production systems water use is greatest for cow-calf production, followed by stocker and feedlot production (Beckett and Oltjen, 1993; Capper, 2012; Rotz et al., 2015). Direct water consumption is estimated to have less variability on overall water use compared with irrigated crops. However, the concentration of cattle in feedlot operations may cause a strain on local water supplies.

Table 1. Water use estimates for meat production.

Reference	L/Kg meat	Details
Robbins, 1987	20,864	
Kreith, 1991	20,559	
Beckett and Oltjen, 1993	3,682	
Capper, 2011	1,763	2007 production year
	2,006	1977 production year
Capper, 2012	4,857	Conventional
	5,725	Natural
	19,572	Grass-fed
Rotz, 2015	2,470	
White 2015	712	
Water Footprint Network, 2015	15,415	Beef
	5,553	Pork
	4,325	Chicken

Quality of Water

Within specific geographic regions (or even at the farm level) water quality can be dramatically different among sources. The majority of groundwater currently used for beef production is considered safe. However, quality of water may be dependent on concentration of livestock, use, management and site-specific factors. Mineral antagonism exists and should be accounted for when developing diets. Recommendations for water tests include specific sources and documenting seasonal and annual trends (Patterson and Johnson, 2003). Groundwater use can vary greatly on individual operations during the year. Irrigation and other activities that use water may concentrate nitrates, minerals and unwanted compounds such as pesticides or herbicides. Surface water that is marginal in quality may be more appropriately used early in the grazing season before evaporation concentrates unwanted nutrients. Regions that are considered to have acceptable water for livestock should be tested to ensure other contaminants are not present.

Water Analysis of Commercial Operations

In 1997, the USDA National Animal Health Monitoring System conducted a Water Testing Survey of cow-calf (n = 498) and feedlot (n = 263) operations (APHIS, 2000 a,b). Water data were collected by region, source (well, spring, municipal, other sources), well depth, age of well and size of operation. The majority of all water samples submitted during this survey had nitrate concentrations that were not detectable or acceptable for beef use (**Table 2**). Older wells surveyed were typically not drilled as deep (< 100 ft) and generally had a greater concentration of nitrates compared with newer, deeper wells.

Table 2. Concentration and effects of nitrate in water for livestock.[1]

Nitrate Concentration, ppm[2]	Effects	Percent Samples: Cow-Calf	Percent Samples: Feedlot
< 10 - 44	Not harmful	80.1	72.1
45 - 132	Safe if diet is not high in nitrates	16.7	23.4
133 - 220	May be harmful if consumed over a long period of time	0.0	0.0
221 - 660	Dairy cattle at risk; possible death losses	3.2	4.2
661 - 800	High probability of death losses; unsafe	0.0	0.0
> 800	Do not use; unsafe	0.0	0.0

[1] Adapted from USDA APHIS, 2000 a,b
[2] Nitrate can be expressed in a laboratory result as: Nitrate, Nitrite, Nitrate-nitrogen, Nitrite nitrogen, Potassium nitrate, and Sodium nitrate. Most laboratory results indicate safe levels for the method used.

Total Dissolved Solids

Total dissolved solids are a measure of all dissolved mineral in water. The most common ions found are calcium, magnesium, sodium, bicarbonate, chloride, and sulfate (Wright, 2007). The National Academy of Sciences (1974) suggest the maximum SAFE level for cattle is up to 0.3%. When sulfates are at MODERATE levels (0.3 to 0.5%) it may cause water refusal or mild diarrhea and HIGH levels (0.5 to 1%) should be avoided for pregnant or lactating cattle. Concentrations > 1.0% are not safe unless additional water sources are blended. Total dissolved solids from the survey suggests the majority of cow-calf (96.2%) and feedlot (97.7%) operations that submitted samples were under the maximum safe level.

Additional Contaminants

Laboratory tests generally quantify the most likely causes for unacceptable water for livestock. Other toxic substances may be present and are not commonly reported on a standard test. These substances are often present in low concentrations and do not pose a health risk for cattle. Limited research is available for some of these substances and the maximum safe limit is not established for cattle (**Table 3**). Herbicides, pesticides, and fungicides may also be present in surface or groundwater supplies. If contamination is suspected of any of these compounds additional tests may be required.

Table 3. Maximum concentration of potentially toxic substances in drinking water.[1]

Substance	Safe Upper Limit, ppm	Substance	Safe Upper Limit, ppm
Alkalinity (carbonate + bicarbonate)	200	Iron	Not established
Aluminum (Al)	5	Iron (B)	5
Arsenic	0.2	Lead	0.1
Barium	Not established	Manganese	Not established
Cadmium	0.05	Mercury	0.01
Chromium	1	Molybdenum	0.5
Cobalt	1	Nickel	1
Copper	0.5	Selenium (Se)	0.5-0.10
Cyanide	Not established	Vanadium	0.1
Fluoride	2	Zinc	25

[1] Adapted from Nutrients and Toxic Substances in Water for Livestock and Poultry (1974).

Sulfates

Total dietary sulfur data has been evaluated to reduce the incidence of polioencephalomalacia (PEM; Nichols et al., 2013; Drewnowski et al., 2014). Compared with feed sources of S, inorganic sources (sulfates) appear to have a greater impact on ruminal hydrogen sulfide concentrations because a portion of the S in feedstuffs bypasses the rumen (Sarturi et al., 2013). McAllister et al., 1997 reported increased ruminal sulfide concentrations and PEM during the summer when an additional well was used at a commercial feedlot to keep up with water needs during higher temperatures. As sulfate increases in water sources, performance decreases for both growing (Patterson and Johnson, 2003) and finishing cattle (Loneragan et al., 2001; **Table 4**).

Table 4. Water sulfate levels for cattle.[1]

Sulfate level (ppm)	Interpretation	
< 500	Safe	
500-1500	Generally safe	Trace mineral availability may begin to be reduced. May decrease performance in confined cattle.
1500-3000	Marginal	May be considered poor for confined cattle during hot weather. Sporadic cases of polio may be seen in confined cattle. Performance may be affected.
3000-4000	Poor water	Sporadic cases of polio are probable, especially in confined cattle. Performance of grazing cattle may be affected.
>4000	Dangerous	Health problems expected. Substantial reductions in performance expected.

[1] Patterson and Johnson, 2003.

Manure and Microbes

Other contaminates such as feces or microbes can impact water intake and performance. When water was offered from a well average daily gains improved 23% compared for cattle consuming water directly from a pond or water that was pumped from a pond into a tank (Williams et al., 2002). When there is direct access to water in a pond, sediment may be disturbed and cattle have the ability to defecate directly into the water (Wright 2007). During a 7 d experiment, cattle were allowed to select from 3 water sources with 0, 0.05 or 0.25 mg feces/g water (Willms et al., 2002). No differences in water selection was observed on d 1 but during the remainder of the experiment cattle selected the water source with 0 mg/g manure more often compared with the treatments that had fecal contamination.

To determine the presence of microbes in water tanks of dairy operations, 435 tanks at 99 different operations were sampled (LeJeune et al., 2001). Total coliform counts were higher in the summer compared with the spring and winter while protozoa counts were lower in the summer compared to the winter or spring. Direct exposure to sunlight which compared tanks outside of barns and inside was associated with lower coliform and E. coli but higher protozoa counts existed. Frequency of cleaning tanks decreased coliform, protozoa and nematodes. Proper management of water tanks will greatly reduce the risk of outbreaks and decreased performance.

Feedlot Water Use

The primary uses of water in beef cattle feedlots are direct water consumption, mitigation to relieve heat stress, and overflow to minimize winter freezing. Direct water consumption by beef cattle is influenced primarily by feed intake and climatic variables (Parker et al., 2007). The current NRC (1996) provides a table of water intake as a “guide only” based on a summary from Winchester and Morris (1956). These data include 4 individual animals at 2 different ambient temperatures. Additionally, NRC data were based on estimates with cattle in chambers under controlled environmental conditions. Hoffman and Self (1972), reported a correlation between ambient temperature and daily water intake in shaded and unshaded pens. Another early contribution to our understanding of water intake by feedlot cattle was developed by Hicks et al. (1988). The authors developed an equation to include maximum temperature, dry-matter intake, precipitation and dietary salt content. Estimates of water intake has evolved in the last decade to include additional factors that help to explain variability of water compared with the current NRC (1996).

A series of 7 experiments that included summer and winter feeding conditions were reported by Arias and Mader (2011). From regression analyses, the best predictors of water intake were the temperature humidity index (THI), ambient temperature, and minimum temperature. Smaller coefficients of determination were observed that included DMI and solar radiation. Additional estimates of water intake as a result of management or environmental factors have been reported. Environmental conditions included in the models of Hicks et al. (1988) and Arias and Mader (2011) suggests mitigation to heat stress may reduce water use if shade is provided. Providing shade was reported to decrease water intake and improve ADG and feed efficiency (Barajas et al., 2013; Gaughan et al., 2014). In contrast to these results Sullivan et al. (2011) reported that water intake increased as heat load increased, but the increase was greater for the shaded cattle due to a greater DMI.

Panhandle Water Research

There is a large range for reported feedlot cattle water intake that is influenced by diet (Fisher et al., 1999; Gaughan et al., 2014; Sexson et al., 2010), management (Mader and Davis, 2004), and environment. More accurate estimates of water use in beef production are needed due to the increased demand in rural and urban sectors. The Panhandle Research Feedlot is equipped with 61 pens that have individual water tanks and lines. Each water line has a flow meter with a data logger

to allow for measurement of water flowing to each pen/hour. Multiple years of these data will be used to create equations for water use and compare with existing models.

Materials and Methods

Animals and Diets- The dataset for model development was derived from an experiment reported by Bremer et al. (2015) that included 320 yearling steers in 40 pens. The objective of the experiment was to determine the effect of feeding de-oiled wet distillers grains plus solubles (WDGS) in dry-rolled corn (DRC) or steam-flaked corn (SFC) diets and also compare the relative feeding value of de-oiled and normal WDGS on performance and carcass characteristics (**Table 5**). The dietary treatments were organized in a 2X3 + 2 factorial arrangement with factors being corn processing method (DRC or SFC) and concentration of de-oiled WDGS in the diet at 0, 17.5, or 35% of DM. Two additional diets containing normal WDGS were fed at 35% of the diet in DRC or SFC basal diets. The two additional diets allowed for the analysis of an embedded 2X2 factorial comparing corn processing method and oil content of the WDGS.

Table 5. Dietary treatments fed to yearling steers during the summer.

	SFC[1]				DRC[1]			
De-oiled WDGS[2]	0	17.5	35	35[3]	0	17.5	35	35[3]
Ingredient								
SFC	74.44	60.75	44.0	44.0	--	--	--	--
DRC	--	--	--	--	74.44	60.75	44.0	44.0
De-oiled WDGS	0.00	17.50	35.00	--	0.00	17.50	35.00	--
Full fat WDGS	--	--	--	35.00	--	--	--	35.00
Corn Silage	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soybean Meal[4]	3.56	0.10	--	--	3.56	0.10	--	--
Urea	1.00	0.65	--	--	1.00	0.65	--	--
Supplement[5,6,7]	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0

[1] SFC= steam flaked corn, DRC = dry rolled corn.
[2] WDGS = wet distillers grains plus solubles.
[3] Full fat WDGS included in diet.
[4] Soybean meal and urea was added to diets containing 0 or 17.5% WDGS via liquid supplement to meet the MP requirements for steers.
[5] Supplement added to diet via micromaching to provide 360 mg/steer Rumensin® and 90 mg/steer Tylan®.
[6] Supplemented formulated to contain, 30 mg/kg Zn, 50 mg/kg Fe, 10 mg/kg Cu, 20 mg/kg Mn, 0.1 mg/kg Co, 0.5 mg/kg I, and 0.1 mg/kg Se.
[7] Supplement formulated to contain, 10670 IU/kg Vitamin A, 1342 IU/kg Vitamin D, and 77 IU/kg Vitamin E.

Water, Environmental, and Statistical Analysis–

Water intake for each pen was recorded using a data-logging system by hour for each pen. A weather station at the facility recorded ambient temperature (Ta), percent relative humidity (RH), solar radiation (SR), wind speed (WS), maximum temperature (Tmax), minimum temperature (Tmin), dry-matter Intake (DMI) and metabolizable energy intake (MEI). To calculate the temperature humidity index (THI), Ta and RH were used based on the equation {$THI = 0.8*Ta + [(RH/100)*(TA – 14.4)] + 46.4$; NOAA, 1976}. Data were analyzed by pen to determine the impact of dietary treatment on daily water intake (DWI) using the model described by Bremer et al. (2015). To determine the influence of climatic variables (Ta, RH, SR, WS, and THI) on water use by hour across all pens a stepwise regression procedure was used with DWI as the response variable. This

same procedure was used to determine DWI for the duration of the experiment using Ta, RH, SR, WS, THI, Tmax, Tmin, DMI and MEI as independent variables.

Results–

Corn processing method did not have an impact on DWI but as level of WDGS increased in the diet less water tended to be consumed (**Table 6**). When oil was removed from the thin stillage to produce de-oiled WDGS, steers tended to consume less water compared with normal fat WDGS (**Table 7**). Hourly water intake was statistically correlated with all variables in the model but the greatest correlation was with Ta (r = 0.95; $P < 0.01$). The majority (74%) of the variability in determining daily water intake across all pens during the experiment was explained by the equation:

$$\underline{DWI = 7.70 + (0.62*Ta) + (0.52*MEI) + (0.03*SR) - (0.15*RH)}$$

Table 6. Effect of corn processing method with increasing concentrations of de-oiled WDGS[1] on water intake.

	DRC[2]			SFC[2]				*P*-values[3]			
Item	0	17.5	35	0	17.5	35	SEM	Int.	CPM	Lin	Quad
Intake, L/d	37.7	29.4	29.0	32.7	34.6	31.4	3.4	0.19	0.70	0.08	0.76

[1] WDGS = wet distillers grains plus solubles.
[2] DRC = dry rolled corn, SFC = steam flaked corn.
[3] Int. = interaction between corn processing method and WDGS type, CPM = main effect of corn processing method (DRC or SFC), Lin = linear effect of WDGS level, Quad = quadratic effect of WDGS level.

Table 7. Daily water intake in de-oiled and full fat WDGS[1] at 35% concentration in DRC[2] and SFC diets.

	DRC		SFC			*P*-values[3]		
Item	De-oiled WDGS	Full Fat WDGS	De-oiled WDGS	Full Fat WDGS	SEM	Int.	CPM	Type
DWI	29.1	34.8	31.4	32.0	3.9	0.14	0.51	0.10

[1] WDGS = wet distillers grains plus solubles.
[2] DRC = dry rolled corn, SFC = steam flaked corn.
[3] CPM = main effect of corn processing method (DRC or SFC), Type = main effect of type of WDGS (de-oiled or full fat fat). Int. = interaction between corn processing method and WDGS type.

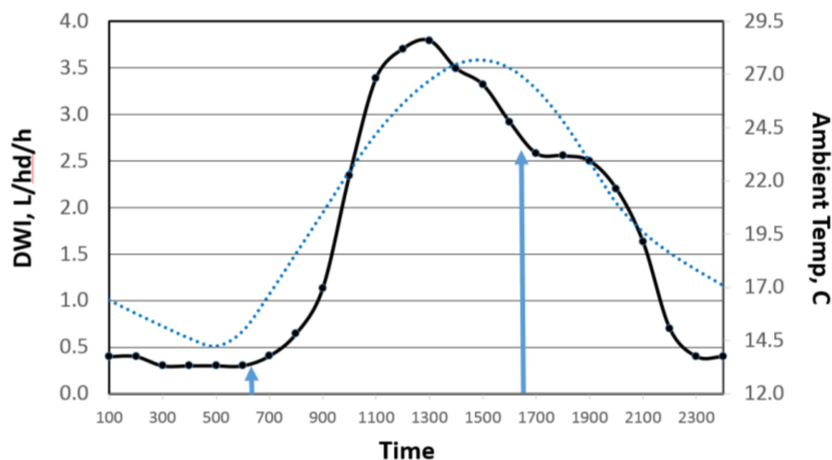

Figure 1. Water intake by hour for 40 pens during the summer. Solid line = water intake (L/hd/h), Dotted line = Ambient temperature (°C). The first arrow (approximately 6:30 a.m.) is the time of feeding. The second arrow (approximately 4:30 p.m.) is when the feedlot crew checks cattle at the end of the day.

Discussion

Feeding diets with more fat or oil should result in a lower heat increment in comparison with carbohydrates and proteins. When energy dense ingredients are fed the efficiency of utilization is greater which results in a decrease in heat production in the rumen (Gaughan and Mader, 2009). Feeding WDGS with a normal oil concentration should lower body temperature and reduce the amount of water intake for steers to maintain thermoneutrality, which was not observed in this study. Similar results were reported by Gaughan and Mader (2009) who fed an increased amount of dietary fat to mitigate heat stress and they observed that water intake increased as well. When SFC is fed compared with DRC, the energy content of the grain increases and we could expect an increase in heat production because the energy source is from carbohydrates and not fat. However, when SFC is fed cattle typically have lower intakes so heat production in the rumen is not increased.

It was first hypothesized by Winchester and Morris (1956) that when diets or ingredients have a greater amount of water that direct water intake would decrease. As level of WDGS increased in the diet water intake decreased linearly. Initially, my hypothesis was that there would not be a change in water intake when WDGS were fed. Although WDGS contains approximately 68% water, the minerals are concentrated 3 times during the ethanol process compared with corn grain. These minerals have an impact on dietary cation-anion difference (DCAD) commonly monitored in the dairy industry. Changes in mineral content (K, Na, S, Cl) or DCAD have an impact on renal function and water intake.

Daily water intake followed ambient temperature throughout the day similar to the observation by Hoffman and Self (1972). At this time we do not know if all of the water intake response is due to temperature or if time of feeding plays a role in water consumption. It is clear that at time of feeding water intake increases. Similarly, when there is another activity such as checking cattle in the afternoon we also see a (smaller) response. Feeding multiple times each day or in the evening may change consumption patterns and what variables we use to estimate water intake.

With this dataset we did not have the same response variables that explained water intake as those described by Arias and Mader (2011) in eastern NE. In eastern NE, the best predictors of water intake were THI, Ta, Tmin, and Tmax. In this dataset Ta, MEI, SR, and RH were the best predictors. Differences in location and climate are shown in **Table 8**. As a generalization, western NE is dryer and cooler compared with eastern NE. Metabolizable energy intake (MEI) was not reported in eastern NE but dry-matter intake can be used as a proxy for comparison.

Table 8. Mean values of DMI and environmental variables in eastern and western NE during the summer[1].

	Climatic Variable							
Location	Ta, °C	DMI, kg	Tmax, °C	Tmin, °C	RH, %	WS, m/s	SR, w/m^2	THI
Eastern NE[2]	21.4	9.57	27.5	15.5	77.7	4.0	221	69.0
Western NE	20.7	12.20	29.1	13.2	63.0	3.5	289	65.8

[1]Ambient temperature (Ta), dry-matter intake (DMI), maximum temperature (Tmax), minimum temperature (Tmin), percent relative humidity (RH), wind speed (WS), solar radiation (SR), temperature humidity index (THI).
[2]Adapted from Arias and Mader (2011).

Conclusions

Water quality is not a concern for most producers in the Great Plains to obtain maximum performance. There are areas within a region that have challenges with water quality that need to be identified. Water samples should be analyzed to determine if there are changes in nutrient

content or if contaminates exist in the water source. Sampling at different times of the year is important based on use of the source to determine if nutrients or contaminants are being concentrated when water use is high. By identifying peak water use during the day, feedlots can plan to have adequate supply when a heat stress event occurs. Different models exist for water consumption by cattle and should be tailored for the region. Additional factors contribute to overall water use and need to be accounted for to ensure adequate supply in the future.

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Agronomic and Nutritional Considerations of Cover Crops in Forage Systems

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Take Home Message

Cover crops are grasses, legumes or small grains grown between regular grain crop production periods for the purpose of protecting and improving the soil, providing additional grazing acres, and/or increasing forage production. In livestock production systems, cover crops represent a significant source of forage as well as soil maintenance and enhancing properties. Cover crop species such as members of the brassica family; including turnips, radish, rapeseed and various hybrid varieties have nearly become synonymous with the idea of cover crops. However, cereal grains such as rye, triticale, barley, and wheat are still important components of a cover cropping system; as are the summer annuals such as millets, forage sorghum, and sorghum x sudangrass hybrids. Although monocultures of many current cover crop species have been thoroughly researched both agronomically and nutritionally over the years; nutritional characteristics and performance curves in both beef and dairy systems using mixes of cover crop species as a forage source is less defined. The focus of this paper will be to synthesize data that is currently available on cover crop monocultures and mixes to provide some baseline knowledge in terms of forage quality of various cover crop scenarios. Additionally, performance responses of both beef and dairy systems to mechanically harvested and grazed cover crop forage are discussed. Functional management considerations in terms of where cover crop systems make the most sense in a cash-crop/livestock system and which cover crop monocultures or mixes work in various situations are addressed.

Introduction

The use of cover crops in commercial crop production systems has grown exponentially over the last decade. The idea of using ‘green chop’ to control erosion has been expanded and improved to incorporate many different species of plant cover to serve a variety of different purposes. Traditionally, cereal grain species such as oats, rye, wheat, or barley were the cover species of choice, largely because of their availability and reasonable cost. Although the primary objective was growing additional forage, soil protection, weed suppression, and nitrogen recycling were added benefits. Often times however, that was limit of the functions cereal species could provide in a cover crop situation.

Renewed interest in sustainable production practices has brought cover cropping systems back to the forefront of production agriculture in an effort to improve overall soil health; citing a lack of supplementary soil organic matter, compaction and poor soil infiltration from conventional tillage systems, and soil erosion as cause for concern. Cover crop species that could address the soil health concerns however, were not available at the time or were not available in sufficient quantities to be useful. More recently, the breeding and commercialization of many types of brassica species for increased yield and tuber size is the most notable advancement in cover cropping technology. These advancements and their incorporation into previous cover cropping management strategies have improved their utility in addressing soil health concerns.

As interest in cover cropping systems has expanded, many crop and livestock producers have realized the possibilities in incorporating cover crops as part of their forage systems; either through chopping, haying or grazing. However, as cover cropping systems, species, and practices have changed; so too have livestock genetics. Therefore, information concerning livestock performance

response to cover crops and cover crop systems in both harvested forage and grazing situations has become increasingly important to the livestock producer.

In the north-central region of the United States, cover crops are primarily used in corn-soybean-cereal grain crop rotations. Operations with livestock generally are interested in not only in the soil health aspects of the cover crop vegetation, but also the forage production. In cereal grain and corn or sorghum silage production, chopping the cover crop for wet feed or grazing are the most common forms of harvest. Although some certainly do put up dry hay if possible; that form of forage harvest is more common in the western Great Plains. In areas where fewer acres are committed to cereal grain production, cover crops may follow corn or soybean harvest where a small amount of very high quality vegetation is expected to improve the quality of the crop residue which is then grazed by beef cows over the winter.

Available cover crop species

There are many vegetation species that can be used in a cover cropping system. **Table 1** is by no means an exhaustive directory of cover crop species, but simply a list of the most common species used in cover crop systems in this region up to this point.

Table 1. Classification and common name of commonly used cover crop species in the North-Central region of the United States.

Brassicas/ Broadleaf	Cereals	Legumes/ Clovers	Summer Annuals	Annual Grasses
Turnips	Wheat	Hairy vetch	Sorghum	Ryegrass
Radishes	Rye	Red clover	Foxtail millet	Italian ryegrass
Winfred	Barley	Sweetclover	Pearl millet	Teff
Austrian winterpea	Oats	White clover	Japanese millet	
Dwarf Essex Rapeseed	Triticale	Burnett	Sudangrass	
Canola		Crimson clover	Sorghum x sudangrass	
Ethiopian cabbage		Chickling vetch		
Cow pea		Common vetch		

Amongst crop and livestock producers, the term cover crop has become almost synonymous with the brassica species; turnips, various varieties of radishes, and several brassica crossbreeds such as Hunter, Winfred, and Dwarf Essex Rape to name a few. However, traditional cover crops such as the cereal grains and summer annuals are still a very important part of the cover crop matrix. The use of cereals and summer annuals is largely determined by the ultimate objectives of the cover cropping system. Legumes and clovers, once considered primary forage crops, are now considered secondary cover cropping species within the overall context of cover cropping systems. The primary goal of including legumes and clovers in cover crops mixes is to add nitrogen to the soil. Although alfalfa remains a forage staple for many livestock producers, many different legume and clover species also are substantial nitrogen fixers and can add a substantial amount of nitrogen to the soil system in a relatively short amount of time. Additionally, annual grass species, both traditional and improved varieties are becoming more popular in cover cropping systems, especially amongst livestock producers. Annual and Italian ryegrasses as well as the warm-season Teff grass are popular components of cover crop plantings that will ultimately be harvested for use by livestock.

Where do cover crops fit in a forage system?

Monocultures

Traditionally, cover crops were planted as monocultures; usually of a cereal grain that would ultimately be harvested for forage. This method of cover cropping generally met all of the management objectives of the time. Currently, monocultures can still be an integral part of an overall cover cropping system, especially when a high quality or high quantity forage crop is the ultimate objective.

When determining how cover crops fit into an existing cropping system, the following considerations should be made to determine the type of cover crops species that should be used and whether a monoculture or mix makes the most sense to achieve management objectives:

1. Method of harvest
2. Nutritional considerations
3. Season available
4. Previous and subsequent crop
5. Cost considerations

Nutritionally, the class of livestock to be fed will have a tremendous impact on not only whether a monoculture or mix of cover crop species should be used, but also the species that will most consistently meet performance and cost objectives. This will be discussed in detail later in this paper.

Cereal grains, annual ryegrass and summer annuals remain the mainstays of monoculture cover cropping. Winter cereals are generally used as monocultures because the very nature of winter cereal permits tremendous flexibility in terms of both seeding and tillage management as well as double cropping possibilities. The following are some general guidelines in terms of expected yields of winter cereals planted in monocultures.

1. Winter rye[1]
    1. Var. Rymin – 3.5 – 4.0 T DM
2. WinterTriticale[1]
    1. Var. Fridge – 3.6 – 4.5 T DM
3. Winter barley[1]
    1. Var. Haybet (awnless/forage) – 3.6 – 4.1 T DM
4. Winter wheat[2]
    1. Var. Ideal – 2.5 – 3.1 T DM
    2. Var. Willow Creek (forage) – 4.0 – 5.0 T DM
    3.

[1]NDSU Variety Trials; [2]Brookings County, SD

Spring cereals and annual ryegrass are generally used in either monocultures or mixes. While spring cereals do preclude some of the advantages of the winter cereals, spring cereals have the flexibility of being planted in the spring or fall and are terminal upon frost. The following are some general guidelines in terms of expected yields of spring oats and annual ryegrass planted in monocultures.

1. Oats[1]
    1. Var. Morton – 2.0 – 3.0 T DM
    2. Var. Everleaf – 3.5 – 4.5 T DM
2. Annual ryegrass[2]
    1. Var. Gulf (diploid) – 1.0 – 2.0 T DM

[1]Brookings County, SD; [2]U of M NCROC data

Summer annuals also can be an important for cover cropping systems, especially on dry or relatively poor soils. Summer annuals are less flexible than spring cereals, but double cropping may be feasible in some situations. The following are some general guidelines in terms of expected yields of summer annual species planted in monocultures.

1. Millet[1]
    1. German (Foxtail) – 4.0 – 6.5 T DM
    2. Pearl – 3.0 – 8.0 T DM
    3. Japanese[2] – 3.0 – 4.0 T DM
2. Forage Sorghum[2]
    1. BMR – 5.0 – 12.0 T DM
    2. Conventional– 7.0 – 15.0 T DM
3. Forage Sorghum x Sudangrass[2]
    1. Conventional – 6.0 – 13.0 T DM
    2. BMR – 4.0 – 9.0 T DM

[1]U of M NCROC data; [2]Hand County, SD data

Mixes

Forage yield data for cover crop mixes are somewhat elusive. Therefore, forage yield data for cover crop mixes will be reported in animal performance studies later in this paper.

Determining where cover crop monocultures or mixes fit into a cropping rotation is a key component to successfully meeting management objectives. Although there are many cover crop species available for a cover cropping system, rarely is it advantageous to get too carried away with the number of species included in a cover crop mix. On the flip side, using too few species in a mix can create management challenges as well. Primarily cost will be one of the major concerns of using too many different species in a cover crop mix. As with most things, there can be a substantial differential in the cost of seed for different cover crop species making mixes with a lot of different species potentially very expensive. Agronomically, too many species in a single seed mix can be challenging as well because of potentially tremendous variation in seed size making seeding calibration a challenge. Therefore, generally speaking, 5-6 of the right species in a cover crop mix should be considered adequate to meet management objectives. The following **Tables 2-4** are example cover crop mixes, seeding rates, seed cost, and suggested harvest method for different practical scenarios where cover crop mixes make good sense in a cropping rotation in this region.

Table 2 demonstrates an example cover crop mix for seeding following a cereal grain crop. The species selected in this mix are ideal for the potential climate conditions that will be encountered by seedlings at that time of year and the seeding method that will likely be used to establish the crop. Generally speaking, this scenario will work best in a no-till system. Although there are certainly individuals whom have cut and baled a mix like this for dry hay, the results are usually highly variable and making dry hay out of this mix is not recommended. It is much better suited to chopping for haylage or grazing to achieve maximum efficiency and nutritional quality.

Table 2. Example cover crop mix for seeding in July or August following a cereal grain crop.[1]

Species	Seeding rate/acre (lb/acre)	Seed cost per acre
Oats	16	$5.00
German millet	5	$5.00
Turnip	3	$9.00
Radish	1	$3.50
Winfred	1	$2.00
Filler	5	$2.50
Total	**45**	**$27.00**

[1]Chop or graze.

Table 3 demonstrates an example cover crop mix for seeding following silage corn. The primary difference is the lack of summer annual in the mix. The length of the remaining growing season would likely not support a tremendous benefit from using a summer annual in the mix and may actually create additional problems depending on the summer annual species used due to the potential for prussic acid in sorghums and sudangrasses and nitrate buildup in millets if conditions are right. Therefore, the producer can minimize risk by excluding such species in the mix that are not likely to add much of a benefit anyway.

Table 3. Example cover crop mix for seeding following silage corn.[1]

Species	Seeding rate/acre (lb per acre)	Seed cost per acre
Oats	16	$5.00
Turnip	3	$9.00
Winfred	2	$4.00
Filler	5	$2.50
Total	**40**	**$20.50**

[1]Chop or graze.

Using a cover crop mix to improve the grazing quality of crop residues is a management strategy that has seen tremendous growth in the last few years. Producers are seeding a cover crop mix into standing cash crop stands prior to canopy. The objective is to use cover crop species, as suggested in **Table 4**, that will develop a high protein concentration profile by the time of livestock turn-out and the yield expectation for the cover crop is usually fairly low. One-half ton per acre of extremely high protein vegetation amongst corn stalk or soybean residue will provide tremendous benefits in terms of animal performance and cost savings because commercial protein supplements will be eliminated.

Table 4. Example cover crop mix for seeding into standing corn or soybean prior to canopy for improving the quality of grazing residue.[1]

Species	Seeding rate/acre (lb per acre)	Seed cost per acre
Annual ryegrass	20	$10.00
Crimson clover	3	$7.50
Dwarf essex	1	$1.00
Filler	4	$2.00
Total	**28**	**$20.50**

[1]Formulated for grazing.

In all of the example scenarios presented, seed cost per acre is $20 to $30 per acre making the application of a cover cropping system extremely cost effective. Economics of cover crop forage systems will be discussed later in this paper.

Forage quality of cover crop species

Only a small amount of work has been reported for forage and nutritional quality of brassica species. Conversely, there are numerous reports concerning forage quality of cereal grain species, summer annuals, and annual grasses. Therefore, this section will focus on some available data for forage quality of brassica species and brassica-based mixes.

Figures 1 and 2 show the whole plant crude protein concentration profile of ungrazed turnips over a roughly 90-day period; where the profile is divided amongst whole-plant aboveground vegetation and the belowground tuber. **Figure 3** shows the aboveground vegetation crude protein concentration profile of grazed turnips over the same period. The comparison of these profiles comparing crude protein concentration of ungrazed vs. grazed turnips is consistent with the profiles reported for other forage species in ungrazed vs. grazed comparisons in that whole-plant ungrazed profiles overestimate the true nutritional quality of a forage species compared to what the grazing animal is actually receiving at a specific point in time. Furthermore, the animal performance data in several

studies that will be discussed in the next section demonstrate the misrepresentation of true nutritional quality of forage species in a grazing situation.

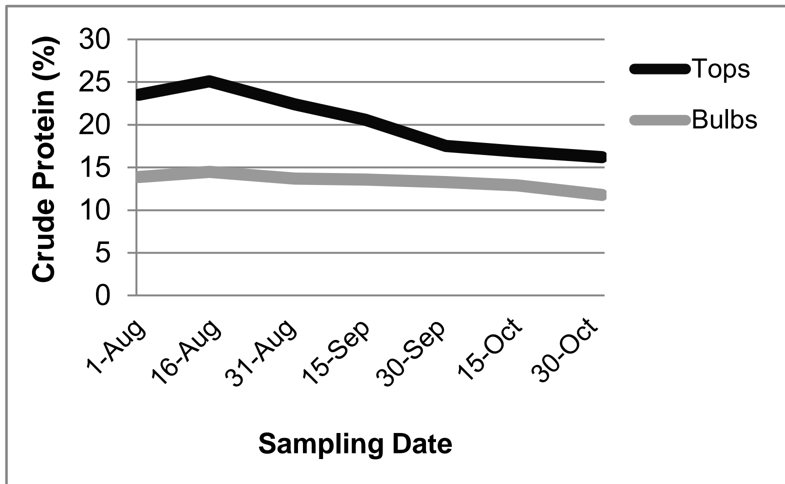

Figure 1. Crude protein (%) content of ungrazed tops and bulbs of Purple Top turnip grown in a monoculture at Grand Rapids, MN in 2014.

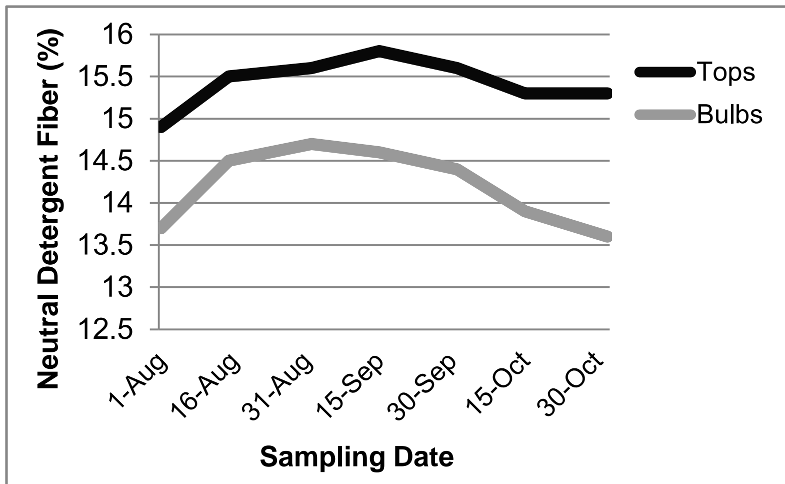

Figure 2. Neutral detergent fiber (%) of ungrazed tops and bulbs of Purple Top turnip grown in a monoculture at Grand Rapids, MN in 2014.

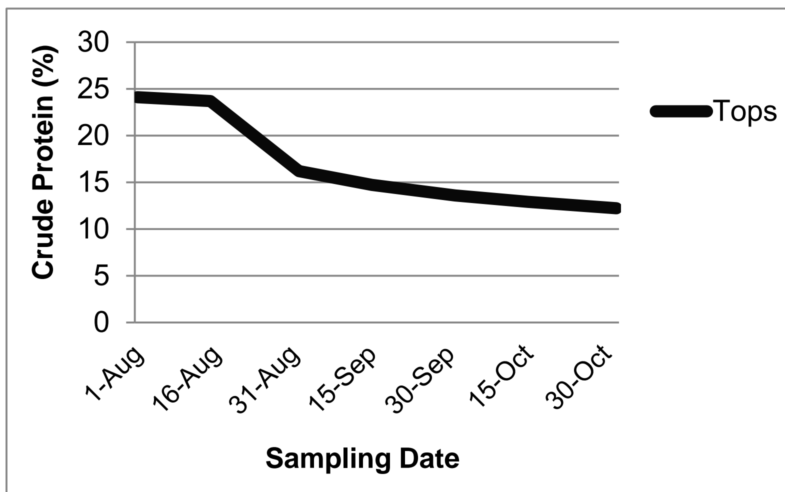

Figure 3. Crude protein (%) content of grazed tops of Purple Top turnip grown in a monoculture at Grand Rapids, MN in 2014.

Table 5. Cover crop yield and forage quality of selected cover crop species in Minnesota (Heins and Paulson 2015).

Cover crop	Dry Matter (kg/acre)	Ton/ acre	Crude Protein	Neutral Detergent Fiber	Lignin	Total Digestible Nutrients
Annual ryegrass	2,183	2.4	21.7%	37.9%	5.4%	60.6%
Berseem clover	1,013	1.1	22.4%	38.5%	6.6%	60.9%
Buckwheat	1,507	1.7	13.6%	42.4%	7.3%	58.0%
BMR sorghum/sudan	4,045	4.5	14.3%	53.7%	2.8%	62.2%
Crimson clover	1,371	1.5	20.4%	38.1%	3.9%	63.6%
Fodder beets	1,266	1.4	24.0%	33.4%	3.7%	66.7%
Forage oats	1,436	1.6	16.6%	51.0%	3.7%	62.2%
Forage peas	2,909	3.2	13.5%	41.1%	7.2%	45.5%
Grazing corn	5,797	6.4	13.4%	32.7%	3.3%	48.4%
Kale	1,239	1.4	23.2%	39.0%	4.5%	65.2%
Lentils	566	0.6	14.8%	49.8%	4.8%	52.2%
Pearl millet	3,066	3.4	15.9%	54.8%	2.6%	60.6%
Phacelia	404	0.4	21.4%	34.2%	4.2%	63.7%
Rox Cane	9,130	10	12.7%	51.3%	3.0%	63.2%
Sorghum-sudangrass	6,580	7.2	10.9%	56.1%	3.3%	58.4%
Soybeans	612	0.7	22.1%	37.9%	4.4%	62.6%
Sugarbeet	2,845	3.1	21.7%	29.3%	3.3%	68.6%
Sunn hemp	1,790	2.0	19.8%	37.6%	4.9%	62.6%
Teff	3,059	3.4	17.7%	59.0%	4.0%	60.2%
Turnip	1,600	1.8	17.2%	28.6%	2.4%	67.8%

Figure 4 shows a summary of the nutritional profile of 4 different ungrazed brassica-based mixes using combinations of cereal oats and German millet in two separate studies in South Dakota and Minnesota. This summary indicates a relatively sharp decrease in moisture concentration and increase in bot neutral and acid detergent fiber concentrations in brassica-based mixes compared to a brassica monoculture as shown in **Figures 1 and 2**. Furthermore, a marked increase in neutral and acid detergent fiber is noted between brassica-millet and brassica-cereal mixes.

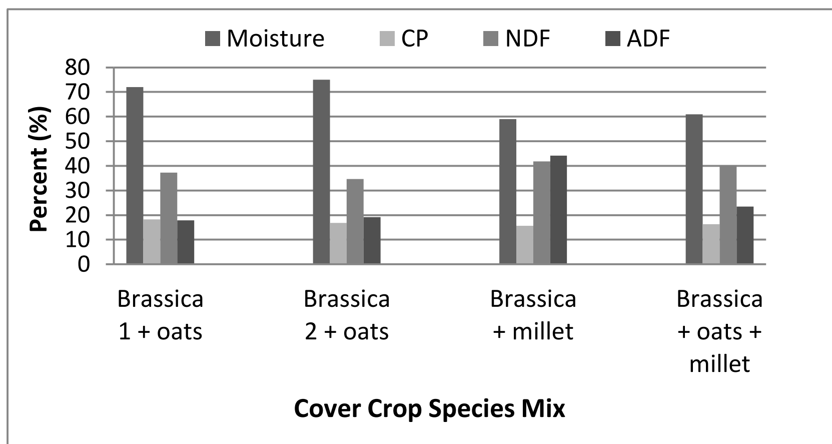

Figure 4. Moisture, crude protein content, neutral detergent fiber, and acid detergent fiber of cover crop mixes at 65 days post germination as a summary of studies conducted at Miller, SD and Grand Rapids, MN.

Brassica 1 + oats – 3 lb Purple Top turnip, 16 lb Mustang oats
Brassica 2 + oats – 2 lb Diakon radish, 16 lb Mustang oats
Brassica 1 + millet – 3 lb Purple Top turnip, 8 lb German (VNS) millet
Brassica 1 + oats + millet – 3 lb Purple Top turnip, 10 lb Mustang oats, 5 lb German millet

Animal performance

Performance and economics of beef cattle grazing cereal species and summer annuals is well established in the literature and will not be reiterated here. The animal performance data in this paper will focus on cover crop mixes and the influence of dry matter composition, digestibility, and yields on performance and cost of feeding beef cattle.

Cover cropping strategies will differ depending on the class of livestock that ultimately will consume the forage harvested. In the case of mid- to late-gestational beef cows who do not require the high nutritional quality of growing cattle or lactating cows; acceptable performance is categorized as maintenance-level and cost is the primary driver of cover cropping decisions. As with most forage species and mixes, crop yield has the principle impact on cost per unit. However, when analyzing the true costs within the system, it is important to derive the costs of the most economically significant unit. In most cropping systems, cost per acre is viewed as the primary cost-based derivative of the system. However, when the crop is feeding livestock, cost per unit of production and cost per unit of livestock become more tangible estimates of economic efficiency. **Table 6** demonstrates the yield and costs associated with grazing 4 different cover crop mixes with mid-gestational, Angus X cows. Cow performance, measured as change in body condition score (data not shown) did not differ for any of the treatments; the principle take-home point is to notice the difference between cost per acre, cost per ton of forage, and cost per cow. Although the initial establishment cost of these forage mixes seems substantial, 3 of the 4 exceeding $100/acre, the cost per ton and cost per cow per month, make them seem much more economically efficient. Obviously in this scenario, the higher yields of the brassica-rye and brassica-millet mixes provide more units of forage to divide the establishment cost over.

Table 6. Dry matter yield (T/acre), cost per acre, cost per ton, and cost per cow per month for mature, mid-gestational, Angus X cows grazing 4 cover crop mixes for 60 days; 1 NOV – 31 DEC at Miller, SD.

	Brassica Mix[1]	Brassica[2] + Winter Rye	Brassica[3] + Annual Ryegrass	Brassica[4] + German Millet
DM Yield (T/Acre)	0.53	2.73	1.96	4.15
Cost/Acre	$46.00	$126.00	$121.00	$111.00
Cost/T	$92.00	$45.81	$61.73	$26.74
Cow/Cow/Month[5]	$35.78	$19.26	$25.96	$11.23

[1]Purple top turnip 3#/ac; Graza radish 1#/ac; Winfred hybrid 1#/ac.
[2]Purple top turnip 3#/ac; Winfred hybrid 1#/ac; Rymin winter rye 1 bu/ac.
[3]Purple top turnip 3#/ac; VNS annual ryegrass 25#/ac.
[4]Graza radish 2#/ac; Winfred hybrid 2#/ac; VNS German millet 15#/ac.
[5]Average monthly cow cost = $64.28; Mousel 2014.

When grazing or feeding growing cattle or lactating cows however, effects of the forage on performance becomes one of the primary foci; as well as cost. As noted previously, most cover crop species in this discussion are relatively high quality forages in terms of crude protein concentration and digestibility. However, moisture content of the forage in a grazing situation can differ dramatically. Brassica species at the time of grazing will normally exceed 95% moisture while cereal species and summer annuals will be 55-70% moisture at the time of grazing. Additionally, the very high digestibility of brassica species makes capturing nutrients by the ruminant animal inefficient. Average daily gains gathered from weanling Angus calves grazing a monoculture of turnips demonstrates the classic performance curve for calves grazing over a period of time where performance increases to a point where either nutritional quality of the forage is inadequate to sustain growth performance or nutrient requirements of calves exceeds the ability of the forage to sustain the previous rates of growth, or both (**Figure 5**). Note the highest level of gain achieved by this group of calves was slightly over 1 lb/day and performance began to drop when calves reached about 550 lb.

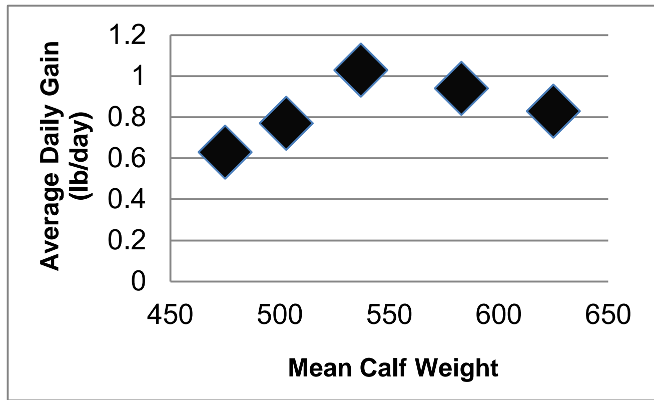

Figure 5. Average daily gain (lb/day) of Angus calves grazing Purple Top turnip monoculture at Grand Rapids, MN in 2013.

Figure 6, however, shows the average daily gain of weaned calves out of the same cut of calves grazing a brassica-millet mix. In this treatment, the highest average daily gain reached by this group of calves was about 2 lb/day and their body weight averaged about 675 lb before performance began dropping off.

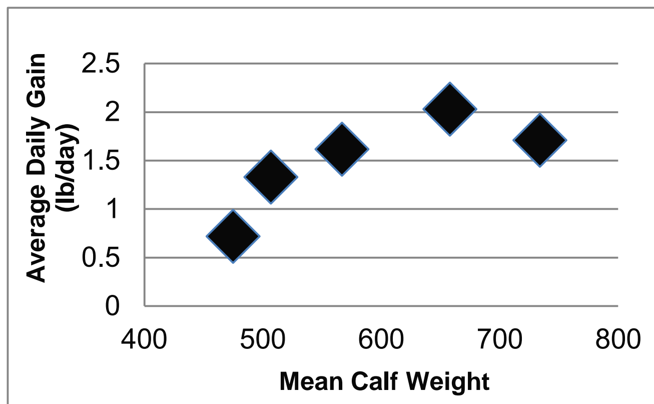

Figure 6. Average daily gain (lb per day) of Angus calves grazing brassica-millet[1] mix at Grand Rapids, MN in 2013.

[1]Purple Top turnip 3 lb, 15 lb. German millet.

Table 7 shows a similar response to different species and species combinations on calf performance and cost. Average daily gain for the overall grazing period for calves grazing a brassica species mix was about half of average daily for calves grazing either cereal oats or German millet. Similarly, when brassicas, cereal species, and millet were mixed in different combinations, average daily gain was higher when the cereal or millet was a higher proportion of the mix than the brassica species and vice-versa.

Table 7. Dry matter yield, crude protein, cost per ton dry matter, average daily gain (ADG), and cost of gain of weaned calves (mean weight = 607 lb) grazing for 45 days (1 SEP – 15 OCT) at Miller, SD.

	DM Yield (T/ac)	Crude Protein (%)	Cost/T DM	ADG	Cost of Gain ($/lb)
Brassica Mix[1]	2.75	18	$46.00	1.37	0.26
Oats (Jerry)	2.31	16	$57.33	2.55	0.18
Millet (VNS German)	4.52	14	$26.39	2.57	0.08
70% Brassica | 30% Oats	2.61	16	$47.70	1.56	0.24
30% Brassica | 70% Oats	2.47	18	$53.93	2.23	0.19
70% Brassica | 30% Millet	3.11	16	$40.11	1.78	0.18
30% Brassica | 70% Millet	3.65	15	$32.27	2.36	0.11

[1]PT turnip 3#/ac; Diakon radish 1#/ac; Dwarf Essex Rape 3#/ac.

Other considerations

Cold tolerance

When incorporating a cover crop system into any forage or cropping system, there are a couple of other considerations that should be taken into account. Although these are more systems oriented things, they are still extremely important management items to return a consistent forage quality and quantity result from the both the cover crop and the cash crops in the system.

Cold tolerance of cover species is paramount when selecting species for either a cover crop monoculture or mix. In our region with a relatively short growing season, cover crop species that are going to be used for forage or grazing later in the season will be subjected to low temperatures and likely light frost at some point during their growth cycle. Additionally, cover crops grown for grazing after the traditional grazing season likely will need to be able to withstand heavy frost and freezing conditions for extended periods of time to remain viable and deliver expected nutritional results.

Figure 7 demonstrates a relative cold tolerance index comparison of common cover crop species used in this region and should be a guide to determine species that will work in a cover crop monoculture or mix depending on the objectives of the system. Brassica species, cereal grains, and to a lesser degree, annual ryegrass are able to withstand extensive cold periods and in certain conditions with withstand freeze-up for extended periods. Conversely, summer annuals will not withstand even a light frost and will cease growth when temperatures fall below 60 degrees F. As a result, a cover crop system should take this into consideration to ensure that planted species will meet management objectives.

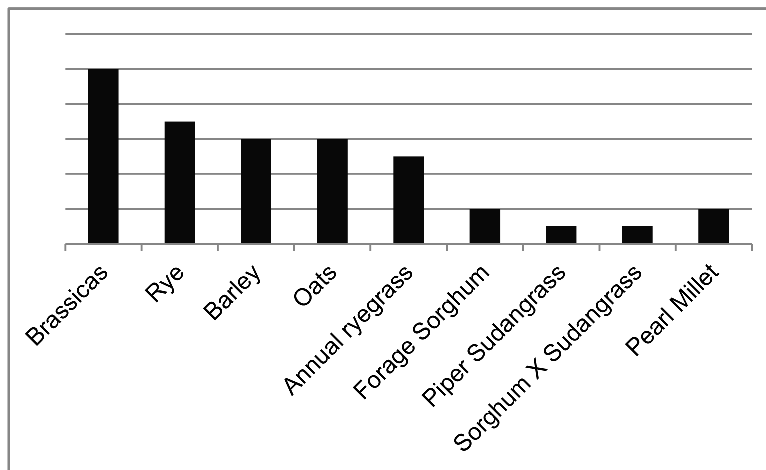

Figure 7. Relative cold tolerance of cover crop species.

Cover crop residue

Another critical consideration from a systems standpoint is residue management of cover crops in grazing scenarios. In many instances, grazing of large forage yielding vegetation is not very efficient and the more rudimentary the grazing system in which grazing pressure is applied, the poorer the grazing efficiency. **Figure 8** demonstrates the harvest efficiency, a proxy measurement for animal intake, and forage wastage of 9 different species grown in monoculture and grazed for 45 days from 1 SEP to 1 NOV by mature Angus X cows. Level of harvest efficiency is heavily influenced by vegetation type whereas harvest efficiency of brassica species, annual ryegrass, and pearl millet was 60% or greater. Harvest efficiency of the cereal species, forage sorghums, and sorghum x sudangrass was less than 40%. The significance of this is the impact large amounts of cover crop residue will have on agronomic and management implications for the subsequent crop. Producers generally assume that grazing will remove most or all of the vegetation residual and little residue will be present when preparations are made for the following crop. However, this is not always the case as tremendous amounts of ungrazed residue may be present following grazing. Therefore, it is

paramount that cover crop systems consider species, harvest method, stocking rates, and forage yields when selecting the most appropriate species or mix of species to address cover crop residual.

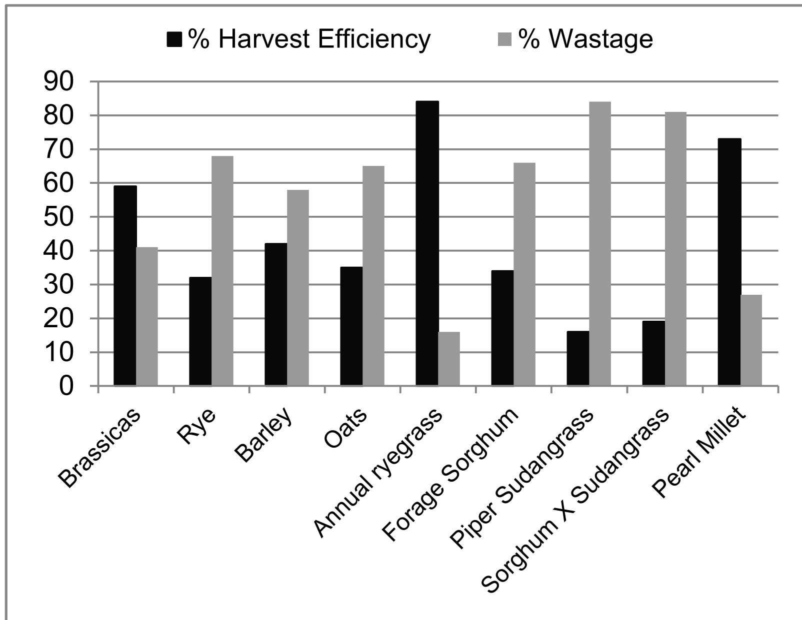

Figure 8. Harvest efficiency (%) and wastage (%) for cover crop species monocultures grazed at 60 days post-germination for 45 days (15 SEP – 1 NOV) by mature Angus X cows at Miller, SD.

New Insights on Nutrition and Feeding of Post-Weaned Dairy Heifers

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Take-Home Message

Proper nutrition of post-weaned heifers is important to continue to promote growth and development of heifers. Numerous recently conducted research studies continue to show the importance of feeding post-weaned heifers quality, grain-based diets as a way to increase growth and improve feed efficiency. Continuing to component feed heifers as they entered the growing phase was found to be advantageous as compared to switching young heifers (~300 lb) onto a TMR feeding system. In addition, continuing to feed diets containing a higher level of grain and concentrates (60:40 grain to forage ratio) was found to improve ADG and growth, while decreasing the costs per pound of gain. Further research has shown that feeding heifers diets containing greater levels of non-fiber carbohydrates (NFC) resulted in greater ADG in heifers from 12 to 28 weeks. Also, the nutritional program of calves was found to impact the growth and development of heifers after weaning. Paying close attention to the diets of post-weaned heifers helps to ensure that the diets they are fed are being utilized efficiently and their growth rates are preparing them for breeding at an early age.

Introduction

Nutrition of dairy heifers is often talked about as a whole without referring to the age and growth stage of the heifer. Even though there is a lot of focus placed on feeding milk-fed calves, little research information is available regarding the best strategies for feeding post-weaned dairy heifers. Paying close attending to the diets of post-weaned heifers helps to make sure they are growing at a rate to make sure that they will be ready for breeding and that they are efficiently utilizing the diets they are fed. As feed costs are the greatest expense for raising dairy heifers, nutritional strategies to encourage growth and development while improving feed efficiency will be beneficial for both the animals and heifer raisers.

Heifer diets are often forage-based diets that are formulated with a goal of being inexpensive. As heifers are fed for approximately 2 years without any economic return, they do comprise a significant cost for dairy operations, and heifers are usually either the second or third greatest expense for dairy herds (Heinrichs et al., 2013). As compared to lactating cattle, dairy heifers have relatively low nutrient requirements and are often fed diets with higher forage levels. However, young heifers require greater dietary nutrient concentration than older heifers and, therefore, need to be fed differently.

Nutrition of dairy heifers is often talked about as a whole without referring to the age and growth stage of the heifer. Similar to lactating cows in various stages of lactation, the nutrient requirements of dairy heifers vary substantially during their 2 years of development. Although milk-fed calves have obviously different feed requirements, the nutrient requirements of heifers continue to change, especially over the 6 months after weaning. It is important to keep in mind calves that were recently weaned have very different nutrient requirements from year old heifers and, thus, need to be fed differently. Starter intake does help to promote the growth and development of the rumen in calves, but making the assumption that weaned calves are fully functional ruminants is not correct. Therefore, continuing to pay close attention to how post-weaned heifers are fed will allow for the rumen to continue to develop and will maximize the growth and development of these heifers.

Intake of Post-weaned Heifers

When formulating diets for heifers, having a knowledge of intake is important to help determine dietary concentrations needed to ensure that the animals are consuming the recommended amounts of nutrients. The current Dairy NRC (2001) model utilizes only $BW^{0.75}$ and NE_m content of the diet when predicting intake of non-pregnant growing heifers and does not consider other dietary or non-dietary factors. Estimates of dry matter intake for large breed heifers according to the Dairy NRC (2001) are 2.8% of body weight or less. In our research, intakes of post-weaned heifers averaged 3.0% or more of their body weight when they were fed diets containing at least 60% concentrate (**Figure 1**).

In a study designed to look at feed delivery methods, diets formulated according to the NRC (2001) requirements for 2.0 lb/d of ADG for Holstein heifers estimated DMI of 13.6 lb/d for heifers at the conclusion of the study. Actual DMI observed at the end of the study averaged 20.6 lb/d among treatments, a 51% increase over the NRC predicted intake. While ADG was similar to NRC predictions in the current study, particularly for heifers fed using a TMR, the gross under-estimation of DMI by the model suggests factors other than dietary energy content are required for more accurate estimations of intake in heifers.

Other estimations for intake of heifers have been made. Hoffman et al. (2008) proposed that replacement heifers will restrict their overall intake to 1.0% of BW as NDF intake; however, in the feed delivery study, NDF intake ranged from 1.3% to 1.4% of BW during the transition period and reached over 2.0% of BW during the grower period. Similarly, NDF intakes ranged from around 1.0 to over 1.6% of BW for heifers receiving different grain to forage ratios (**Figure 2**), suggesting that factors other than total dietary NDF have the potential to influence intake in replacement heifers. However, when just forage NDF intake was determined as a percentage of BW, heifers did not consume above 1% of BW (**Figure 3**), indicating that forage NDF and not total NDF may be a better estimator of intake in younger heifers.

Results from various recent heifer studies, done using both pen fed and individually fed animals, indicate that the current models are not accurately estimating intakes for this group of post-weaned heifers. An increased understanding of the factors that regulate intake in post-weaned dairy heifers would help in both formulating diets and determining optimal feeding strategies for this group of animals.

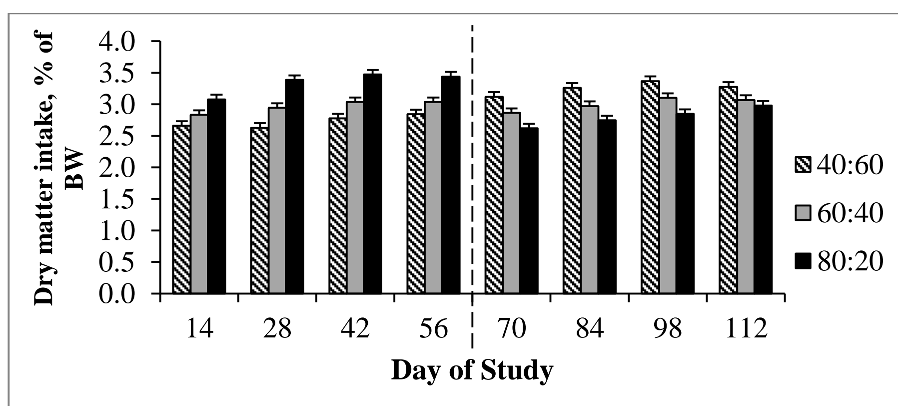

Figure 1. Effects of increasing grain inclusion during the treatment period followed by a rapid switch to a common diet on DM intake as a % of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. Treatment differences were not apparent overall (P = 0.18), however a treatment × time interaction was observed (P < 0.01), as heifers fed 40:60 consumed the least amount of DM during the treatment period as a % of BW compared to heifers fed 80:20, but consumed the most DM during the grower period compared to 60:40 and 80:20.

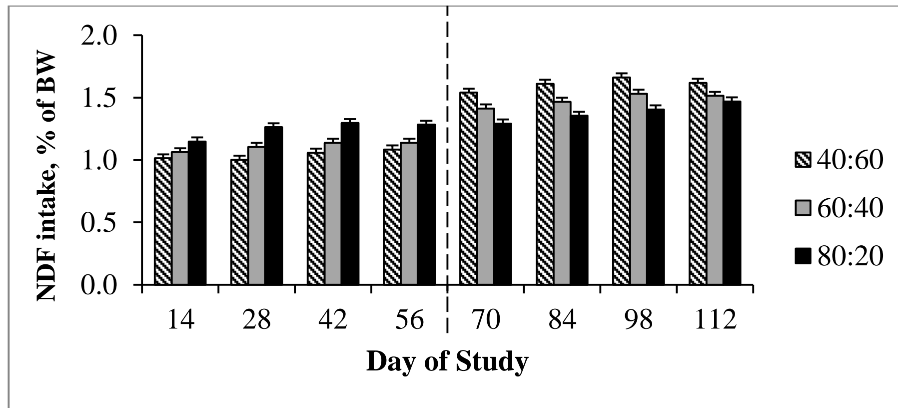

Figure 2. Effects of increasing grain inclusion during the treatment period followed by a rapid switch to a common diet on NDF intake (DM basis) as a % of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. There were no overall treatment differences (P = 0.46), however a treatment × time interaction was observed (P < 0.01), as heifers fed 40:60 consumed the least amount of total NDF during the treatment period as a % of BW compared to heifers fed 80:20, but consumed the most total NDF during the grower period compared to 60:40 and 80:20.

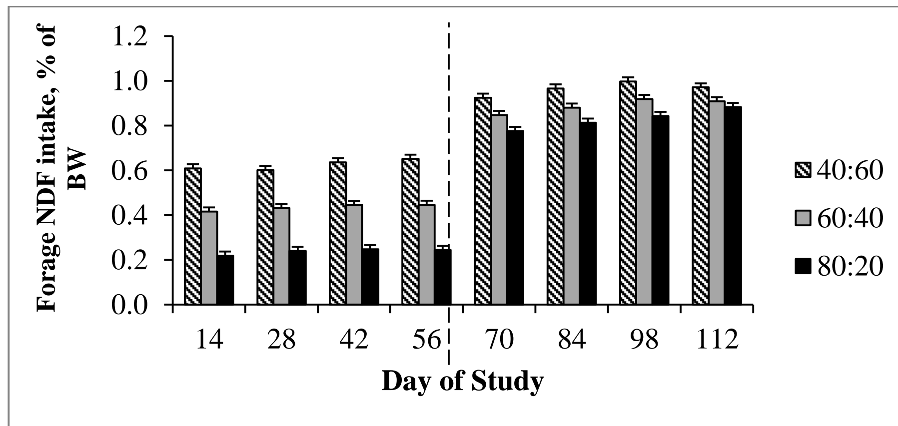

Figure 3. Effects of increasing level of concentrate inclusion during the treatment period followed by a rapid switch to a common diet on forage NDF intake (DM basis) as a % of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. Forage NDF intake increased linearly overall as grain inclusion was reduced in the treatment period (P < 0.01), and a treatment × time interaction was also observed overall (P < 0.01). As expected, forage NDF intake linearly increased as grain inclusion decreased; however, forage NDF intake was greatest throughout the grower period for heifers previously fed 40:60.

Feeding Strategies for Post-Weaned Heifers

Feed delivery methods for post-weaned heifers

Dietary composition is an important aspect of feeding heifers, but the delivery method can also have an impact when feeding heifers. A study was conducted to evaluate the effects of feeding heifers a total mixed ration (TMR), feeding them concentrate and hay side-by-side in a feed bunk (SBS), or feeding grain in a bunk and hay in a feeder (HF) on growth and intake of post-weaned heifers (**Table 1**). In this study, heifers fed using HF were significantly heavier ($P \leq 0.05$) than heifers fed using SBS from d 49 throughout the end of the study. Delivering feed using HF resulted in heifers that were, on average, 12.1 lb and 7.3 lb heavier than heifers fed using SBS and TMR, respectively, over the course of the study. Heifer weights at the conclusion of the grower period were 605, 576, and 575 lb for HF, SBS, and TMR, respectively.

Table 1. Body weight, intake, and skeletal measurements of prepubertal dairy heifers fed common diets using different feed delivery methods.

Item[1]	HF	SBS	TMR	SEM	*P*-value
Body weight, lb					
d 28[2]	396.5	391.6	387.6	4.45	0.37
d 133	605.3[a]	575.7[b]	575.1[b]	4.45	<0.01
ADG[3], lb/d					
d 0 to 28	2.29	2.09	1.96	0.121	0.21
d 29 to 133	2.05[a]	1.83[b]	1.85[b]	0.064	0.06
d 0 to 133	2.09[a]	1.90[b]	1.87[b]	0.055	0.02
Hip height, in					
d 133	47.6	47.8	47.9	0.25	0.81
Heart girth, in					
d 133	58.8[a,x]	57.8[b]	58.1[b,y]	0.28	0.03
DMI[4], lb/d					
d 0 to 28	9.57	9.08	9.72	0.223	0.15
d 29 to 133	18.04[a]	17.00[b]	16.96[b]	0.209	<0.01
d 0 to 133	16.16[a]	15.26[b]	15.34[b]	0.176	<0.01
Feed efficiency[5]					
d 0 to 28	0.224[a]	0.228[a]	0.188[b]	0.010	0.03
d 29 to 133	0.114	0.111	0.109	0.003	0.58
d 0 to 133	0.124[ab]	0.127[a]	0.115[b]	0.004	0.10

[1]HF = hay feeder; SBS = side-by-side; TMR = total mixed ration; SEM = standard error of the mean.
[2]Day of study.
[3]Average daily gain.
[4]Dry matter intake.
[5]Feed efficiency expressed as lb of ADG per lb of daily DMI.
[ab]Means differ at $P < 0.05$ level.

Average daily gains did vary depending on the time period of the study, as heifers fed using a TMR had lower ADG from d 7 to 14 ($P = 0.05$) and d 14 to 21 ($P = 0.07$) compared with HF and SBS, but higher ADG compared to SBS from d 21 to 28 ($P = 0.03$). These results suggest that post-weaned heifers require more time to adjust to new diets when feeding a TMR compared with component-feeding.

During the grower period, heifers fed using HF averaged 1.1 lb/d more DMI compared with SBS and TMR ($P < 0.01$). However, heifers fed using a TMR consumed more DMI daily from d 63 to the conclusion of the study. The results of this study suggest that, along with responses in ADG, component-fed heifers maintained intake and weight gains when transitioning to a new diet, while TMR-fed heifers caught up in terms of ADG and efficiency towards the end of the transition period and throughout the grower period. This study indicates that there may be a certain point during the growth of a heifer when it is ideal to be able to switch over to feeding a TMR.

Feeding hay or ensiled forages

Forages are an important component of heifer diets. However, little research has looked at how well post-weaned dairy heifers are able to utilize ensiled forages as compared to dry forages. A study was done to evaluate the performance of post-weaned dairy heifers that were fed either dry hay or baleage. In this study (Dennis et al., 2012), heifers fed a diet containing either 40% of their dietary DM as hay or baleage for a 28 d transition period had improved ADG, and the increase in ADG continued when heifers were fed the dry hay at 60% of the dietary DM for an additional 56 d grower period (**Table 2**). Interestingly, the DMI of the heifers during the transition period was not decreased; thus, the decreased gain was not a result of lesser intakes. During the grower period, the DMI was

decreased for heifers fed baleage though there was still an overall tendency for improved feed efficiency for heifers fed dry hay.

Table 2. Body weight, intake, and feed efficiency of prepubertal dairy heifers fed either Hay or Baleage for 28 d Transition Period followed by a 56 d Grower Period (Dennis et al., 2012).

Item[1]	Hay	Baleage	SEM	*P*-value
Transition Period				
Initial BW[2], lb	290.7	313.1	3.99	0.93
Final BW, lb	374.9	368.2	3.99	0.26
ADG[3], lb/d	2.23	1.96	0.088	0.04
DMI[4], lb/d	11.0	11.2	0.031	0.44
NDF Intake, lb/d	3.20	3.31	0.049	0.14
Feed efficiency[5]	0.205	0.178	0.012	0.14
Grower Period				
Initial BW, lb	373.5	369.6	3.99	0.47
Final BW, lb	482.2	467.5	4.37	0.02
ADG, lb/d	1.39	1.23	0.044	0.04
DMI, lb/d	12.5	11.9	0.15	<0.01
NDF Intake,	5.78	5.71	0.035	0.25
Feed efficiency	0.113	0.107	0.002	0.06

[1]Hay or Baleage fed at 40% of diet DM in the Transition Period and 60% of diet DM in the Grower Period.
[2]Body weight.
[3]Average daily gain.
[4]Dry matter intake.
[5]Feed efficiency expressed as lb of ADG per lb of daily DMI.

The results of this study indicate that feeding ensiled forages to post-weaned dairy heifers may result in decreased feed efficiency. In this study, the heifers fed hay were apparently able to better utilize the forage in their diet. Although measurements of rumen development were not determined in this study, it may be possible that the rumen of the post-weaned heifers was still undergoing development and the ensiled forage was not able to be fully utilized at that point in their development.

Grain and forage ratios

In most dairy systems today, calves are fed *ad libitum* amounts of palatable grain-based starters within a few days of birth. As calves grow, they continue to increase their starter intake until they are to the point where they are able to consume enough nutrients from the starter to support their growth without consuming milk. Once calves are weaned, their starter intake continues to increase substantially to make up for the nutrients that are no longer being consumed through milk and to cover the increased nutrient needs of the calf as they continue to grow. At this time, calves are often fed a diet that consists of only starter or starter and some forage. The timing as to when calves should begin to receive forage, the type of forage they should receive, and how much of that forage they should be given is still of some debate. Some recommendations are that calves do not need to receive any forage until a couple of weeks after weaning, though there is some evidence that having some forage available at weaning may be beneficial (Bach study, 2011). In addition, information as to how to continue transitioning these heifers to higher forage diets has been even less available.

Research was conducted at Purdue University to look at different grain to forage ratios to help determine the best strategy for feeding post-weaned dairy heifers. Heifers began the study when they were approximately 330 lb and 4.5 months of age and were assigned to diets containing either

80, 60, or 40% concentrate (on a DM basis) for 56 days before abruptly being switched to a common diet that was 40% concentrate.

In this study, increasing grain inclusion from 40 to 80% of the dietary DM resulted in a linear increase in BW (**Table 3**). Total BW gain during the treatment period averaged 76.8, 104.9, and 136.0 lb for heifers fed 40:60, 60:40, or 80:20, respectively, whereas total gain on the common diet averaged 108.2, 106.9, and 96.4 lb for heifers previously fed 40:60, 60:40, or 80:20, respectively. Average daily gain was improved overall for heifers fed 80:20 during the treatment period compared with heifers fed 40:60 or 60:40, though following a diet change, ADG was improved for heifers previously fed 40:60 or 60:40 compared to heifers fed 80:20. Frame growth exhibited similar responses to those observed for BW and ADG. Hip heights, heart girth circumference, and body condition score linearly increased with increasing grain inclusion ($P < 0.01$) during the treatment period, resulting in higher growth overall during the study for heifers fed 80% grain during the treatment period. In 1993, Peri et al. reported increased BW for dairy heifers fed *ad libitum* compared to restricted energy diets. However, Buskirk et al. (1996) fed early-weaned beef heifers either a moderate- or high-energy diet and reported similar ADG and skeletal growth, most likely due to increased intake for heifers fed the moderate-energy diet, resulting in similar energy intake between treatments.

Table 3. Weight, skeletal measurements, and intake responses of prepubertal dairy heifers fed increasing levels of grain during the treatment period then switched to a common diet.

Item[1]	40:60	60:40	80:20	SEM	*P*-value
Body weight, lb					
d 57[2]	369.2^{c}	398.6^{b}	428.8^{a}	6.01	< 0.01
d 112	476.1^{c}	504.7^{b}	524.9^{a}	6.03	< 0.01
ADG[3], lb/d					
d 0 to 56	1.37^{c}	1.87^{b}	2.29^{c}	0.088	< 0.01
d 57 to 112	1.94^{a}	1.92^{a}	1.72^{b}	0.064	0.07
d 0 to 112	1.65^{c}	1.90^{b}	2.07^{a}	0.042	< 0.01
DM intake, lb/d					
d 0 to 56	9.3^{c}	10.7^{b}	12.7^{a}	0.198	< 0.01
d 57 to 112	14.3	14.1	13.7	0.291	0.31
d 0 to 112	11.8^{c}	12.4^{b}	13.2^{a}	0.165	< 0.01
DM intake, % of BW					
d 0 to 56	2.73^{c}	2.96^{b}	3.35^{a}	0.044	< 0.01
d 57 to 112	3.26^{a}	3.00^{b}	2.80^{c}	0.062	< 0.01
d 0 to 112	2.99^{xy}	2.98^{y}	3.07^{x}	0.035	0.18
Feed efficiency[4]					
d 0 to 56	0.147^{c}	0.178^{b}	0.196^{a}	0.008	< 0.01
d 57 to 112	0.136	0.139	0.128	0.005	0.31
d 0 to 112	0.142^{b}	0.158^{a}	0.161^{a}	0.004	0.02
Hip height, in					
d 56	43.7^{c}	44.4^{b}	45.1^{a}	0.13	< 0.01
d 112	45.8^{c}	46.8^{b}	47.2^{a}	0.13	< 0.01
Heart girth, in					
d 56	51.3^{b}	52.6^{a}	52.9^{a}	0.29	< 0.01
d 112	55.6^{b}	57.1^{a}	57.4^{a}	0.29	< 0.01

[1]Grain:forage ratio.
[2]Day of study.
[3]Average daily gain.
[4]Feed efficiency expressed as lb of ADG per lb of daily DM intake.
^{abc}Means with differing superscripts are significantly different at $P \leq 0.05$ level.
^{xy}Means tend to differ at $0.10 \geq P > 0.05$ level.

Feed costs per lb of DMI averaged $0.11, $0.12, and $0.13 for heifers fed 40:60, 60:40, and 80:20, respectively, during the treatment period (**Table 4**). Daily feed costs per hd were 44.7% and 21.9% greater for 80:20 than 40:60 and 60:40, respectively, on d 14 of the trial and subsequently increased with increased DMI. On d 56 prior to switching to a common diet, feed costs per hd were 68.1% and 32.5% greater for 80:20 than 40:60 and 60:40. Feed costs per lb of ADG were lowest for 60:40 heifers over the duration of the study compared to heifers fed 40:60, though they were statistically similar to the feed costs for the 80:20 heifers. When heifers were fed 60:40 or 80:20 during the treatment period, savings were $0.24 and $0.22 per lb of ADG compared to heifers fed 40:60.

Table 4. Daily feed costs for heifers fed increasing levels of concentrate during the treatment period (d 0 to 56) followed by a common diet (d 57 to 112).

Item[1]	40:60	60:40	80:20	SEM	*P*-value
Daily feed cost per hd[2]					
d 0 to 56[3]	1.03[c]	1.29[b]	1.67[a]	0.024	< 0.01
d 57 to 112	1.48	1.45	1.41	0.030	0.31
d 0 to 112	1.26[c]	1.37[b]	1.54[a]	0.018	< 0.01
Cost of gain[4]					
d 0 to 56	0.96[a]	0.73[b]	0.73[b]	0.061	0.03
d 57 to 112	0.82	0.79	0.87	0.052	0.62
d 0 to 112	0.89[a]	0.76[b]	0.80[ab]	0.040	0.10

[1]Grain:forage ratio.
[2]All values given in US dollars ($).
[3]Day of study.
[4]$/lb of average daily gain.
[abc]Means with differing superscripts are significantly different at $P \leq 0.01$ level.

This study demonstrated that feeding higher grain levels to post-weaned dairy heifers can improve growth and can actually decrease the cost of gain over higher forage diets. In addition, it reinforced that heifers fed high grain levels can be negatively impacted by abrupt changes to higher forages diets, with the heifers on the 80:20 treatment showing a definite decline in intake when they were switched to a 40:60 diet that took some time to recover from (**Figure 1**).

Non-fiber carbohydrates in heifer diets

Even though previous research found that feeding higher concentrate diets improved gain and feed efficiency, the concentrate portion of the diet may be made up of a wide variety of different ingredients and nutrient compositions. Understanding the best strategies for designing the concentrate portion of the diet could further help to improve the gains and feed efficiency of dairy heifers.

Previous research has found that butyrate and propionate are the most important volatile fatty acids for developing the rumen in young heifers (Lesmeister and Heinrichs, 2004; Tamate et al., 1962). Therefore, diets that provide greater amounts of readily fermentable substrates could potentially increase the production of butyrate and propionate in the rumen and may help to further promote rumen development and increase the growth and development of heifers.

In order to evaluate the effects of the composition of the concentrate portion of the diet on heifer growth, intake, and feed efficiency, studies were conducted to look at the effects of feeding concentrates that were formulated to provide either high or low levels of non-fiber carbohydrates (NFC). In the first study, heifers were fed a low NDF diet (LNFC), a high NFC diet (HNFC), and a low NFC diet with added fat (LNFC+) formulated to provide the same amount of Mcals of energy as the HNFC diet.

Heifers fed LNFC+ were heavier on d 56 and d 112 of the study compared to heifers fed LNFC (**Table 5**). Heifers on the HNFC diet were intermediate and tended to be lighter on d 56 and d 112 compared to heifers fed LNFC+. Overall, heifers fed LNFC+ gained 19.4 lb more BW than heifers fed LNFC during the study ($P = 0.05$). Average daily gain in the first 56 d was 14.9% and 8.9% greater for heifers fed LNFC+ compared to heifers fed LNFC ($P < 0.01$) or HNFC ($P = 0.05$), respectively. Several studies have illustrated increased growth rates with increasing energy concentration for growing dairy heifers (Radcliff et al., 1997; Davis Rincker et al., 2008), though increased body condition likely accounted for some of the differences in this study as energy intake increased.

Table 5. Weight, skeletal measurements, and intake responses of prepubertal dairy heifers fed diets containing high non-fiber carbohydrate (NFC), low NFC (LNFC), or LNFC with added fat (LNFC+) grain fractions.

					P-value[1]	
Item	HNFC	LNFC	LNFC+	SEM	T	T×S
BW[2], lb						
d 56[3]	437.5^{ab,y}	431.3^{b}	447.6^{a,x}	4.15	0.02	--
d 112	552.4^{ab,y}	543.5^{b}	562.9^{a,x}	4.15	<0.01	--
ADG[4], lb/d						
d 0 to 56	2.14^{b}	2.03^{b}	2.34^{a}	0.062	0.02	0.01
d 56 to 112	2.05	2.01	2.05	0.073	0.86	< 0.01
d 0 to 112	2.09	2.01	2.21	0.057	0.13	< 0.01
DM intake, lb/d						
d 0 to 56	12.68	12.61	12.86	0.146	0.45	0.01
d 56 to 112	16.54^{a}	15.30^{b}	15.44^{b}	0.353	0.06	< 0.01
d 0 to 112	14.60	13.96	14.16	0.225	0.15	< 0.01
DM intake, % of BW						
d 0 to 56	3.26	3.24	3.22	0.038	0.73	0.03
d 56 to 112	3.25^{a}	3.03^{b}	2.96^{b}	0.045	< 0.01	< 0.01
d 0 to 112	3.25^{a}	3.14^{b}	3.09^{b}	0.032	< 0.01	< 0.01
NDF intake, % of BW						
d 0 to 56	1.15^{b}	1.42^{a}	1.42^{a}	0.015	< 0.01	< 0.01
d 56 to 112	1.34^{b}	1.41^{a}	1.39^{a}	0.021	0.09	< 0.01
d 0 to 112	1.25^{b}	1.42^{a}	1.41^{a}	0.014	< 0.01	< 0.01
Feed efficiency[5]						
d 0 to 56	0.166^{ab,y}	0.161^{b}	0.181^{a,x}	0.006	0.06	0.20
d 56 to 112	0.123	0.132	0.133	0.007	0.52	0.10
d 0 to 112	0.144	0.146	0.157	0.004	0.12	0.07
Hip height, in						
d 56	44.8^{ab}	44.7^{b}	45.1^{a}	0.13	0.06	--
d 112	47.6^{a}	47.2^{b}	48.0^{a}	0.13	< 0.01	--

[1]T = treatment effect; T×S = treatment × time interaction.
[2]Body weight.
[3]Day of study.
[4]Average daily gain.
[5]Feed efficiency expressed as lb of ADG per lb of daily DM intake.
^{abc}Means differ at $P \leq 0.05$ level.
^{xy}Means tend to differ at $0.10 \geq P > 0.05$ level.

During the first 56 d, treatment tended to affect feed efficiency (FE), as heifers fed LNFC+ were 12.7% more efficient than heifers fed LNFC and 9.3% more efficient than heifers fed HNFC, with a trend ($P = 0.07$) towards improved feed efficiency for LFC+ from d 0 to d 112 as compared to HNFC.

Net efficiency of fiber utilization, whether from forage or non-forage sources, is generally lower than that of starch and fat (VandeHaar and St-Pierre, 2006), though there were not any differences between the feed efficiency of high and low NFC diets in this study. However, there was an advantage in feed efficiency when fat was added to the higher fiber diet during first half of the study when heifers were younger.

During the NFC study, heifers fed LNFC maintained the lowest cost per heifer/d throughout the study as was expected due to the high inclusion rates of by-product feeds. However, feed costs per lb of ADG were lowest for heifers fed LNFC+ compared to HNFC, resulting in a cost savings of $0.12 per lb of gain. However, feed costs per lb of ADG were similar among treatments overall. In our study, a larger proportion of the HNFC diet included corn and DDGS, resulting in greater costs per ton for the grain mix, especially due to higher corn prices from the 2012 crop year. Paired with increased DMI for heifers fed HNFC, our data suggests that alternative energy sources, such as supplemental fat, may be more cost-effective for feeding growing heifers.

A second study was conducted to evaluate the effect of NFC level in the diets of post-weaned heifers after being started on either a conventional (22:20) or higher plane of nutrition (28:20) milk replacer. One of the goals of this study was to determine if how a calf was raised pre-weaning affects subsequent heifer growth and performance. In this study, animal receiving the HNFC diet had greater weight gain during the growing period from 12 to 28 weeks. Interestingly, when the animals were started on a higher plane of nutrition during the milk feeding period and subsequently fed LNFC diets, their body weight gain was significantly decreased as compared to animals that were started with a convention milk replacer program (**Table 6**). This study indicates that when calves are started on diets with a higher level of nutrition, maintaining a greater level of nutrition into the growing period may be even more important than when calves are started on a conventional milk feeding program.

Table 6. Weight and skeletal growth responses of dairy heifers and steers at 28 wk of age fed a milk treatment (MILK) of either conventional milk replacer (CONV) or high nutrition plane milk replacer (HIGH) and fed a grower diet (GRWR) of high non-fiber carbohydrate (HNFC) or low NFC (LNFC) post-weaning grower diets from 12 to 28 wk of age.

	CONV		HIGH			*P*-value[1]		
Item	HNFC	LNFC	HNFC	LNFC	SEM	MILK	GRWR	MILK × GRWR
BW[2], lb								
28 wk[3]	516.4[a]	503.0[ab]	522.1[a]	494.8[b]	7.98	0.88	< 0.01	0.04
ADG[4], lb/d								
0 to 28 wk	2.12	2.03	2.14	1.98	0.053	0.95	0.01	0.49
Hip height, in								
28 wk	47.6	47.2	47.4	47.3	0.22	0.91	0.24	0.60
Hip width, in								
28 wk	13.9[ab]	13.9[ab,x]	14.1[a]	13.7[b,y]	0.10	0.85	0.15	0.08
Heart girth, in								
28 wk	56.1	56.5	56.7	56.5	0.39	0.34	0.90	0.59

[1]MILK = effect of pre-weaning milk treatment; GRWR = effect of post-weaning diet; MILK × GRWR = interaction of milk treatment vs. post-weaning diet effects.
[2]Body weight.
[3]Weeks of age.
[4]Average daily gain.
[ab]Means with differing superscripts significantly differ at $P \leq 0.05$ level.
[xy]Means with differing superscripts tend to differ at $0.10 \geq P > 0.05$ level.

Conclusions

Using the best feeding strategies for post-weaned dairy heifers allows heifers to continue to meet their growth potential while reducing costs per lb of gain and reducing the overall costs of raising dairy heifers. Continuing to feed heifers high levels of grain post-weaning provides them with a digestible source of nutrients that facilitates growth and improves feed efficiency. At young ages, heifers appear to continue to need readily available energy sources as their rumen continues to develop. Realizing that post-weaned heifers are still developing and are not yet ready to be fed like cows facilitates an understanding that specific feeding strategies need to be developed to allow for optimal growth and development of these heifers.

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Fats and Fatty Acids in Animal Nutrition

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Abstract

Nutritionists attempt to meet nutrient requirements and regulate energy balance through dietary interventions. More digestible concentrates are commonly fed to increase the energy density of a diet, but in ruminants this increases fermentation acid production and decreases fiber digestibility. Fat supplementation increases dietary energy density without increasing diet fermentability. Nearly all dietary ingredients contribute some fat to the diet and ingredients with a low fat intake that are fed at high rates are commonly overlooked, but contribute greatly to fat intake. Feeding high fat byproducts and the development of varieties selected for a specific fatty acid profile is quickly changing lipid nutrition of both ruminant and non-ruminants. Fatty acids are well known to be bioactive nutrients that modify metabolism and physiology. The use of lipids as an energy source, substrate for cellular membrane synthesis, substrate for signaling factor synthesis and their bioactivity make determination of "requirements" difficult. Important aspects to consider are that fat supplements increase the energy density of the diet, but intake and digestibility must be maintained to increase daily energy intake. At the molecular level fatty acids interact with a number of transcription factors and signaling molecules that have large effects on metabolism, including the rate of lipid synthesis and fatty acid oxidation. Future research spanning applied nutrition and molecular biology will be required to understand both the optimal fatty acid requirement and the mechanisms involved.

Take Home Message

- Nearly all ingredients in a diet contain fat, but in different forms and with different fatty acid profiles.
- Fatty acids are a concentrated source of energy, but also are bioactive nutrients that modify metabolism.
- The requirements for essential fatty acids are difficult to predict because of overlapping functions and limited synthesis of elongated and further desaturated products.
- Literature in both experimental models and production animals provide insight into the molecular mechanism of dietary fatty acids, although the specific effect of each fatty acid in each tissue is not yet clear.

Dietary Fat

Dietary fatty acids (**FA**) serve a number of functions in animal nutrition. Traditionally, fat has been considered an energy source, providing energy required for maintenance and production of tissue and product. Dietary FA also serve as integral structural components of cellular membranes and regulatory molecules. More recently, FA are appreciated as modifiers of physiology and metabolism, making them bioactive compounds. Animals experience dynamic metabolic states during different growth and lactation phases and dietary FA serve different roles during these states. It is reasonable to expect that response to a fat source will depend on FA profile, animal physiological state, and their interaction.

Fat supplementation in animal nutrition is not a new area of investigation. Palmquist and Jenkins (1980) provided a short history of fat research in ruminants staring with a 1907 review of the effect of fat on milk and milk fat yield (Kellner, 1907). Dairy nutrition research in the late 1920's to early

1940's consistently observed a 2 to 10% milk production response to increased dietary lipids. In a 1960 review, Warner (1960) discussed reduced fiber digestion and milk production with fat supplementation, leading to the conclusion that fat was rarely superior to cereal grain. Palmquist and Jenkins (1980) focused their review on the renewed interest in using fat supplementation to increase dietary energy density, without increasing dietary starch content, to support energy requirements of high producing cows. Recently, dietary fat has also gained interest for increasing reproductive efficiency (Staples et al., 1998), and changing the FA profile of animal products (Glasser et al., 2008, Shingfield et al., 2013). Consumer interest in FA profile is dynamic with continued, but waning, interest in decreased saturated fat and increased interest in increased omega-3 FA. Dietary manipulation allows designing FA profiles of meat and milk products to meet consumer demands, although the opportunity is more limited in ruminants than non-ruminants due to ruminal biohydrogenation. Lastly, FA have a profound effect on animal physiology including metabolic signaling and gene transcription that may have application to increase production and efficiency.

Sources of Dietary Fat

Nearly all diet ingredients contain lipids. The sources vary in type and FA profile and have different feed values and effects in the rumen. Lipids can be simply categorized as glycerol or non-glycerol based. The non-glycerol based lipids, such as waxes, have little to no nutritive value. Glycerol based lipids include triglycerides, phospholipids and glycolipids. The energy concentration of the glycerol based lipids varies depending on the proportion of FA with triglycerides being the most energy dense. Ether extract includes all lipids and is not as useful in determining nutrition value compared to determination of FA concentration.

Although cereal grains and forages contain low concentrations of fat they contribute greatly to dietary fat intake since they are a large proportion of the diet. This is especially true in low total fat diets. Forage lipids are found predominantly in the plant leaf, mostly in the form of glycolipids and some as phospholipids (Harfoot, 1981). The high concentration of glycolipids may cause an overestimation of the energy value based on ether extract. Generally, forage FA composition is highly unsaturated, normally containing over 70% linoleic and linolenic acid.

Grain supplements vary in their FA concentration, profile, and availability. Corn grain FA content varies with variety including specially bred high-oil corn. We have recently characterized corn grain and corn silage FA concentration and profile in test plots during two growing seasons. Moderate variation in both total FA concentration and FA profile were observed with a 10th and 90th percentile of C18:2 in corn silage of 0.94 and 1.60% of DM (Baldin et al., 2015). Additionally, we characterized that over 92% of the C18:1 and C18:2 are in the kernel in corn silage. Cereal grain byproducts also commonly provide a considerable amount of lipid and can vary greatly with processing method, source, and batch. Solvent extracted soybean meal contains minimal residual fat, but expeller extracted retains significant oil and may have phospholipids added back during processing. Corn distiller's grains contain variable concentrations of FA depending on the processing method and efficiency of lipid removal.

Oilseeds contain a high concentration of lipid in the form of triglycerides and can increase the dietary lipid concentration even at low inclusion rates (2-12% of DM). Cottonseed, soybean, canola, and flaxseed are common oilseeds fed based on price and interest in increasing omega-3 FA intake. Most oilseed FA are in the form of triglycerides that are contained inside the seed coat and are adsorbed to the seed components. The seed must be mechanically broken down by processing, chewing, or digestion to release the triglycerides. Some oilseeds have been extensively selected for FA profile using traditional selection and genetic modification. For example, canola is a low erucic acid (C22:1) rapeseed. Low linolenic acid (18:3) soybean oil became popular to increase the frying life and oxidative stability of soybean oil during the movement away from partially hydrogenated vegetable oil. More recently, high-oleic acid soybeans (>70% C18:1) have become commercially

available and are expected to be quickly adopted after approval in major export countries. These changes have created more variability in FA profile in traditional commodities.

Animal and vegetable fat by-products provide an economical source of FA. Liquid fats can be used in small quantities to control dust and improve feed quality and in higher concentrations as an energy source. These fat sources vary in their FA profile and quality (rancidity) and the source should be considered. Importantly, the FA profile of many animal fats has changed with the increased feeding of high fat byproducts (DDGS) in poultry, swine, and cattle, which results in increased linoleic acid (C18:2) and reduced saturated and mono-unsaturated FA. Animal and plant oils can be processed to provide highly saturated FA that are naturally rumen inert. The massive growth of the palm oil industry stimulated by interest in biofuels has provided additional byproducts that are high in saturated FA and specifically high in palmitic acid.

Metabolic Utilization of Absorbed Fatty Acids

Dietary FA are a concentrated source of energy and early research recognized the increased energy value of fat, assigning it a physiologic fuel value 2.25 times that of protein and carbohydrates (Stipanuk, 2000). This is the result of increased efficiency during digestion, oxidation, and tissue deposition. Fatty acid digestion in the small intestine results in roughly 80% absorption of available FA in the cow (Drackley, 2000), although this varies considerably in the literature. Fatty acid digestibility is also efficient in other production animals, but is more dependent on FA profile as monoglycerides and unsaturated FA play an important role in micelle formation. The cow relies heavily on lysolecithin for micelle formation and maintains higher digestibility with saturated FA. The metabolism of FA yields energy for maintenance and production through complete oxidation or partial oxidation and ketogenesis. Finally, transferring dietary fat to product is very energetically efficient as preformed FA can be directly deposited in adipose or milk and do not have to enter synthesis pathways that result in energy loss.

Biological systems are engineered to use fat for insulation, cushioning, cellular structure, long-term storage of energy, and production of second messengers. Animals can synthesize FA *de novo* from nutrients such as protein and glucose. However, the ability to produce unsaturated FA are limited. Mammals can desaturate FA but cannot synthesize double bonds in the omega-6 and omega-3 positions. Therefore, the omega-6 and omega-3 FA are essential in the diet as they are required for normal formation of cellular membranes and synthesis of key regulatory molecules such as prostaglandins (Sardesai, 1992).

Fatty Acid Requirements

The many roles of FA and their bioactivity complicate the determination of dietary FA requirements. This highlights the ambiguous nature of defining nutrient and animal requirements. The terms dispensable and indispensable are used to categorize amino acids. Reeds (2000) discussed application of these categorizes in protein metabolism and highlighted dependence on their definition that may change from a nutritional, metabolic, or functional perspectives. Likewise, the same concept has been applied to FA, categorizing each as essential or nonessential based on the animals capacity to synthesize or conserve the required amounts (Cunnane, 2000). Some consider the very long chain omega-3 FA (e.g. EPA and DHA) to be conditionally essential as they can be synthesized by elongation and desaturation, but the capacity of their synthesis is highly limited in most production animals. Additionally, there is some overlap in the ability to utilize omega-3 and omega-6 FA in some pathways. However, signaling molecules originating from omega-3 and omega-6 FA differ in their functional capacity. This overlap and competition for elongation and desaturation has led to the concept of omega-3 to omega-6 ratios, although the importance of these measures is still uncertain.

Animal requirements are difficult to quantify as they may be defined as the substrate required for maintenance and sustained production, or nutrient concentrations that stimulate maximum

production through changing physiology and metabolism. The first definition employs simple accounting and a factorial approach to first calculate expenditure in maintenance and production activities, and then determines required intake based on biochemical assumptions of efficiency and metabolic conversion. A FA requirement is thus the amount of the FA secreted in milk, retained in tissue, and oxidized for energy. The second definition recognizes that absorbed nutrients change physiology and metabolism that determine animal response. Through this definition, FA requirement depends on the amount and profile of FA that directs nutrients to lactation and increases efficiency through gene regulation and endocrine stimulation. Recognizing the second level of complexity demands research into not only the energy value of dietary nutrients consumed, but also the physiological and metabolic effect of individual FA.

Essential FA have been a subject of conversation in many species, including ruminants, for many years. Although essential FA flow to the duodenum is severely limited in ruminants, there are no reports of classical FA deficiency in adult ruminants. (Mattos and Palmquist, 1977) measured linoleic acid biohydrogenation and transfer to milk fat in cows fed a high grain diet, and observed linoleic acid available at twice the requirement for female weanling rats on a metabolic body weight basis. In addition, ruminants may be adapted to sparing unsaturated FA, preserving them for required purposes. Essential FA are less available for oxidation since they are highly incorporated into phospholipids and cholesteryl esters (Drackley, 2000). The slow turnover of phospholipids and cholesteryl esters pools ensure retention of the essential FA. Dietary FA are also incorporated into milk and tissue, however the efficiency of conversion of dietary unsaturated FA to milk is lower than saturated FA (Chilliard, 1993). A final conservation method for unsaturated FA is lower oxidation. Reid and Husbands (1985) observed lower linoleic acid oxidation in cultured hepatocytes, and Leat et al. (1975) showed a 25-40% lower oxidation rate for linoleic acid than stearic and palmitic acid. Using the factorial approach it appears that essential FA are normally available in adequate concentrations, however there may be benefits to FA supplementation to health including improving reproductive efficiency and immunology that are not directly apparent in when determining requirement based on the factorial approach.

Fatty Acid Digestibility

Fat supplements must be efficiently digested and absorbed for maximal efficiency. In the ruminant, lipid complexes are hydrolyzed in the rumen delivering nonesterified FA to the small intestine, in contrast to the esterified FA flow in non-ruminants. Salivary, gastric, and pancreatic lipases and bile are essential for intestinal digestion of triglycerides, formation of micelles, and absorption of FA (Drackley, 2000). In the ruminant, lysolecithin aids formation of micelles and replaces monoglycerides and unsaturated FA that are key to micelle formation in nonruminants (Doreau and Chilliard, 1997). In the ruminant there is a large decrease in total tract digestibility when saturated FA are fed in the esterified form as they are more resistant to ruminal and intestinal lipolysis than unsaturated TG (Elliott et al., 1994, Elliott et al., 1999).

Dietary Fatty Acid Effects on Intake

Fatty acid supplements increase the energy density of the diet, but daily energy intake depends on energy concentration and dry matter intake. Intake is highly regulated by animal nutrient requirements and metabolic state, and also by the type and temporal pattern of fuels absorbed (Allen, 2000). Fatty acid supplementation can cause hypophagia, and fat source, form and type are significant predictors of intake response. In the dairy cow, calcium salts of palm oil linearly decreased intake with increasing dietary concentration while saturated FA had no effect on intake (Allen, 2000). Benson et al. (2001) summarized 11 infusion studies representing 26 treatment groups showing intake depression with all but two treatments; regression analysis revealed a negative relationship between infused C18:1 and C18:2 FA concentration and intake, with C18:2 creating greater intake depression. Abomasal infusions of unsaturated FA with a lower C16:C18 FA ratio decreased DMI and digestible energy intake (Drackley et al., 1992), and DMI and gross energy intake (Christensen et al., 1994). Bremmer et al. (1998) demonstrated a negative relationship

between intake and unsaturated FA with the same C16:C18 FA ratio. Oleamide consistently decreased intake compared to free oil and linearly decreased intake with increasing inclusion rate (Jenkins, 2000, Jenkins et al., 2000). Finally, four-day continuous intravenous infusion of both palmitic and oleic acid significantly decreased intake, while stearic acid only numerically decreased intake (Vandermeerschen-Doize and Paquay, 1984). Recent work with enriched palmitic acid supplements have observed decreased intake compare to no fat controls (Lock et al., 2013, Rico et al., 2014), although the mechanism has not been investigated.

Ruminal Metabolism of Fatty Acids

Metabolism of FA by rumen microbes has an impact on the profile of FA absorbed from the intestine and subsequently animal physiology and production and FA profile of edible products. Duodenal FA are more saturated than dietary FA and include many FA isomers from incomplete biohydrogenation and odd-carbon and branch-chain FA from microbial synthesis. The importance of biohydrogenation has been highlighted in dairy production with the development of the biohydrogenation theory of milk fat depression, where specific biohydrogenation intermediates pass from the rumen and impact mammary capacity for lipid synthesis [See reviews by Harvatine et al. (2009) and Bauman and Griinari (2001)]. However, ruminal biohydrogenation also greatly limits our ability to improve the omega-3 FA status of the cow, which may benefit reproductive efficiency and immune function, and increase the unsaturated and omega-3 FA concentration of meat and milk.

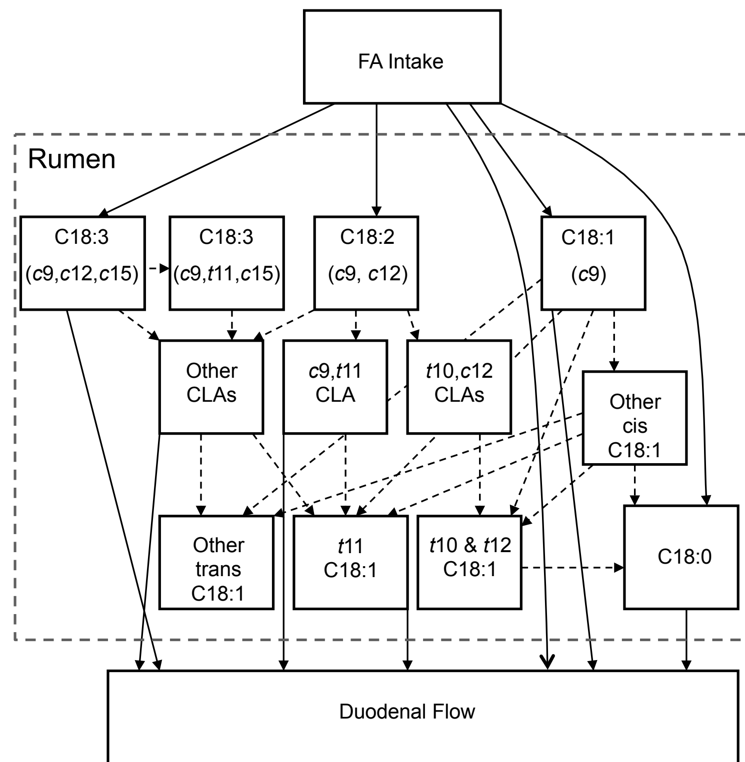

Figure 1. Fatty acids enter the rumen through intake and isomerization and biohydrogenation and fatty acids are available for passage from the rumen.

Ruminal biohydrogenation is a dynamic multistep process (**Figure 1**) that is dependent on the microbial population present in the rumen and amount of unsaturated FA available for biohydrogenation. Any factor that modifies the rumen environment or changes substrate for fermentation has the potential to change the rate or pathways of biohydrogenation. This provides a rich list of factors to investigate including the FA concentration and profile of the diet, diet macronutrient composition, and animal factors impacting rumen function among many others. A complete discussion is beyond the scope of this paper, but is subject of many reviews including those by Harfoot (1981), Harfoot and Hazlewood (1988), Fievez et al. (2007), Jenkins et al. (2008), and Lourenco et al. (2010).

Dietary FA can alter microbial growth and have profound associative effects on ruminal fermentation. Palmquist and Jenkins (1980) concluded inhibitory effects of FA on microbial activity changes competitiveness of some species and shift the microbial population, especially causing a decrease in protozoa and cellulolytic bacteria. Inhibitory effects of FA on fiber digestion can be partially alleviated by metal cations (e.g. Ca salts), which are insoluble salts that block FA metabolism by

microbes and unsaturated FA inhibition of microbial growth (Palmquist and Jenkins, 1980). Increasing saturation and chain length of the FA increases the amount and strength of salt formed (Jenkins and Palmquist, 1982). The formation of the metal salts is determined by the binding affinity of the cation and the dissociation constant of the FA. Fatty acid binding to metal cations is partially dependent on pH of the rumen and the pK_a of the FA. Sukhija and Palmquist (1990) determined the pK_a for calcium salts of stearate, tallow, palm FA and soy oil to be 4.5, 4.5, 4.6 and 5.6 respectively. These pK_a values are misleading because they were determined for a FA mixture. Soy oil contains a much higher concentration of unsaturated FA than the other treatments and demonstrates the high pK_a of unsaturated FA.

Unsaturated FA change ruminal biohydrogenation in two ways. First, they are substrate for biohydrogenation and increased intake of unsaturated FA can overrun the capacity to biohydrogenate. Secondly, unsaturated FA are toxic to some rumen microbes and result in a substantial shift in the rumen microbial population. This associative effect can decrease biohydrogenation capacity or change the predominant pathways.

Methodology Considerations for Fatty Acid Analysis

Analysis of the FA concentration and profile includes sample collection, drying and grinding, FA extraction and derivatization, and quantitative separation of FA. Unsaturated FA are sensitive to oxidation and isomerization, and migration of double bonds. These processes are increased by light, increasing temperature, and the presence of oxygen. These factors should be considered at all steps. Important aspects impacting FA profile will be briefly covered below.

Collection of a representative sample is the first consideration in all nutrition experiments. Oxidation of unsaturated FA should be of concern when drying samples, as unsaturated double bonds are susceptible to oxidation. The concern for oxidation greatly increases with increase unsaturation of the FA, especially if in free FA form. Stewart et al. (2003) compared the effect of forced air convection drying on unsaturated FA in soybeans and found degradation of unsaturated FA with drying time being more important than drying temperature. For example, linoleic acid content was greater with drying to 13% moisture at 100ºC than at 60ºC (0.91 vs. 0.38 mg/g dry mass of soybean). In research laboratories freeze-drying is recommended, but is not practical in other settings. Samples should be stored at low temperatures (-20°C), protected from light, and under nitrogen if containers have headspace.

Synthesis of FA methyl esters (FAME) is the most common derivation method for analysis of FA profile. There are many methylation procedures, but the standard chemistry is either base catalyzed or acid catalyzed. Additionally, methods either extract FA and then methylate FA or methylate FA and then extract the resulting FAME [See Reviews by (Christie, Liu, 1994)]. Extraction and purification of the organic solvent soluble components before methylation has the advantage of reducing artifacts formed by the interaction of the methylation reagent with the sample matrix (e.g. carbohydrates and proteins), but has a lower yield resulting in under-estimation of FA concentration and the possibility of biasing FA profile. In direct methylation procedures the methylation reagent is added directly to the sample. Extraction of the FAME are subsequently conducted and is more efficient as FAME are easily solubilized in organic solvents, complex lipids in plant structures are digested during methylation, and the high temperature incubation of the methylation reaction softens the sample matrix. However, this procedure results in artifact peaks that may be over 20% of the total area of the chromatogram and must be excluded during integration. These artifacts are especially problematic if they co-elute with a FA of interest. Increasing reaction time, temperature, and catalyst concentration can also increase the stringency of methylation procedures, but this also increases the opportunity for synthesis of artifacts and isomerization and oxidation of FA. The high temperature acid methylation procedure of Palmquist and Jenkins (2003) is commonly used for methylation of feeds and feces in digestion studies as these samples have a high concentration of free FA and identification of *trans* isomers is not needed. Dual methylation using base catalyzed transmethylation followed by a short duration acid methylation has been widely adopted for direct

methylation of samples containing *trans* and omega-3 FA that have a high risk for isomerization and oxidation (Park and Goins, 1994, Kramer et al., 1997, Jenkins, 2010) as the procedure provides efficient methylation with minimal risk of isomerization. Lastly, individual FA are quantified by chromatography and a large number of options are available. Traditionally, GLC with flame ionization detection was used and modern columns provide separation of most FA including the major *trans* C18:1 isomers. Advances in mass spectroscopy have provided a large number of options for FA analysis. The specifics of these approaches are beyond the scope of this paper, however, it should be noted that MS technology and methods differ greatly and specialized approaches are needed to distinguish *cis* and *trans* bond configuration.

Regulation of Metabolism by Fatty Acids

It is well recognized that individual FA have bioactive roles through modification of multiple cellular signaling pathways. The omega-3 FA are the most studied in humans, rodents, and cell culture (reviewed by Sampath and Ntambi, 2005, Jump, 2008) and *trans*-10, *cis*-12 conjugated linoleic acid (CLA) has received considerable attention in dairy research because of its role in diet-induced milk fat depression (reviewed in Harvatine et al., 2009). Although many factors interact to determine tissue rates of lipid synthesis, expression of the genes for key enzymes and proteins in the process are highly regulated by a few well characterized transcription factors known as “master regulators” including sterol response element binding protein 1c (**SREBP1**), thyroid hormone responsive spot 14 (**S14**), and a number of members of the nuclear hormone receptor family that are briefly discussed below.

Sterol response element-binding protein 1 is expressed as two isoforms with SREBP1a predominantly involved in regulation of cholesterol metabolism in the liver while SREBP1c predominantly regulates FA synthesis in lipogenic tissues. SREBP1c signalling is inhibited by polyunsaturated FA in cell culture and rodent models and this reduction mediates a major portion of the anti-lipogenic response (Hannah et al., 2001; Moon et al., 2002). SREBP1 is highly expressed in the bovine mammary gland where it is highly correlated with the expression of fatty acid synthase and lipoprotein lipase and is decreased during diet-induced milk fat depression (Harvatine and Bauman, 2006).

The molecular activation of SREBP1 is well described (reviewed by Goldstein et al., 2006). Briefly, the full-length inactive SREBP1c protein is complexed with the SREBP chaperone protein (**SCAP**) and anchored in the endoplasmic reticulum through association with a third protein, either insulin-induced gene 1 or 2 (**INSIG1 or INSIG2**). SREBP is activated by dissociation of INSIG from the SREBP/SCAP complex, allowing translocation to the Golgi where it is proteolytically cleaved to nuclear SREBP1 (**nSREBP1**), the transcriptionally active fragment. nSREBP1 translocates to the nucleus where it binds to sterol-regulatory elements (**SRE**) in the promoter/enhancer regions of target genes, recruits coactivators, and stimulates transcription of genes involved in lipid synthesis. While the sequence of SREBP1 activation and the ability of PUFA to effect this activation are well established, the initial steps in interaction of unsaturated FA and the SREBP1 complex are not characterized.

Thyroid hormone responsive spot 14 gene encodes a nuclear and cytoplasmic protein that is closely associated with the regulation of lipid synthesis in lipogenic tissues (Cunningham et al., 1998), including the bovine mammary gland. We identified S14 as a *trans*-10, *cis*-12 CLA responsive gene in microarray analysis of bovine mammary cultures (Harvatine and Bauman, 2006). Furthermore, we established that expression of S14 in the bovine mammary gland is down-regulated in both CLA-induced and diet-induced MFD. Although its exact biochemical function is not known, S14 is found in the nucleus and is a putative transcriptional coactivator (Chou et al., 2007, Chou et al., 2008) that is highly responsive to pro-lipogenic signals including SREBP1 activation (Martel et al., 2006). The expression of S14 is positively associated with conditions of excessive lipid synthesis, including human obesity, chicken lines selected for increased growth and adiposity, muscle of cattle selected for marbling, and high lipogenic cancers (summarized in Harvatine and Bauman, 2006). Of special

interest in considering possible relevance to milk fat synthesis, S14 knock-out mice had a 62% reduction in mammary lipogenesis and a 26% reduction in milk clot triglyceride concentration that was predominantly due to decreased de novo fatty acid synthesis; however, activities of mammary lipogenic enzymes were unaltered (Zhu et al., 2005).

Genes from the nuclear hormone receptor (**NR**) family are also central regulators of metabolism. The peroxisome proliferator-activated receptors (**PPARs**), in particular, have been well investigated in liver and adipose tissue. Cellular free FA are natural ligands for the PPARs and CLA is a potent agonist of PPARα and PPARγ. The nuclear receptors have tissue specific expression. For example, HNF4α is predominantly expressed in the liver (15,500-fold higher in bovine liver than lactating mammary tissue). In the case of the PPARs, PPARα is predominantly expressed in tissues with high rates of FA oxidation (e.g. liver, muscle, heart), PPARγ is predominantly expressed in adipose tissue, and PPPARß/δ is expressed in similar concentration in most tissues. Nuclear receptor activity and function is modified by ligand binding, post-translational modifications, and association with various co-repressors and co-activators (Feige et al., 2006). For example, ligand activation of PPARα and PPARß/δ increases FA oxidation and ligand binding of PPARγ increases FA transport and lipogenesis. Ligand dependent and independent repressor mechanisms are also well described for the PPARs and function primarily to reduce inflammatory and immune responses (Ricote and Glass, 2007).

Conclusion

Dietary fat and FA metabolism continues to be an active and important area of ruminant and non-ruminant nutrition. The interaction of FA with important growth, lactation, and health processes provide the opportunity to modify production efficiency and animal health through nutrition. Future work is expected to clarify FA requirements and allow develop of more precise FA feeding strategies.

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Milk Fat Depression—Causes and Solutions

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ABSTRACT

Diet-induced milk fat depression (MFD) continues to have major economic impact in the dairy industry and a priority for finding solutions. Current thinking links MFD with the formation of bioactive *trans* fatty acid intermediates produced from biohydrogenation of unsaturated fatty acids by the rumen microbial population. The most potent biohydrogenation intermediates linked to MFD include several conjugated linoleic acid (CLA) isomers. Formation of these CLA milk fat inhibitors (CLA_{MFI}) has been associated with several dietary risk factors including source and amount of grain, source and amount of fat, fiber source, and animal management factors. Since CLA_{MFI} overproduction in the rumen leads to MFD, excess CLA_{MFI} and therefore MFD can be controlled by paying close attention to managing these nutritional risks. This paper outlines these risks and thus grants the nutritionist control of milk fat synthesis. Solutions to solving MFD are complicated by interactions that often exist among two or more risk factors, making the process of reversing MFD often times slow and frustrating. In most cases, no single dietary factor is responsible for MFD, and interactions among various dietary components can increase the rumen outflow of CLA_{MFI}. A subtle change in one nutritional parameter, even within accepted guidelines, can imbalance the whole rumen environment and cause accumulation of CLA_{MFI}. Thus, if you are within the proper guidelines, but still have MFD, then the overall balance of all parameters has been upset. All risks have to be considered with regard to the combination of factors at play in a given ration formulation and with regard to the limitations of management and physical plant. An improved understanding of these events will provide the critical framework with which to better troubleshoot MFD.

INTRODUCTION

Sustained drops in milk fat yield translate into significant economic loss on a dairy because milk pricing is based on components in most Federal Orders. Fortunately, many producers experience few problems with MFD because their nutritionists have developed and maintain a consistent, well formulated feeding program. Even the best nutritionist, however, can fall victim to MFD after responding to changes in feed prices, limited availability of some feed ingredients, or unexpected changes in nutrient composition of feed ingredients. Apparently logical changes in the feeding program can drop milk fat several fractions of a percentage point to more than a full percentage point in a short period of time. It can take several weeks to months to identify the nutritional cause and return milk fat to normal.

MFD is caused by nutrition-driven changes in the rumen. Lipids in feed are metabolized by the rumen microbial population, which leads to the formation of bioactive lipids. “Bioactive” means the lipids affect living cells and tissue. These bioactive lipids are referred to as conjugated linoleic acid or CLA. Microorganisms in the rumen produce more than twenty types of known CLA but three have been shown to cause MFD. This discussion will refer to these three as CLA_{MFI}, because these CLA act as milk fat inhibitors. The CLA_{MFI} produced in the rumen travel via the blood to the mammary gland, where they inhibit the synthesis of milk fat by impairing the production of several enzymes essential for fat synthesis in the mammary gland. CLA_{MFI} are also present in cows that produce acceptable milk fat levels, but at concentrations too low to cause MFD.

The bottom line is that the type of feed the cow consumes affects rumen conditions, which in turn affects the amount and type of CLA produced. Since CLA_{MFI} overproduction in the rumen leads to MFD, excess CLA_{MFI} and therefore MFD can be controlled by paying close attention to several key

nutritional risks. This paper outlines these risks and thus grants the nutritionist control of milk fat synthesis.

NUTRITIONAL FACTORS THAT CAN CAUSE MFD

Five independent nutritional factors are currently targeted for influencing rumen production of CLA_{MFI} and development of MFD. Each will be discussed. More is known about the influence of forages, starch, and fat in the diet. These factors will receive more detailed consideration in this paper than yeast and management influences, which have been less tested and documented.

Too Much Unsaturated Fat

Too much fat in the diet of dairy cows is a classic cause of MFD. Nutritionists are keenly aware that fat must be limited to lower levels than protein or carbohydrate to avoid impaired rumen fermentation, reductions in feed intake, and MFD. It is tempting to push the limit on feeding fat when prices are favorable for high-fat byproducts, when grain prices reach record levels making commercial fats more competitive, or when the farm has access to (perceptually inexpensive) high-fat waste products from a nearby food processing plant. The key to preventing MFD from these high-fat ingredients is to fully understand the nutritional and chemical impact these ingredients have on both the rumen microbes and the cow, and to choose a feeding rate that will provide the most benefit with the least risk of detriment to the production of milk and components.

Fat supplements pose different degrees of MFD risk. Low-risk fats are those that cause little disruption of the microbial population in the rumen and thus maintain normal fermentation and limited production of $CLA_{MFI.}$ Low-risk fats are generally characterized by high saturated fatty acids or calcium salts of fatty acids. Most commercial bypass fats are based on one or both of these characteristics, so the risk of MFD is low. Bypass fat feeding rate is usually limited by cost and availability. In addition, bypass fats are dry solid products, rather than liquid fats, and therefore easier to package, transport, and mix on the farm without specialized equipment. Bypass fats are also called rumen-inert fats to emphasize their lower risk to disrupt the rumen.

High-risk fat supplements contain more unsaturated fatty acids (**Table 1**) that are typically found in forages, cereal grains, and oilseeds (cottonseed, soybeans, canola, sunflower, etc.). A high concentration of unsaturated fatty acids in the rumen from one or more of these sources can inhibit some microbial species in the rumen. This change can favor species that produce $CLA_{MFI,}$ the accumulation of which can lead to MFD. These unsaturated high-risk fat supplements are referred to as rumen-active fats to emphasize their tendency to disrupt rumen conditions.

Table 1. Individual and total unsaturated fatty acid (UFA) values for fat sources used as energy supplements in cattle rations.

Fat	Oleic	Linoleic	Linolenic	Total UFA
	---------------------- % of total	--------------------		
Tallow	42	3		45
Animal-vegetable	34	16	2	52
Palm	43	10		53
Poultry fat	41	19	1	61
Restaurant grease	48	20	3	71
Cottonseed	19	53		72
Soybean	25	53	7	85
Corn	29	55	1	85
Canola	60	20	10	90

A convenient tool to monitor risky unsaturated fatty acid intake is called RUFAL or Rumen Unsaturated Fatty Acid Load. RUFAL reflects the total unsaturated fatty acid supply entering the rumen each day from feed. RUFAL accounts for unsaturated fatty acids from all feed ingredients rather than fatty acids only from fat supplements. RUFAL may better indicate potential rumen fermentation disruption than simply calculating the percentage of fat added to the diet. Studies show that increasing RUFAL causes fermentation disruption, which can hurt animal performance. Excessive RUFAL can lead to MFD. Although a single RUFAL cutoff to prevent MFD has not been established, values below 3% of the TMR (DM basis) are viewed as low fat intakes while those above 3% of the TMR (DM basis) indicate fatty acid intakes that may be at risk of being too high. It should be noted that herds with milk fat above 3.8% have fed RUFAL in excess of 3%, so the tool only suggests a guideline for identifying diets low or high in fat.

Of the many strategies to feeding fat to dairy cows, perhaps the most important, yet most elusive, is the proper amount to feed. A proper feeding rate can usually prevent MFD associated with fat supplements. To effectively use the vast array of fat products available, practical guidelines must be developed that match sources of fat with proper supplementation. Many recommendations to limit rumen-active fats suggest a single feeding rate for added fat in dairy rations. These single numbers are easy to remember and calculate, but don't account for fatty acid contributions from the basal diet or adjust fat feeding rates in relation to fat supplement composition. An alternative approach includes the following two calculations:

1. Limit the total fat consumed from all sources (basal ingredients plus fat supplements) so that

   lb total fatty acid intake = lb milk fat produced

2. Limit rumen-active fats so that

$$\text{lb rumen-active fatty acids} = \frac{4 * NDF * DMI}{UFA * 100}$$

   Where,

   NDF is % of the dairy TMR

   DMI is dry matter intake of cows in lb/day

   UFA is % unsaturated fatty acids in the rumen-active fat supplement

Too Much Starch

High grain diets are also known to cause MFD. Rapid fermentation of starch can cause acid accumulation and lower pH in the rumen. Factors that can result in marked changes in rumen pH through any 24-h period include: dietary carbohydrate profile and rates of degradation of the carbohydrate fractions as affected by source, processing, and moisture; physically effective NDF (peNDF) supply as affected by source and particle size; and production of salivary buffers as a function of peNDF supply and source. Despite our general understanding of these factors, the degree and duration of low rumen pH required for accumulation of CLA_{MFI} in the rumen is not known. Although data are limited, rumen pH changes are most likely associated with MFD because they alter bacterial populations by favoring those that have alternative pathways of biohydrogenation.

Studies show that low pH alters the microbial population in the rumen and causes accumulation of CLAMFI. In a study by Fuentes et al. (2009), the pH of rumen cultures was lowered from 6.5 to 5.5, causing a shift in CLA production that included increased CLA_{MFI} (**Figure 1**). Although milk fat percentages often decline as rumen pH values decrease, there is still a lot of variation seen as scatter around the line in **Figure 2**. This indicates that rumen pH is not the only factor controlling CLA_{MFI} and milk fat percentage. Therefore, rumen acidosis should not be viewed as a prerequisite for MFD.

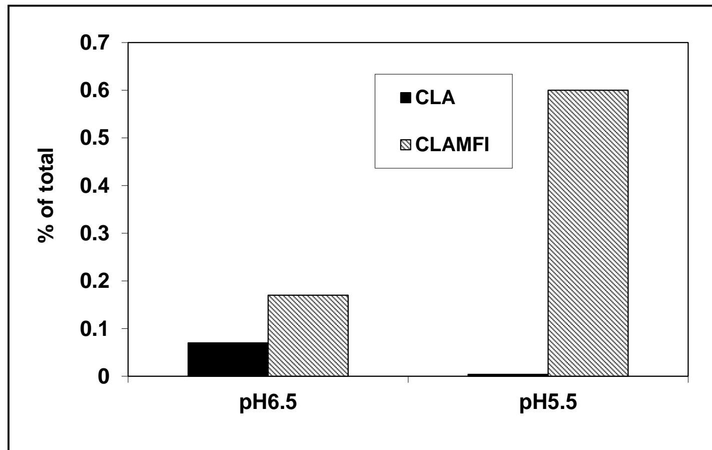

Figure 1. Rumen culture data taken from Fuentes (2009) showing an increase in CLA_{MFI} (cross-hatched bars) but a decrease in an alternative CLA (solid bars) as pH declined from 6.5 to 5.5.

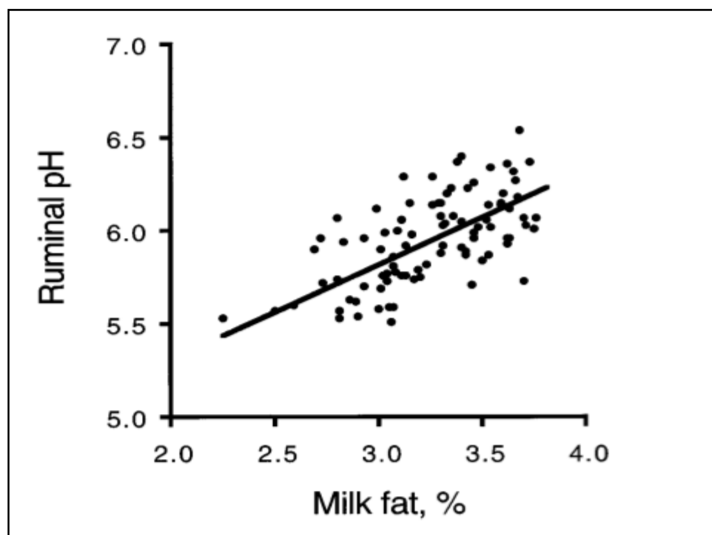

Figure 2. Relationships between rumen pH and milk fat % as reported by Allen (1997).

The rate of degradability of the starch fraction in grains also determines risk for MFD. Field observations and inferences from studies indicate that rapid rates of starch fermentability are linked to a greater risk of MFD. When processed corn having greater than 80% starch fermentability in a 7 h in vitro test was added to continuous cultures the daily production of CLA_{mfi} increased compared to the addition of unprocessed corn with 48% starch fermentability (Young et al., 2015).

Fermented feeds with high grain content such as corn silage and high moisture corn carry the highest risk. Differences in corn varieties, silo storage time, and climate conditions for plant growth can all lead to rapid rates of starch degradation in silage and high-moisture corn. Longer storage can lead to higher rates of starch degradability. A study by Newbold et al. (2006), using an in vitro test in rumen fluid over three hours, found a 30% increase in degradability in corn silage stored for 2 months vs. 10 months. If high rates of starch degradability in forages are suspected as a cause of MFD, usually there is little that can remedy the situation. One option is to dilute the forage with less degradable feed, but often that is not available. An alternative option is to focus on other risk factors (such as rumen pH and dietary fat) to minimize CLA_{MFI} production.

Forage Considerations

Maintaining adequate forage levels in dairy diets decreases the risk of MFD (**Figure 3**). As explained previously, forage can help maintain rumen pH and limit the synthesis of CLA_{MFI}. This approach

emphasizes peNDF to sustain cud-chewing and production of salivary buffers. Nutritionists use specific forage guidelines tailored for specific dairies with individualized forage needs. Within those guidelines, however, maintaining a consistent forage program is the first line of defense against problems with MFD. Again, the rate of starch degradability in forage also affects CLA_{MFI} production. High rates of starch degradability in silage has been associated with an increased risk of MFD, which means that silage NDF alone, as a proxy of forage level and assumed peNDF, is not enough to explain all occurrences of MFD. A lesser known and often ignored attribute of forages related to MFD is their contribution to the cow's total fat intake. For example, fatty acids in corn silage typically average around 1.5 to 2.0% of DM, but can reach 3.5% or higher. It is important to remember that fatty acid content is not the same as crude fat content when requesting a forage analysis (**Figure 4**). Fat content has traditionally been determined as the ether-extractable component of the feed. In addition to extracting fat, ether also extracts some carbohydrate, vitamins, and pigments. Therefore, crude fat in cereal grains, forages, and the total mixed ration often contain less than 60% fatty acids. Forage containing 3.5% total fatty acids could contain 5 to 6% crude fat.

- High fat content with fatty acids 2.5% or more of plant DM.
- High free fatty acids reaching 50% or more of total fatty acids.
- High rates of starch degradability reaching 85% or more in a 7-hour in vitro test.
- High yeasts and molds. Alarms go off with yeast counts approaching 1 million cfu/g.

Figure 3. Characteristics of corn silage often associated with increased risk of MFD.

Crude Fat
- estimated by extracting a ground feed sample with organic solvents
- low cost and AOAC approved
- higher than fatty acid values because includes fatty acids plus other nonlipid contaminants such as pigments, carbohydrates, and some vitamins.

Total Fatty Acids
- isolates only the fatty acid fraction in feed lipids using gas chromatography
- higher cost and not AOAC approved
- lower values than a crude fat analysis because includes only fatty acids and no other contaminants.

Figure 4. Key differences between a lipid analysis of forages by crude fat vs. total fatty acids.

Given the large quantities of corn silage fed to cows in some operations, this amounts to significant fat intakes just from silages alone. High fatty acid intakes have also been reported in grazed forages, but again challenges face proper analysis. Ryegrass at Clemson University grazed by cows November through March 2009 had an initial fatty acid content of 6.8% of DM and fell to 4.7% by the end of grazing (Freeman-Pounders et al., 2009). Importantly, hay analysis does not represent grazing intakes. Cutting and drying plant material during haymaking causes extensive loss of fatty acids and other nutrients because plant metabolism continues for a time after the grass is cut. To best represent what a cow consumes during actual grazing, ryegrass samples in the Clemson University grazing study were clipped and immediately immersed in liquid nitrogen to stop all plant metabolism. Then, samples were freeze-dried and kept frozen before analysis.

Other Factors that can Influence Milk Fat

Yeasts/molds and management factors are both regarded as significant risk factors for MFD, but little is known about exactly how they affect rumen function and the accumulation of CLA_{MFI}. Speculative theories about molds and yeasts suggest they may produce antimicrobial substances as part of their metabolism, which in turn may negatively impact the rumen microbial population; however much remains to be proven in this regard. High yeast and mold counts in fermented feeds is undesirable, not only for risk of MFD, but also because it can reduce feed intake, negatively effect

animal health, and decrease overall lactation performance, in addition to incurring additional feed losses through ‘shrink.” In well preserved silage, yeast counts below 10,000 CFU/g are common. Counts that effect animal health and performance poorly are not well defined and likely depend on the specific strain of yeast or mold infecting the plant. As a general rule, yeast counts at or above a million CFU/g should cause concern.
A number of management factors also have been connected with increased risk of MFD. Among these are bunk space, stocking density, and mixing of the TMR. These factors can all cause sorting and slug-feeding of grain resulting in low rumen pH and subsequent production of CLA_{MFI} in the rumen. In general, all attempts to maintain cow comfort and maintain good overall herd management will minimize the risk of MFD.

INTERACTIONS AMONG RISK FACTORS

A subtle change in one nutritional parameter, even within accepted guidelines, can imbalance the whole rumen environment and cause accumulation of CLA_{MFI}. Thus, if you are within the proper guidelines, but still have MFD, then the overall balance of all parameters has been upset. For example, cultures of rumen microorganisms were fed either a high corn or high barley diet along with the presence or absence of soybean oil (0 and 5%) and of the presence or absence of monensin (0 and 25 ppm). A lipid compound called *trans*-10 18:1 was monitored as a proxy for CLA_{MFI} (the production of the two are highly related and *trans-10* was more reliably analyzed at the time of this study). The addition of soybean oil increased *trans*-10 18:1 concentrations in the cultures for both the corn and barley diets (Jenkins et al., 2003). To a lesser extent, monensin also increased *trans*-10 18:1 for both corn and barley. However, when monensin and soybean oil were both added to the diets the combination interacted. Adding monensin with soybean oil did not elevate *trans*-10 18:1 when the diet was corn-based. When the diet was barley-based, adding monensin with soybean oil elevated *trans*-10 18:1 more than either risk factor alone.

A similar grain, monensin and fat interaction was examined in lactating dairy cows (Van Amburgh et al., 2008). Eighty Holstein cows were assigned either a high (27.7%) or low (20.3%) starch diet for 21 days, followed by the addition of monensin (13 ppm) or corn oil (1.25%) for an additional 21 day. Then, cows were switched to diets with opposite corn oil levels for a final 21 day period, providing eight treatments. Oil level was a higher risk factor for MFD compared to monensin: corn oil decreased milk fat from 3.32 to 2.99% versus 3.20% to 3.11% for monensin (**Figure 5**). Feeding high-starch diets had borderline effects on MFD: milk fat declined from 3.25 to 3.06%. Starch degradability may have contributed to MFD in this study because the diets contained steam-flaked corn, which has an inherently fast rate of rumen starch degradation. Therefore degradability, compounded by high dry matter intake, may be a more potent MFD risk factor than starch intake alone.

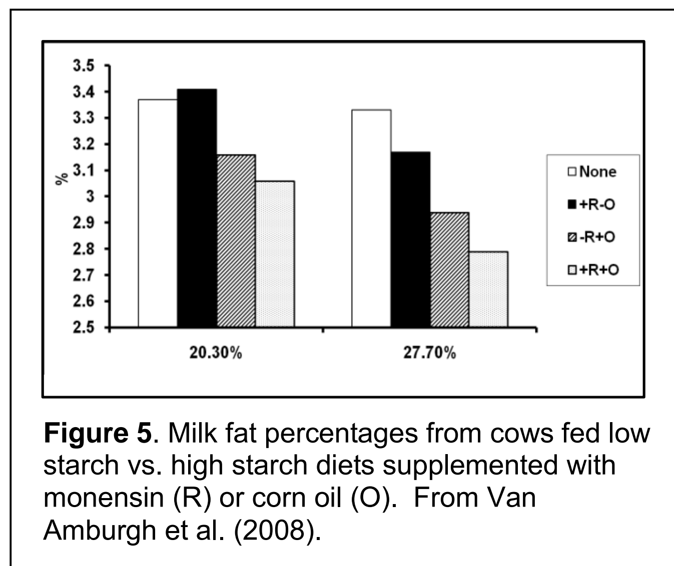

Figure 5. Milk fat percentages from cows fed low starch vs. high starch diets supplemented with monensin (R) or corn oil (O). From Van Amburgh et al. (2008).

TAKE-HOME MESSAGE

The breakthrough in MFD occurred with the discovery that it was linked to CLA production in the rumen. Feeding management controls MFD by limiting accumulation of CLA_{MFI} in the rumen. In general, no single dietary factor is responsible for MFD, and interactions among various dietary components can increase the rumen outflow of CLA_{MFI}. All risks have to be considered with regard to

the combination of factors at play in a given ration formulation and with regard to the limitations of management and physical plant. Further research is required to better understand the rumen conditions that promote the formation of CLA_{MFI} that may trigger MFD. An improved understanding of these events will provide the critical framework with which to better troubleshoot MFD.

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Metabolic Fate of Palmitic and Stearic Acids in Dairy Cows

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Take Home Message

- The saturated fatty acids, palmitic (C16:0) and stearic (C18:0), have a key role in lipid metabolism and response to feeding is affected by the cow's energy balance.
- Palmitic acid has a positive effect on increasing milk fat percentage and yield from both synthesis and dietary contribution.
- Palmitic acid is the primary fatty acid synthesized in adipose cells, but a buildup of C16:0 in cells has negative consequences on cell membranes and integrity. The unsaturated from of C16:0, palmitoleic acid (C16:1), regulates lipogenesis, desaturation and apoptosis in adipose cells.
- Stearic acid is ubiquitous and appears to support a broad range in productive responses (milk, milk component and body weight) through either energy metabolism or direct contribution.
- Very little C18:0 accumulates in adipose or mammary cells as active desaturase enzymes convert C18:0 to C18:1 (oleic) to maintain cell and milk fat fluidity.
- The specific, yet synergistic, functions of these two fatty acids indicate feeding a combination of the two fatty acids will result in the best utilization and optimal performance of dairy cows.

Fat supplementation of diets for lactating dairy cows is generally done to increase energy intake and thereby increase milk yield and/or limit mobilization of body lipids. The fatty acid (FA) composition of the fat supplement, amount of supplement fed and energy balance of the cow have a significant effect on the cow's response to the added dietary energy. The two primary saturated FA in lipid nutrition of dairy cows, palmitic (C16:0) and stearic (C18:0), appear to have significant metabolic roles in determining body weight, milk yield and milk composition responses to dietary fat supplementation. Increased knowledge of how C16:0 and C18:0 are involved in lipid metabolism and regulation in dairy cows will aid in strategically feeding FA supplements for improved production performance and efficiency.

Palmitic (C16:0) and C18:0 acid are similar chemically, both being a saturated FA and only differing by only 2 carbon units. Palmitic acid is a common saturated FA found in plants, animals and many microorganisms. Palmitic acid is usually the FA found in the highest quantity in milk fat. Stearic acid also is prevalent in nature and generally associated more with animal fats than vegetable fats. Palmitic and C18:0 are considered rumen inert fats with little or no effect on rumen microbiology because of being saturated. Quantities of C18:0 leaving the rumen are many fold higher than the amount fed, whereas C16:0 amounts leaving the rumen are similar to the amount fed (Wu et al., 1991). Biohydrogenation of long chain polyunsaturated FA, oleic (18:1), linoleic (C18:2) and linolenic (C18:3) make a significant contribution to the amount of C18:0 leaving the rumen. The amount of C18:0 leaving the rumen is often greater than 40% of the total FA flow into the small intestine. As a result, digestibility of C18:0 will be greatly under estimated and C18:1 (oleic), C18:2 (linoleic) and C18:3 (linoleic acid) overestimated when only amounts fed and excreted are considered. Several papers and reviews have arrived at relatively similar digestibility coefficients for C16:0 and C18:0, ranging in the mid 70 percent, and determined there is no difference in digestibility of C16:0 and C18:0 (Loften et al., 2014). However, a few studies have reported a lower digestibility for C18:0 than C16:0. Glasser et al. (2008b) found absorption of C18:0 was a quadratic function of its duodenal flow with decreasing apparent absorption when intake of C18:0 was greater than 50 g/kg of DM intake. Lysolecithin availability and micelle formation (Freeman, 1969; Doreau, 1992) or possible saturation

of intestinal absorption sites (Kucuk et al., 2004) are possible reasons for the diminishing digestibility of C18:0 at high intakes.

A high percentage of the FA entering the small intestine of ruminants are saturated and unattached to a triacylglycerol. Various isomers of C18 FA formed from incomplete biohydrogenation of long chain polyunsaturated FA in the rumen also exit the rumen as free FA. These isomers of C18 are important lipid fractions that are very bioactive and interact with C16:0 and C18:0 metabolism in adipose tissue and milk fat synthesis. Only free FA are absorbed from the intestine. Once absorbed across the intestine, FA circulate as free FA bound to serum albumin or incorporated into very low-density lipoproteins (VLDL), high-density lipoproteins (HDL) or chylomicrons. Lipoprotein lipase (LPL) releases FA from these lipid moieties in tissues and cells. Adipose, mammary gland, heart and skeletal muscle have high activities of LPL (Drackley, 2000).

Adipose Tissue Metabolism

Ruminant adipose tissue is not static and is metabolically active in both lipogenesis (synthesis and storage of FA in fat tissue) and lipolysis (release of FA for oxidation during negative energy balance). The FA found in highest concentration in adult ruminant adipose tissue are oleic acid (C18:1) followed by C16:0 and then C18:0 (Choi et al., 2013). Leat (1975) showed the FA composition of fat depots in Jersey cattle changed from birth to 2 years of age. Biopsies of the same cattle from birth to two years of age found C18:0 constant at 20% of the FA in adipose tissue the first year of life and then declined to 5% between 1 to 2 years of age. The percentage of C16:1 and C18:1 in adipose tissue increased as C18:0 decreased.

The FA in adipose cells come from both diet and de novo synthesis. Fatty acids of diet origin in adipose tissue are short and medium chain FA up to C16:0. Dietary C18:0 is very poorly absorbed into adipose cells and is a poor substrate for esterification or synthesis of FA in cells (Sampath and Ntambi, 2005). De novo synthesis of FA in adipose cells begins by adding a carboxyl group to acetyl-CoA to form malonyl-CoA. Fatty acid chain length occurs through two carbon unit additions (acetyl-CoA units) to malonyl-CoA yielding even numbered chain FA. Acetate is the origin of acetyl-CoA. Dietary short chain FA can be directly utilized in adipose cells for FA synthesis and are the primary source of de novo synthesized odd chain number and short to medium chain FA. However, C16:0 is the main FA synthesized in adipose cells. Synthesis of FA beyond C16:0 does not occur, but through a family of elongation enzymes (ELOV), C18:0 is produced from C16:0. Stearic acid is then desaturated to C18:1 by the enzyme stearoyl-CoA desaturase. The primary purpose of desaturation is to regulate fluidity of adipose cells from a buildup of high melting point (solid) C18:0 and loss of membrane integrity. Thus, C18:1 is the predominant FA stored in ruminant adipose tissue.

Membrane integrity and fluidity are also maintained in adipose cells through desaturation of C16:0 to C16:1 (palmitoleic acid). Guo et al. (2007) found a buildup of C16:0 in mouse adipose tissue caused endoplasmic reticulum stress and apoptosis (cell death) of cells that was reduced, but not eliminated, by desaturation of C16:0 and C18:0. Burns et al. (2012) found similar results in ruminant adipose tissue cultures and established C16:1 as a regulator of lipogensis, desaturation and apoptosis in adipose cells. The practical implication of decreased adipogenesis with higher amounts of C16:0 through either dietary sources and/or de novo synthesis in adipose cells is potential weight loss. In short term feeding studies, both Warntjes et al. (2008) and Piantoni et al. (2013) reported numerical decreases in body condition score of cows fed C16:0 compared to cows fed control diets.

Another factor entering into the complexity of FA synthesis in adipose tissue is the role of *trans* isomers of conjugated linoleic acid (CLA). Both Kadegowda et al. (2013) and Choi et al. (2014) showed 18:2 *trans*-10, *cis*-12 CLA depressed de novo FA synthesis in adipose tissue, but not gene expression for FA synthesis. Similarly, Wang and Jones (2004) reported various isomers of CLA can reduce fat deposition and body fat content in animals and specifically the *trans*-10, *cis*-12 isomer may induce insulin resistance and fatty liver in mice. The effects of the CLA isomers on FA synthesis

are important as diets favoring incomplete biohydrogenation of unsaturated FA in the rumen may work in conjunction with high C16:0 to depress FA synthesis in adipose tissue.

Non Esterified Fatty Acids (NEFA) and Liver Metabolism

At and following parturition, cows are in a negative energy balance. To meet energy requirements, lipoprotein lipase and triglyceride lipase are activated to release FA from adipose tissue. A logical assumption would be plasma NEFA profile would reflect the FA composition of adipose tissue. However, C18:0 was reported by Contreras et al. (2010) to be the highest FA in NEFA at about 41 g/100 g with C16:0 being about 28 g/100 g followed by C18:1 at about 8 g/100 g. In contrast, Douglas et al. (2007) observed weight percentages of C16:0, C18:0 and C18:1 in plasma were similar during the dry period, but following parturition and during negative energy balance, C16:0 and C18:1 increased whereas C18:0 decreased. Reason for the differences in FA composition of NEFA are unknown, but the increase in circulating saturated FA has been suggested by White et al. (2011) to increase expression of pyruvate carboxylase mRNA. Pyruvate carboxylase plays an essential role in many metabolic pathways including gluconeogenesis, lipogenesis, amino acid metabolism and neurotransmitter synthesis. Stearic acid particularly is a major regulator of pyruvate carboxylase promoters and changes in plasma C18:0 concentrations at calving may contribute to increased pyruvate carboxylase, enhancing gluconeogenesis. These data suggest that C18:0 contributes to the partitioning of energy during periods of negative energy balance.

Grummer (1993) showed that prior to and shortly after parturition, plasma NEFA concentrations lead to increased hepatic uptake of FA, their subsequent esterification, and accumulation of triacylglycerols in the liver. The metabolism of NEFA in the liver is primarily controlled by supply of NEFA to the liver and the uptake of NEFA through the activity of carnitine palmitoyl transferase (Drackley, 1999). Accumulation of triacylglycerol in hepatocytes inhibits glucose synthesis. Rukkwamsuk et al. (2000) showed concentrations of C16:0, C18:1 and C18:2 in liver increased significantly during the first week postpartum of cows fed a high energy diet (23 Mcal NE-L/day) prepartum. Liver C16:0 concentrations decreased in week 3 postpartum of cows fed a restricted energy diet (10.5 Mcal NE-L/day) prepartum. Conclusion from the Rukkwamsuk et al. (2000) study was concentrations of C16:0, C18:0 and C18:1 increase in blood and then in liver, with the exception of C18:0, during early postpartum negative energy balance. Litherland et al. (2011) found feeding of moderately excessive energy diets (150% of requirement) early in the dry period affected C16:0 metabolism in the liver at parturition. Cows fed the moderate energy diet early in the dry period had a decreased capacity for C16:0 oxidation in the liver at parturition favoring deposition of C16:0.

Mashek and Grummer (2003a) observed no net uptake of C18:0 in caprine liver when C16:0 and C18:0 were infused into the liver. However, C16:0 uptake was significantly increased compared to C18:0. Mashek and Grummer (2003b) also observed a two-fold increase in C16:0 metabolism when C18:0 was added to bovine liver cell cultures compared to C16:0 alone. Thus, added C18:0 may aid in the removal of excess C16:0 in liver cells. Sato and Inoue (2006) observed similar increases of C16:0 in liver, subcutaneous adipose, and perirenal adipose tissues of cows with fatty liver, with C18:0 decreasing in liver and adipose tissue following parturition. These data indicate that C18:0 does not accumulate in tissues of cows in negative energy balance and cows metabolize C18:0 for energy, e.g. beta oxidation, in the liver and muscle and/or secrete large proportions of C18:0 through milk as both C18:0 and C18:1.

During the early postpartum period when cows are in negative energy balance, feeding a lipogenic (fat promoting) diet may contribute to significant negative health effects, notably fatty liver syndrome. Such an effect was found by van Knegsel et al. (2007) when a lipogenic diet (included 2% C16:0 in concentrate DM) was fed pre- and post-parturition compared to a glucogenic (glucose promoting) diet. The glucogenic lactation diet was 26.6% starch and 3.1% fat compared to 10.4% starch and 5.0% fat for the lipogenic diet. Multiparous cows fed the lipogenic diet had similar plasma NEFA, plasma BHBA, plasma cholesterol, and liver triacylglycerols in the prepartum period as cows fed the glucogenic diet. Following parturition, cows fed the lipogenic diet had higher plasma NEFA, plasma

BHBA, plasma cholesterol, and liver triacylglycerols than cows fed the glucogenic diet. Higher concentrations of fat metabolites and liver triacylglycerol in cows fed the lipogenic diet were characteristic of cows with fatty liver (>100 mg/g of wet liver weight) and ketosis, which were observed in the lipogenic fed multiparous cows, but not in cows fed the glucogenic diet. Milk production was not different between the two diets, but milk fat percentage was higher for cows fed the lipogenic diet compared to the glucogenic diet.

Stearic acid may be better oxidized by the liver or used as an energy source during late prepartum and early postpartum periods than C16:0. Karcagi et al. (2010) reported that feeding a diet containing hydrogenated palm oil triacylglycerol, which was high in C18:0 (69% C18:0 and 23% C16:0), provided a better energy supply for high-yielding dairy cows in negative energy balance than calcium salts of palm oil FA (33% C16:0 and 4% C18:0). Diets were fed for 25 d prepartum and at 5 d prepartum, there was significantly less triacylglycerol accumulated in the liver of cows fed the palm oil high in C18:0 than either the control (no fat) or calcium salt of palm oil FA. At 5 d postpartum, liver triacylglycerols were 6 times higher in control (no fat) fed cows, 5 times higher in cows fed calcium salt-FA with C16:0, but only 2 fold higher in cows fed the high C18:0 fat supplement then values measured 25 d prepartum. At 25 days in milk, liver triacylglycerols were approximately one-half the concentration measured 5 d postpartum with no difference between cows fed the two fat supplements. However, cows fed the palm oil high in C18:0 consumed an average of 2.1 kg/d more diet DM and produced 7.6 kg/d more 4% FCM during the first 100 d postpartum than cows fed the calcium salt C16:0 fat supplement.

Milk and Milk Fat Synthesis

Very few studies have looked at feeding only a single purified form of either C16:0 or C18:0 to lactating dairy cows. A summary of the lactation study results with feeding either C16:0 or C18:0 are in a companion paper "Lactation Responses to Dietary Supplementation with Palmitic or Stearic Acid" by J. Loften and M. Sellers in this conference proceeding. Readers are referred to this paper for lactation production information. The metabolic role C16:0 and C18:0 have in production of milk and milk fat will be briefly reviewed in this paper.

Fifty percent of the FA in milk are synthesized de novo, 40 to 45% are diet origin, and less than 10% are from adipose tissue and NEFA. Short to medium chain FA (C4 to C14) are synthesized de novo in the mammary gland from blood acetate and beta hydroxyl butyrate (BHBA). Palmitic acid can be both synthesized de novo or taken up directly from the blood. Long chain FA, predominantly C18 FA, in milk fat are derived from plasma triacylglycerols (Moore and Christie, 1979; Palmquist et al., 1969).

Palmitic acid and C18:0 are involved in the synthesis of milk and/or milk fat. Both FA can be oxidized to supply energy for overall synthesis of milk and milk components. Actual and quantified contributions of these 2 saturated FA to the general energy supply for milk synthesis has not been shown. However, indirect evidence through variable responses across studies in milk yield, (increased: Piantoni et al., 2013; Steele, 1969; or no change: Steele and Moore, 1968; Lock et al., 2013) and body weight/body condition score (no change: Rico et al, 2013; Piantoni et al., 2015; decrease: Piantoni et al., 2013) provide strong evidence bioactivity of these two saturated FA exists beyond changes in milk fat. One such effect proposed is the oxidation of FA for energy, sparing glucose for increased milk (lactose) synthesis. The partitioning of FA into different metabolic processes is complexed by carbohydrate level in diets and energy balance status of the cow. Stearic acid is likely to have a stronger glucose sparing effect as it has 2 more carbons than C16:0 and will yield about 14 more molecules of ATP through complete beta-oxidation than C16:0. An extensive review of the exchange between glucose and FA metabolism has been done by Hue and Taegtmeyer, 2009.

The concentration and composition of FA in milk can be altered by feeding C16:0 and C18:0 (Moate et al., 2007). Several early studies found a significant increase in the concentration of C16:0 in milk

fat when a highly concentrated source of C16:0 was either fed (Steele and Moore, 1968a; Noble et al., 1969) or infused (Enjalbert et al., 1998, 2000). Corresponding to the increase in C16:0 were decreased concentrations of de novo synthesized FA, C18:0, C18:1, C18:2, and C18:3 in milk fat. Recent studies from Michigan State University (Lock et al., 2013; Piantoni et al., 2013; Rico et al., 2013) have shown similar reductions in concentrations and/or yields in de novo synthesized FA and preformed (C18:0 and greater) FA in milk fat with feeding C16:0. Consistent in all studies where high concentration sources of C16:0 have been fed is an increase in C16:0 and C16:1 in milk fat. Because C16 in milk can originate from de novo synthesis as well as transfer from blood (dietary source), the efficiency of dietary C16:0 for milk fat synthesis is difficult to quantify. The most recent studies from Michigan State University indicate a diet to milk transfer efficiency between 17 (Piantoni et al., 2013) and 27% (Lock et al., 2013).

When only C18:0 is fed as a fat supplement, C16:0 in milk fat is decreased compared to a control-unsupplemented diet (Moore et al., 1969; Noble et al., 1969; Piantoni et al., 2015) with de novo synthesized FA unaffected (Piantoni et al., 2015) or decreased (Noble et al., 1969). Supplementing C18:0 into diets, increases C18:0, and C18:1 in milk fat (Noble et al., 1969; Piantoni et al., 2015) compared to a control-unsupplemented diet. Determining the efficiency of C18:0 transfer from diet to milk fat is complicated by the conversion of C18:0 to C18:1 in the mammary gland and proportions of C18:0 and C18:1 coming from biohydrogenation of unsaturated long chain FA in the rumen. Piantoni et al. (2015) calculated a dietary transfer of C18:0 into milk fat C18:0 + C18:1 of 8.2%, which is significantly lower than the 50% shown by Enjalbert et al. (2000) with intestinal infusion of C18:0. Both size and hardness of the FA prill can affect digestibility and as noted by Piantoni et al. (2015), the larger prill size and likely hardness of the purified source of C18:0 fed in their study reduced digestibility and calculated transfer values below those reported in other studies.

The changes in milk fat composition with feeding C16:0 and C18:0 are important indicators of how these two saturated FA are utilized in dairy cows. The research by Rico et al. (2014) where C16:0 and C18:0 were directly compared illustrates their difference in metabolism. Negative energy balance was not a factor in this experiment as cows averaged 143 days in milk. This study also was conducted as a crossover experimental design with a short term experimental period (21 day treatment period) which makes changes in body weight and condition score difficult to detect. Each of the two FA were fed at 2% of their respective dietary treatment dry matter. Dry matter intake (DMI) and milk production were not different ($P > 0.2$) between the treatments (**Table 1**). Feeding a highly concentrated C16:0 fat supplement increased milk fat percentage and as a result increased (1.48 vs. 1.40) feed efficiency (3.5%FCM/kg DMI) with no difference in milk or DMI. Palmitoleic (C16:1) was increased with feeding C16:0 and, as described earlier in this paper, this FA causes apoptosis in adipose cells which correlates with the higher NEFA and a tendency for a decline in body condition score and body weight in C16:0 fed cows. A higher diet to milk fat transfer coefficient for C16:0 (≈ 22%) compared to C18:0 (≈ 8%) indicates greater excretion of dietary C16:0 into milk fat and less metabolic utilization compared to C18:0. Feeding C18:0 did not support as high of milk fat percentage as C16:0 and therefore, cows fed C18:0 had a lower calculated feed efficiency. Applying the same metabolism logic to C18:0 as was used for C16:0 suggests C18:0 is likely used more for energy throughout different tissues in the body than C16:0 as indicated by less direct incorporation into milk fat, the tendency for body weight and condition increase and a higher milk protein percent. The higher milk protein suggests C18:0 may have spared glucose in energy metabolism allowing more to be utilized in other functions such as milk protein synthesis. The lower ($P < 0.04$) plasma glucose concentration (55.7 vs. 56.6 mg/dL) in C18:0 fed cows compared to C16:0 fed cows may or may not support this utilization theory.

Table 1. Production results from Rico et al. (2014) comparing purified sources of palmitic and stearic acid in diets of lactating dairy cows.

	Fat Supplementation – 2% diet DM		
Item	Palmitic Acid (C16:0)	Stearic Acid (C18:0)	P-value
Dry matter intake, kg/d	32.1	32.3	0.39
Milk yield, kg/d	46.6	45.8	0.22
Milk fat, %	3.66	3.55	0.01
Milk fatty acid (g/100 g)			
C16:0	36.7	31.2	0.001
C16:1 *cis*-9	1.81	1.62	0.001
C18:0	7.39	8.72	0.001
C18:1 *cis*-9	16.7	17.9	0.001
De novo, total	28.0	30.5	0.001
Milk protein, %	3.24	3.29	0.01
Body weight, kg	720	723	0.12
Body condition score	2.93	2.99	0.11
NEFA, µEq/L	96.3	88.2	0.008

Summary

Fatty acid utilization in lactating dairy cows is complex and complicated by the many metabolic functions within the cow and changes in these functions as the cow transitions from negative to positive energy balance. The two most prominent saturated FA (C16:0 and C18:0) in dairy cow metabolism have separate and synergistic functions in supporting the biology of the dairy cow. Increases in milk fat percent and yield and a likely decrease in fat synthesis within adipose tissue are the most commonly observed responses to feeding a diet high in C16:0 to lactating dairy cows. Stearic acid appears to have a lower magnitude of response in production functions, but has a broader utilization as an energy source, or glucose sparing, in several metabolic reactions (milk synthesis, milk components and body weight gain). Hence, feeding a combination of C16:0 and C18:0 seems warranted to optimize milk production and overall performance of the dairy cow.

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Lactation Responses to Dietary Supplementation with Palmitic or Stearic Acid

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Take Home Points

- C16:0 supplementation to lactating cow diets has increased milk fat % and decreased DMI in 50 percent of recent trials.
- C16:0 supplementation results in increased C16:0 yield in milk, but reduces yield of de novo synthesized short chain FA as well as yield of preformed long chain FA.
- C16:0 in excess may alter lipogenesis in the mammary gland and adipose tissue by inhibiting key enzymes involved in de novo fatty acid synthesis.
- C18:0 supplementation to lactating cows has demonstrated increased DMI in recent trials.
- C18:0 may partition energy via sparing glucose and positively influencing gluconeogenesis, resulting in increased glucose availability for lactose production and milk yield.
- C18:0 may aid in clearance of C16:0 in hepatocytes, which may decrease duration of satiety signals that result from excess hepatic oxidation, resulting in improved DMI.
- Feeding a combination of C16:0 and C18:0 may be warranted to optimize overall dairy cow performance.

Energy intake will continue to be the major nutritional challenge to lactation productivity of dairy cows. Dairy producers and nutritionists have increased the use of high energy feed ingredients such as fat in lactating dairy cow diets to meet this demand for more energy. Dry ruminally inert fat supplements have become common feed ingredients in diets because of their energy content and versatility of use on farms in grain mixes, mineral mixes, etc. Dry rumen inert fats usually contain high concentrations of long chain fatty acids (LCFA) with the most common being palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2). FA are not just a high quality source of energy, but have metabolically different functions in the cow and contribute to the productive function of cows in different ways. Research in recent years has begun to focus on feeding individual FA, many times without a firm understanding of the functions each FA has within the metabolism of the cow. C16:0 and C18:0 have received the majority of the interest since they are abundant in nature and one can acquire concentrated sources to conduct research trials. This paper will concentrate on the results of production trials where highly concentrated forms of C16:0 and C18:0 were tested. **Table 1** shows the results of several trials where highly purified palmitic acid was fed to lactating dairy cows with variable results.

As shown in **Table 1**, feeding highly concentrated C16:0 resulted in a decrease in DMI in 50% of the published trials. Milk production was significantly increased in only 1/3 of the trials. Milk fat (MF) % was significantly increased in only 50% of trials, while milk protein (MP) % was unchanged. The most concerning observation in these trials was the length of the experimental periods. Six of the trials used 21 days or less as experimental periods. One trial used 35-day periods and observed a significant decrease in MF% when highly concentrated C16:0 was compared to the control (3.60% vs. 3.75%). This trial also includes the most cows per treatment at 324. When the means were weighted based on the sample size for each trial, DMI was increased by 1.1 lb/d.

Table 1. Effect of feeding palmitic acid supplements to lactating cows on DMI, milk production and milk composition.

Study	DMI, lb/d	Suppl.[1] C16:0, g/d	Milk, lb/d	Milk fat, %	Milk protein, %	Cows/ treatment	Study length, d
Mosley et al. (2007)							
Control	51.3[a]	0	68.0[a]	3.44[a]	2.98	18	16
Treatment	58.1[b]	414	74.8[b]	3.93[b]	2.97	18	16
Warntjes et al. (2008)							
Control	57.6	0	80.7	3.75[a]	2.96	324	35
Treatment	58.1	384	83.6	3.60[b]	2.99	324	35
Rico and Harvatine (2014) Low cows							
Control	55.7[a]	0	63.4	3.86	3.19	24	14
Treatment	50.6[b]	394	63.8	3.92	3.14	24	14
Rico and Harvatine (2014) High cows							
Control	62.3[a]	0	91.3	3.14	3.14	24	14
Treatment	58.1[b]	449	92.4	3.22	3.17	24	14
Lock et al. (2013) Dry corn treatment							
Control	54.3[a]	0	70.4	3.88[a]	3.33[a]	16	25
Treatment	61.3[b]	361	70.4	4.16[b]	3.28[b]	16	25
Piantino et al. (2013)							
Control	61.2	0	98.8[a]	3.29[a]	3.11	32	21
Treatment	61.2	545	101.2[b]	3.40[b]	3.09	32	21
Garver et al. (2015)[2]	High	Starch	32%				
Control	58.3	0	84.5	3.93	3.29	32	21
Treatment	57.0	330	82.6	3.97	3.30	32	21
Garver et al. (2015)[2]	Low	Starch	16%				
Control	56.8		84.0	3.96	3.26	32	21
Treatment	59.0	342	85.1	4.06	3.34	32	21
Weighted Means							
Control	57.2	0	80.6	3.69	3.15	392	21
Treatment	58.3	398	82.1	3.79	3.15	392	21

[1]Intake of supplemented C16:0. All supplemented sources of C16:0 were > 85% C16:0.
[a,b]Means within a study and within a response category with different superscripts are different ($P < 0.05$).
[2]There was a significant interaction ($P<0.05$) between main effects starch level and fat supplementation for DMI, MF yield, MP% and MP yield, 3.5% FCM and ECM.

Mosely et al. (2007) observed a 7.1 lb/d increase in DMI when 500 g/d of C16:0 was fed, but higher feeding amounts of C16:0 (1,000 g/d and 1,500 g/d) did not increase DMI (54.3, 52.4, and 51.3 lb/d for control, 1000 g/d and 1500 g/d, respectively). The weighted means in **Table 1** for MY show a 1.5 lb/d increase, and MF% was 0.10% higher for the C16:0 supplemented diet when 398 g/d was added. That's over 1 lb/d of 85% C16:0 and is over twice the amount added to most commercial lactating cow diets. The transfer efficiency observed across these trials in **Table 1** is 16% (63.5 g more FA in MF yield vs. 398 g/d fed). Additionally, the Mcal required for 1.5 lb/d of MY is 0.53 Mcal in the 2001 NRC. Let's assume that each lb of purified C16:0 contains 2.7 Mcal of NE_L. The calculation would account for an additional 92 g of the C16:0 intake. That leaves 242.5 g of C16:0 intake per day unaccounted for, or 61% of the total intake. Where did the remainder go? We will discuss the possibilities a little later.

The increase in C16:0 in MF when highly purified C16:0 is fed was proposed to be due to an increase in de novo MF production as discussed by Hansen and Knudsen (1987). In their study, they found adding C16:0 to washed mammary tissue resulted in a sharp increase in de novo FA synthesis as measured by carbon labeled acetate incorporation into milk triglycerides (TG). However, Loften et al. 2014, proposed that mammary tissue attempts to maintain milk TG fluidity by increasing esterification of low melting point FA in the sn-3 position on the TG. That helps in explaining why C18:0 and C18:1 did not cause an increase in de novo synthesis due to the mammary tissue conversion of C18:0 to C18:1 by stearoyl CoA desaturase (SCD) in their study. **Table 2** shows the changes in FA yield in milk for the above trials where milk FA were measured. In all of the trials where highly purified C16:0 was fed, proportions of de novo FA (C4:0-C14:0) and C18 FA were decreased, while C16:0 concentration in milk increased markedly. However, the yield of de novo milk FA are not appreciably changed when highly purified C16:0 is fed.

Table 3 shows a more recent trial, De Souza et al. (2015) where different prill sizes of highly purified C16:0 were fed to lactating cows at 2% of the diet DM. No difference was observed on the effects of prill sizes, so the data was condensed for **Table 3**. These researchers observed no differences in DMI or MY, an increase in MF% of 0.26% and a decrease in MP% of 0.04%, $P<0.01$. De novo and preformed FA yield were significantly decreased by the addition of highly purified C16:0, while the yield of C16 FA were significantly increased, $P<0.01$. Total tract digestibility of total FA and C16:0 was decreased significantly when highly purified C16:0 was added to the diet at 414 g/d as expected. However, the digestibility of C18 FA was increased significantly with C16:0 supplementation, $P<0.04$.

Trials where purified C18:0 were fed are scarce. Boerman and Lock, 2014 fed highly purified C18:0 at 0, 0.8%, 1.6%, and 2.4% of the diet DM. They observed a significant linear increase in DMI, without changes in MY, MF%, MP%, or yield of milk components. The important observation in this trial was the increase in DMI when cows were supplemented with C18:0. We will discuss this result later. In another trial where highly purified C18:0 was fed (98% purity) to high producing dairy cows, surprising observations were made. DMI, MY, FCM, ECM, MF yield, MP yield, and lactose yield were all significantly improved at the $P=0.02$ level or less when 522 g/d of 98% C18:0 was fed. In fact, DMI was increased by 2.0 lb/d and MY was increased by 3.7 lb/d. It was also observed that the higher producing cows improved in MY more than the lower producing cows. Interactions indicated that differences between treatments for intakes and absorption of FA in cows with lower milk production were less than for cows with higher milk production (Piantoni et al., 2015). Milk FA yields for denovo FA, mixed C16 FA, and preformed FA were all significantly improved, unlike with high C16:0 supplementation trials. Total FA digestibility and C18:0 digestibility were significantly decreased with C18:0 supplementation.

Table 2. The effects of feeding supplements high in palmitic acid on milk fatty acid yields.

Study	Milk FA		Treatment Control	Treatment C16:0 Fed	+/-	P Value
Mosley et al. (2007)						
	C4-C15	g/d	212	241	29	NS
	C16:0+C16:1	g/d	321	521	200	<0.001
	C18:0+C18:1	g/d	314	340	26	NS
	Total	g/d	847	1102	255	
Warntjes et al. (2008)						
	C4-C15	g/d	238	237	-1	NS
	C16:0+C16:1	g/d	347	399	52	NS
	C18:0+C18:1	g/d	502	473	-29	NS
	Total	g/d	1087	1109	22	
Piantoni et al. (2013)						
	C4-C15	g/d	408	395	-13	0.05
	C16:0+C16:1	g/d	472	565	93	<0.001
	C18:0+C18:1	g/d	373	369	-4	NS
	Total	g/d	1253	1329	76	
Lock et al. (2013)						
	C4-C15	g/d	287	267	-20	NS
	C16:0+C16:1	g/d	419	548	129	<0.001
	C18:0+C18:1	g/d	341	335	-6	NS
	Total	g/d	1047	1150	103	
All trial summary						
	C4-C15	g/d	286	285	-1	
	C16:0+C16:1	g/d	390	508.25	119	
	C18:0+C18:1	g/d	383	379.25	-3	
	Total	g/d	1059	1172.5	114	

Table 3. The effects of prill sizes and C16:0 supplementation on milk yield, milk components, milk FA yields and nutrient digestibility. (Adapted from De Souza, et al., 2015)

	Control	Fat	P <
C16:0 fed, g/d	0	414	--
Milk Production			
DMI, lb/d	58.5	57.0	0.26
Milk Yield, lb/d	77.0	77.0	0.98
Milk Fat, %	3.99	4.25	0.01
Milk Protein, %	3.38	3.34	0.01
Milk FA Yield, g/100g FA per day			
de novo synthesis (C4 - C14)	297	251	0.01
mixed origin (C16:0, C16:1)	378	457	0.01
Preformed (C18 - C22)	430	383	0.01
Total Tract Digestibility, %			
Total FA	80.3	76.6	0.01
C16	79.1	72.3	0.01
C18	81.6	82.5	0.04

Table 4. The effects of stearic acid supplementation on milk yield, milk components, milk FA yields, and nutrient digestibility. (Adapted from Piantoni, et al., 2015)

		Treatment			
Item		Control	Stearic Acid[a]	+/-	P LEVEL
DMI	lb/d	55.4	57.4	2.0	<0.01
Yield					
Milk	lb/d	84.7	88.4	3.7	0.02
Milk fat	g/d	1350	1420	70	<0.01
Milk protein	g/d	1140	1190	50	0.02
Lactose	g/d	1870	1960	90	0.02
3.5% FCM	lb/d	84.0	89.1	5.1	<0.01
ECM	lb/d	84.0	88.2	4.2	<0.01
Composition					
Milk fat	%	3.60	3.59	-0.01	NS
Milk protein	%	3.00	2.99	-0.01	NS
Lactose	%	4.83	4.86	0.03	NS
Milk FA					
C4-C15	g/d	344	359	15	<0.0001
C16:0+C16:1	g/d	451	461	10	<0.01
C18:0+C18:1	g/d	352	393	41	<0.001
Total	g/d	1147	1213	66	<0.01
Transfer efficiency	%		12.9%		
Total FA digestibility	%	76.1	56.6	-19.50	<0.0001
16 C	%	76.2	75.8	-0.40	0.79
18 C	%	79.1	55.3	-23.80	<0.0001

[a]Included into diet at 2% of the DMI or 522 g/d of 98% C18:0 per day

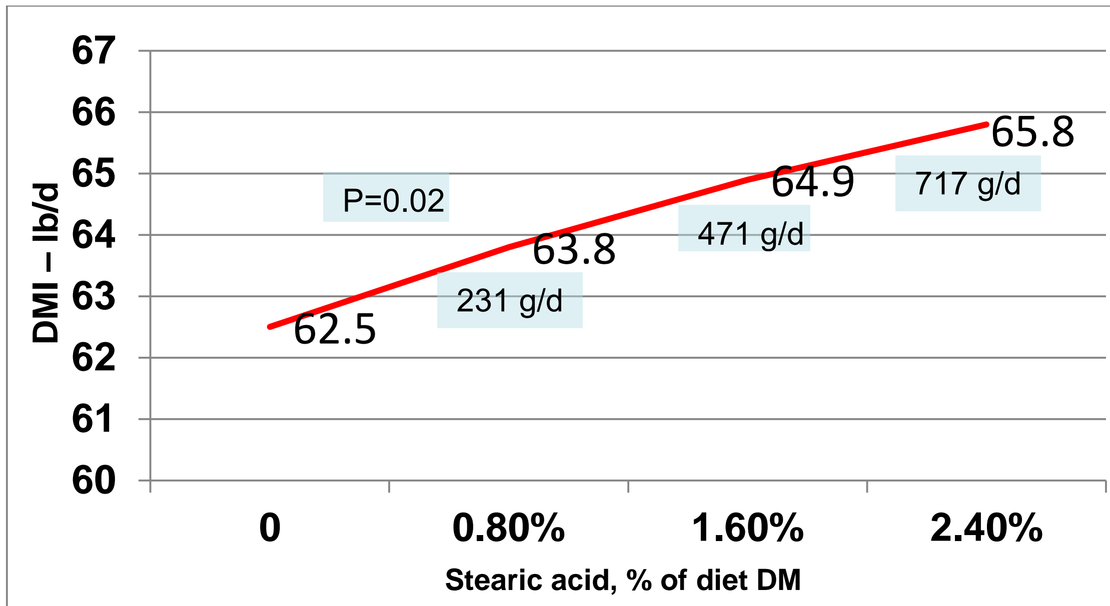

Figure 1. The effects of supplementing highly purified stearic acid on dry matter intake in lactating cows. (Adapted from Boerman and Lock, 2014).

Relationship between palmitic and stearic acid metabolism and production trial results

The production trials using highly purified palmitic acid have demonstrated improvements in MF% and MF yield in roughly 50% of trials. The C16:0 in milk comes from both mammary de novo synthesis as well as from exogenous sources. From the production trials, the increase in C16:0 in milk fat does not appear to be driven by increased de novo synthesis. Therefore, the increase in palmitic acid in milk fat must be primarily derived from exogenous sources. Each milk TG must have a melting point at or below the temperature of the cow. Noble et al. (1969) proposed that excess C16:0 decreases availability of de novo synthesized FA by inhibiting acetyl-CoA carboxylase (ACC) and by inhibiting fatty acid synthase (FASN) activity (Burns et al., 2012). Since C16:0 has a transfer efficiency from diet to milk FA of approximately 20% and approximately half of palmitic acid intake cannot be accounted for, the remainder must be either transported to adipose tissue for deposition or to the liver to be oxidized as fuel. Palmitic acid, fed in excess, has been shown to inhibit lipogenesis in adipose tissue by inhibiting ACC and FASN activity (Burns et al., 2012). Palmitic acid decreases activity of SCD in adipose tissue, which when combined with decreased de novo synthesis, results in reduced ability to maintain membrane fluidity. In turn, decreased membrane fluidity leads to increased stress on the endoplasmic reticulum and cell death via apoptosis. Palmitic acid appears to have upper thresholds in concentration of adipose tissue due to its inhibition of several lipogenic enzymes. The remainder of C16:0, that cannot be used by either mammary or adipose tissue must therefore be transported to the liver for oxidation or to other tissues. In the liver, increased C16:0 oxidation can lead to intake depression in accordance with the Hepatic Oxidation Theory (HOT) as proposed by Allen et al. (2009). Interestingly, Pu et al. (2011) observed that C16:0 increases translocation of the GLUT4 glucose transporter to the cell surface in skeletal muscle cells, resulting in increased glucose uptake in skeletal muscle tissue. A decrease in blood glucose means less glucose is available for lactose production in the mammary gland, and milk yield will be decreased. Decreased glucose availability means that tissue must oxidize FA for energy, resulting in reduced body fat reserves and body condition score, which have been documented in the field.

The C18:0 production trials have demonstrated increased DMI. White et al. (2011) proposed circulating FA that are characteristically increased in transition cows may contribute to increased expression of pyruvate carboxylase (PC) to stimulate gluconeogenesis. Stearic acid was shown to regulate PC promoters P1, P2, and P3 in various tissues. Stearic acid, therefore, appears to be glucose sparing, while C16:0 appears to act conversely by increasing glucose uptake by skeletal

muscle cells. These data suggest that C18:0 contributes to the partitioning of energy during periods of upregulated gluconeogenesis, increased hepatic FA supply, or both (Loften et al., 2014). Mashek and Grummer (2003) observed a two-fold increase in C16:0 metabolism when C18:0 was added to bovine cultures compared to addition of C16:0 alone. This may aid in removal of excess C16:0 from hepatocytes, potentially alleviating the negative effects of C16:0 oxidation on DMI by decreasing the duration of the satiety signal to the brain. By sparing glucose and improving DMI, C18:0 may improve MY, lactose yield, as well as milk FA yield. Because C16:0 may improve MF% and may reduce DMI, the supplementation of C18:0 in conjunction with C16:0 provide optimal results in terms of DMI, MY, and components.

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