Detecting and Abating Heat Load in Dairy Cows

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Summary

Heat stress is widely understood to be an important, practical challenge in dairy production. Two key aspects of managing this challenge on farm are discussed: detecting increased heat load and abating it before consequences occur. We focus on tools for use on farms, based on research and insights from the cows. We suggest that monitoring the responses of mature animals informs best management practices across all types of dairy systems.

Introduction

Heat stress is a challenge for both cows and producers. Many dairy farms are located in regions that regularly have warm weather conditions for several months of each year. In the summer or warm conditions, cows will accumulate heat load, and if they are unable to dissipate this heat, consequences include reduced milk production, impaired fertility, and in extreme cases, death (St-Pierre et al., 2003). In addition to these well-studied biological and financial implications, there is also evidence that the animals face other welfare challenges, including pain associated with higher rates of lameness in summer months (DeFrain et al., 2013), higher somatic cell counts, and impaired immune function. It is also possible that cattle experience breathlessness during periods of rapid breathing and panting (Mellor and Stafford, 2004), although this potential aspect has received little attention to date. Finally, there are also longer-term implications for other animals on the farm. Calves are affected when dams experience high heat load during gestation.

Heat stress results from an accumulation of heat load within the animal. An animal-centered approach to understanding heat stress provides insights specific to the management and conditions of each farm, in all types of dairy systems, regardless of how abatement is provided. Rather than an endpoint- is she heat stressed or not? - we find it useful to think about the progression of responses. The exact timing and thresholds for each response are often not known, but in general, cows try to dissipate heat by increasing respiration rate, sweating, seeking shade or other abatement, spending more time standing, eating less and drinking more (Table 1). If these responses are unsuccessful, then consequences set in: body temperature rises, often above fever thresholds, milk production is reduced and fertility is compromised. Often, the consequences, like a drop in bulk tank milk or pregnancy rates, tells the producer that a problem has already occurred. Cows were not able to stay cool. By focusing on the initial responses instead, we are able to prevent longer-term consequences for the producer and cows.

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Detecting Heat Load

Body temperature

Elevated body temperature is a clear sign that cattle are accumulating heat load. Monitoring tools, such as intra-vaginal or rumen-based loggers, can provide insights into "pinch points" for heat stress on a dairy. Loggers may be incorporated into regular monitoring systems on the farm, or a consultant or veterinarian may conduct a heat stress evaluation for the farm using this type of technology. Monitoring body temperature over several days in the summer provides valuable information and is justified for several reasons. First, measuring rectal temperature once or a few times per day does not capture what happens for a cow over a 24-h period (Tresoldi et al., 2019). We find that more frequent monitoring, at least every 2 hours, is required to capture a fuller picture. Secondly, using loggers to gather body temperature information allows us to collect data over days, even at night, when cows may be less likely to be observed by the producer or employees. This fuller picture from the body temperature loggers allows us to identify when cows accumulate heat load and tells us what and where abatement is needed. An example of 24-hour body temperature patterns from the UC Davis dairy are provided in Figure 1.

Examples of specific "pinch points" could include, but are not limited to:

- Body temperature rises when cows wait for milking, but not at other times, then investment in cooling in the crowd pen may be warranted,
- Cows are not cooling down overnight, then next step may be to examine cooling in the home pen, where the cows rest, and
- In pasture-based systems, the walk to or from the parlor could be identified as an area that needs attention, based on a rise in body temperature during this activity.

Temperature thresholds that define fever are as low as 102°F/38.9°C (Hillman et al., 2005) to as high as 104°F/40°C (Burfeind et al., 2012; Pohl et al., 2014), encompassing values within this range: 102.6°F/39.2°C, 102.9°F/39.4°C, 103.1°F/39.5°C, and 103.5°F/39.7°C (summarized by Tresoldi et al., 2019). These fever thresholds aid interpretation of data collected with loggers. It may be useful to look at if fever levels are reached, due to heat stress, and how many hours cows experience these levels of elevated body temperature.

Respiration rate and signs of panting

Increasing respiration rate is a flexible response cows use to reduce heat load. It can be measured by counting flank movements, that is, one full breath includes both the inward and outward motion. Various tools, including the free Thermal Aid app from University of Missouri (thermalnet.missouri.edu/ThermalAid) facilitate taking this information. We have found that we need to measure respiration rate every 90 min over the hottest part of the day to have a full picture of the heat load experienced by lactating dairy cows. Interpreting respiration rate is straightforward at the extremes. Thirty breaths/min is a cool cow; 100 breaths/min indicates she is hot. It is more difficult to interpret the values in between extremes. Information about when cows will choose to use heat abatement and when body temperature begins to rise inform our understanding of respiration rate: these changes occur between 50 and 80 breaths/min.

What is clear is once signs of panting are involved, cows are at the upper end of their attempts to cope and reduce heat load. Panting involves breathing with the mouth open. The tongue may or may not extend out of the mouth.

On California dairy farms, panting is seen at approximately 100 breaths/min (Tresoldi et al., 2016). Another component of panting is drool. We often see stringy drool before open-mouth panting begins. We think that it may be an indicator that cows are trying to cope and that it may be easier to measure than either respiration rate or body temperature. Current work at UC Davis is investigating the use of early signs of stringy drool as a measure of heat load in dairy cattle.

Environmental monitoring

To supplement an animal-centric approach, we can also measure aspects of the environment. Many recommendations about environmental monitoring focus on ambient air temperature, humidity, solar radiation (or black globe air temperature) and wind speed. The combination of these four measures (heat-load indices, HLI) or of air temperature and humidity (temperature-humidity indices, THI) are often referenced in literature about heat stress in dairy cattle. Authors often delineate clear thresholds using these metrics. The trend across the literature is that accumulated heat load begins to affect dairy cow behavior and production as soon as 71 to 73°F/22 to 23°C or THI of 65 to 68. The challenge with this type of environmental monitoring is that the key parameters (temperature, humidity, wind speed and solar radiation) are rarely all monitored on farm. This type of information about the environment is often available and useful in terms of predicting and preparing for heat-wave events in a given region. As technology becomes less expensive, it will become easier to incorporate these metrics into controllers for soakers, fans or other forms of cooling on individual dairy farms.

On farms, several other aspects of environmental monitoring are valuable to consider in an assessment of the dairy's heat stress management. Taking ground or bedding temperatures, with a point-and-shoot infrared gun or an infrared camera, can be useful. Bedding in freestalls with direct sun, for example, can get very hot and increase the "effective" stocking density of the pen in those hours by reducing the number of usable stalls or amount of usable space. Knowing this and raising the producer's awareness of this type of issue can inform how the beds are managed. In drylot dairy farms, we have found that the dirt surface, in full sun, can easily exceed 120°F/49°C. Unsurprisingly, cows in these systems spend less than 2 minutes out of the shade, likely because this unprotected environment is inhospitable (Tresoldi et al., 2017). Infrared images of cows before and after cooling can also be influential visual aids for understanding how well cooling strategies work.

Finally, as a consultant in these matters, it may be helpful to also evaluate how climate is taken into account for soakers or fan activation. If a person turns soakers on or off, discussions comparing the human vs. bovine thermal comfort zones (83 to 90°F/28 to 32°C for humans vs. 41 to 68°F/5 to 20°C) may be useful. By the time a human feels hot, a cow has already begun to accumulate heat and invest energy into dissipating it. Alternatively, if soakers or fans are controlled with a thermostat, it may be helpful to compare the microclimate of the controller location to where the cows are located. If the controller is located in a cooler corner of the barn, adjustments may need to be made to the activation temperature to match what the cows experience.

Abating Heat Load: What Cows Tell Us

Milk production and fertility: the problem has already occurred

When cows cannot dissipate heat load effectively, they produce less milk, their fertility

is impaired and in extreme cases, they die. By the time these consequences are apparent, the problem has already occurred. This is costly for the dairy producer in several ways: the direct cost of the problems described above, as well as indirect costs associated with reduced feed intake and efficiency and possibly also in terms of higher levels of lameness or claw lesions seen in summer. Higher culling rates associated with low milk production, failure to become and remain pregnant and other health issues are also an indirect cost. The cows also pay a price. They rest less, because they spend more time standing, possibly to increase air flow around their body. It is possible that resting less plays a role in predisposing cows to the higher rates of lameness seen in summer. Lameness is painful. Reduced immune function and higher somatic cell counts are related to other painful conditions, like mastitis. Little is known about what cows experience while panting or with high respiration rates. We have documented that they will assume a statue-like, inactive position at higher respiration rates (Tresoldi et al., 2017), but it is unknown what cows experience during this time.

Water cooling and shade: when cows prevent the problem

If cows have a choice, they will prevent heat load accumulation. This has been well described for shade. More recently, in several studies where we gave cows control over cooling with water, they began to use either soakers or a cow shower, when their respiration rates were 50 to 60 breaths/min (Legrand et al., 2011; Chen et al., 2013). By doing this, cows prevented the rise in body temperature seen in their counterparts that did not have access to or control over their cooling. Similarly, we have begun to monitor when cows stand at the feedbunk, fitted with soakers, but do not eat. More than 80% of cows at the UC Davis dairy are eating when they are at the bunk overnight or in the early morning, but in mid-afternoon and evening, when ambient conditions are warmest, our cows only feed about 50% of the time they are at the bunk (Tresoldi et al., 2019). These findings bolster what we already knew about shade: cows will seek cooling with water too, especially when combined with shade. Taken together, all of this information indicates that cows have "heat load" intelligence. They will prevent buildup of heat if we give them the opportunity.

Conclusion

An animal-centered approach to heat stress assessment will work across dairy types and climatic conditions. By focusing on responses like respiration rate, panting, cow behavior and body temperatures, we can optimize heat abatement on farms. Consultants and veterinarians providing this type of value-added service will benefit cows and the producer's bottom line.

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Table 1. Responses to dissipate heat and consequences if heat load continues to accumulate in dairy cattle.

Cattle responses to dissipate heat	Consequences if heat load accumulates
Increase respiration rate, pant[1]	Body temperature rises above fever levels[1]
Sweat	Milk production drops
Seek shade or other abatement[1]	More lameness, claw lesions; higher SCC, impaired immune function
Spend more time standing up, less active	Impaired fertility
Drink water	Death
Eat less[1]	

[1]Promising measures to use in an animal-centered approach to heat stress management

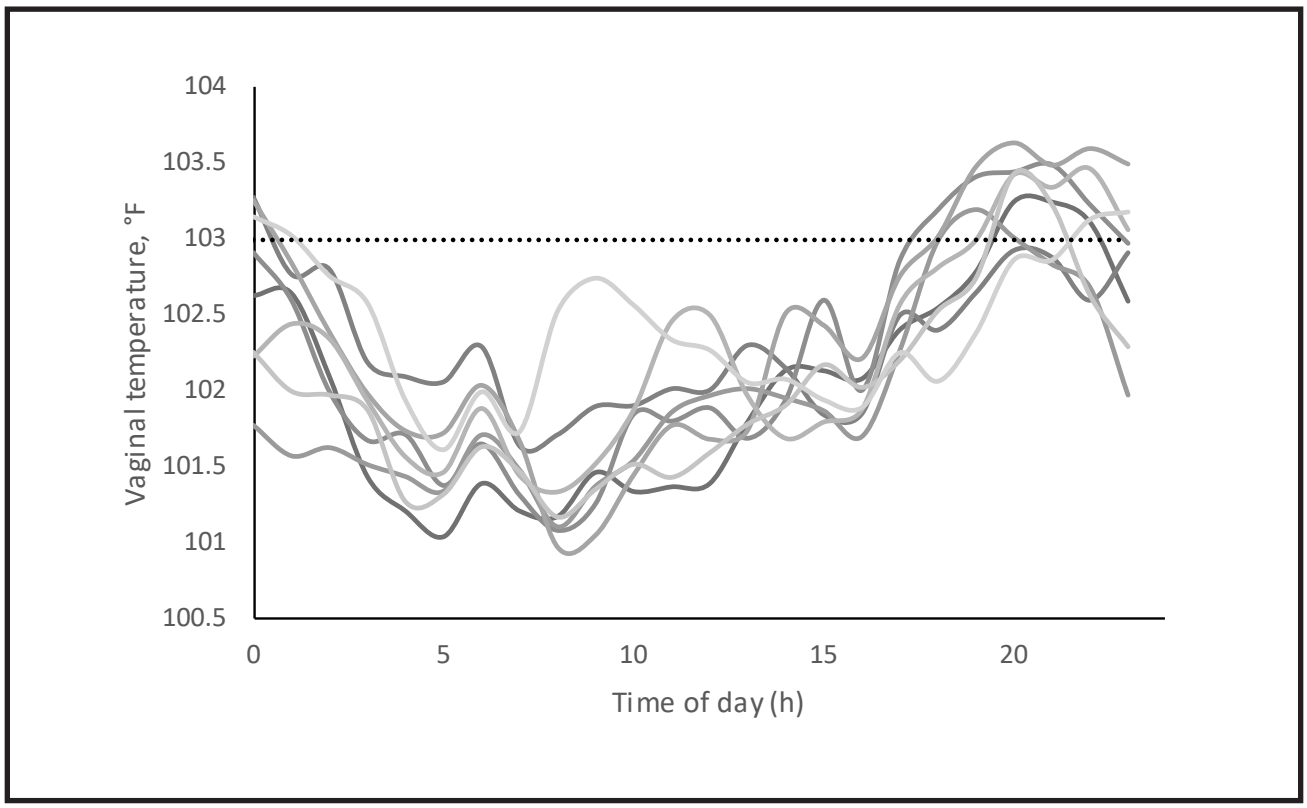

Figure 1. Vaginal temperatures (°F) for 8 cows over 24-hours on the UC Davis dairy. Each solid line represents an individual lactating cow. Several patterns can be seen in this graph. Cows cooled down by early morning and the effect of heat abatement (fans and water spray) while waiting to be milked (black arrow) is evident; all animals were milked at the same time. Depending on the fever threshold used, for example 103°F or the dashed line, some of these cows would be considered hot in the late afternoon or evening. Indeed, only some of the cows received adequate cooling in the evening because of experimental heat abatement treatments.

Optimizing the Role of Starch as an Energy Source for Dairy Cows

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Summary

Although starch is not considered a required nutrient, it is often a topic of discussion, primarily due to its high concentration in corn grain and silage. The objectives of this article are to present and discuss: 1) the effects of dietary starch concentration throughout the lactation, and 2) potential strategies to optimize digestibility of starch in feedstuffs and its utilization by lactating dairy cows. Potential negative effects on either milk yield or feed efficiency when reducing dietary starch exist and underscores that monitoring income over feed costs is recommended rather than price per unit of diet dry matter (**DM**) when corn prices are high to fully assess economic benefits of reduced-starch diets. Many factors alter starch digestibility of feedstuffs; mean particle is the most important factor in corn silage, corn grain, and high-moisture corn. However, on farm assessment is advised.

Introduction

Compared with other nutrients, starch was the most under evaluated research topic in dairy nutrition for many years. Consequently, starch recommendations for dairy cows were not established by the NRC (2001). Recently, improvements in the use of starch by lactating dairy cows garnered much interest by dairy farmers and their nutritionists; particularly over the past decade with the 2-fold rise in corn prices. Although starch is still not considered a required nutrient, it was highlighted as a very important factor for diet formulation during the 28th ADSA Discovery Conference – Starch. But despite not being considered a required nutrient, starch is often a topic of discussion, primarily due to its high concentration in corn grain (approximately 70% on a DM basis). Although other carbohydrates can be fed to dairy cows to supply and meet energy demands, carbohydrate sources differ in fermentation end-products produced by rumen microorganisms. Starch is rapidly fermented by rumen microorganisms into propionate. Propionate is absorbed into the bloodstream and transported to the liver, and later, it is used as a precursor for glucose. If not digested in the rumen, starch reaches the small intestine and is digested by pancreatic amylase directly into glucose. Thus, despite starch not having established requirements, its supplementation directly affects glucose supply and thereby, lactation performance of dairy cows. Consequently, starch utilization by lactating dairy cows became an important research topic. Thus, the objectives of the present article are to present and discuss: 1) the effects of dietary starch concentration throughout the lactation, and 2) potential strategies to optimize digestibility of starch in feedstuffs and its utilization by lactating dairy cows.

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Starch Concentration in the Diet

Although starch can be used throughout the entire lactation, its concentration or potential replacement viability is dependent of the stage of lactation. These effects are related to energy demand and metabolism during each stage. The most controversial period is the early lactation; few studies were conducted with fresh cows compared with the abundant available data for mid-lactation cows. During early lactation, cows require a diet balanced to support the extreme metabolic adaptations they undergo through calving. Briefly, there is a major limitation in feed consumption which severely reduces the energy available to meet the requirements of high-producing animals. Thus, it would be coherent to increase dietary starch concentration to minimize the period by which dairy cows remain in negative energy balance. However, in several herds, cows are fed controlled-energy close-up diets, which if combined with a fresh cow diet of high-starch concentration may negatively affect rumen health and metabolism. Based on 3 studies conducted in the northeast of the United States, McCarthy et al. (2015) suggested that perhaps the difference in starch levels between pre- and post-partum diets may be more important than specific dietary starch levels fed to fresh cows. In addition, it is important to formulate lower starch diets during the early lactation period with digestible carbohydrates so they do not limit intake because of gut fill or through the hepatic oxidation pathway (Allen et al., 2009).

As dairy cows reach the peak of their milk production and continue throughout their mid-lactation, energy requirements are still high, but the metabolic constraints of feed consumption are no longer a concern. Unless limited by gut fill, cows would adjust their consumption levels to attend to their energy demands. For example, a reduction in feed consumption and milk production were observed when corn silage partially replaced dry ground corn in the diet (26 vs. 32% of dietary starch, respectively) which is indicative of increased gut fill (Weiss et al., 2011). In contrast, studies replacing dry ground corn with soy hulls revealed similar milk production but greater intake for cows fed the reduced-starch diets; this is indicative of adjusted consumption to achieve the required energy intake.

A recent review used a meta-analysis approach to evaluate the effect of dietary starch on lactation performance by dairy cows (Ferraretto et al., 2013). Dietary starch values were considered for this study but not the specific type of carbohydrate used to replace starch. Starch concentration in the diet did not affect intake and this was thought to be related to 2 opposing effects: rumen fill limitation (Mertens, 1987) and increased ruminal propionate concentrations with corresponding decreased meal size (Allen et al., 2009) when corn grain was partially replaced by forage and non-forage fiber sources, respectively. Although milk yield increased 0.08 kg/day per %-unit increase in dietary starch content, feed conversion was unaffected by dietary starch. In addition, increased dietary starch concentration enhanced milk protein content. Reduced milk protein content for cows fed reduced-starch diets are related to lower starch intake reducing ruminal microbial protein production (Oba and Allen, 2003). Alternatively, lower amount of starch reaches the small intestine mediating milk protein content through alterations in arterial insulin concentrations (Rius et al., 2010). Conversely, however, milk fat content decreased as dietary starch content increased. Milk fat depression in high-starch diets is likely related to greater starch and lower NDF intakes (Jenkins and McGuire, 2006). The MUN concentration was also reduced by increasing dietary starch concentrations. Overall, these data suggest better

ruminal nitrogen utilization (NRC, 2001) as starch in the diet increases.

Another result of interest highlighted by the meta-analysis of Ferraretto et al. (2013) is the effect of dietary starch concentration on in vivo NDF digestibility. The digestibility of dietary NDF decreased 0.61%-units ruminally and 0.48%-units total-tract per %-unit increase in dietary starch content. Similarly to milk fat depression, decreased fiber digestibility may be partially explained by a decrease in rumen pH as a consequence of greater amounts of starch being digested in the rumen as starch intake increases. Low rumen pH is known to affect microbial growth and bacterial adherence and thereby fiber digestion. Also, the inherently high fiber digestibility of non-forage fibrous by-products used to partially replace corn grain in reduced-starch diets may be partly responsible. A meta-analysis by de Souza et al. (2018) used individual animal data instead of treatment means and observed a similar reduction of 0.59%-units in total tract NDF digestibility for each 1%-unit change in dietary starch. An exercise presented by Weiss (unpublished) during the 28th ADSA Discovery Conference on Starch for Ruminants calculated the effects of a 0.5%-unit change in total tract NDF digestibility for each 1%-unit change in dietary starch content on dietary energy values. In the Weiss exercise, a 5%-unit increase in dietary starch content (e.g., 30 vs. 25%) would increase diet NE_L content by 6.5% without accounting for adverse effects of dietary starch on total tract NDF digestibility. However, it was revealed that the reduction of 2.5-% units (46.5 to 44.0%) in total tract NDF digestibility would alter this scenario to a 5.3% increase in diet NE_L content. Further incorporation of these effects on models are warranted. However, other factors should also be considered to enhance future predictive equations. For example, grass inclusion in the diet and intake (expressed as percentage of BW) altered total tract NDF digestibility in the study by de Souza et al. (2018). White et al. (2017) observed greater effects of intake than starch concentration on total tract NDF digestibility and suggested that the potential negative effects of starch on consumption may attenuate its effect on NDF digestibility when gut fill is not a constraint.

Perhaps to separate the specific feed ingredients used to replace starch in dairy cattle diets could be an important step. Reduced-starch diets could be formulated by partially replacing cereals grains with high-fiber, low-starch byproduct feedstuffs (e.g., soy hulls, citrus pulp, whole cottonseed, beet pulp, cottonseed hulls, wheat middlings, etc.), high starch forages (i.e. whole-plant corn silage), or high-sugar ingredients (i.e. molasses, whey, sucrose). However, although these varied carbohydrate sources can be used for energy, their ruminal fermentation by microorganisms yields different fermentation end-products than starch, which in turn alter metabolism and performance by dairy cows. Fredin (2015) conducted a meta-analysis to identify which of these feeding strategies could mitigate potential negative effects of feeding reduced-starch diets to lactating dairy cows. Milk yield was decreased when starch was replaced by either non-forage fiber sources (0.16 kg/day per %-unit decrease in dietary starch) or forage (0.32 kg/day per %-unit decrease in dietary starch). Reduced intake and ruminal degradation of forage NDF compared to non-forage NDF (Allen, 1997) were thought to induce greater reduction in milk yield when dietary starch was replaced by forage in the study by Fredin (2015). However, Fredin (2015) highlighted that 24 out of 61 treatment means for milk yield were greater for reduced-starch compared to high-starch diets, suggesting that positive lactation performance can be achieved when feeding reduced-starch diets. Milk component yields were also reduced when dietary starch was replaced.

Potential negative effects on either milk yield or feed efficiency underscores that monitoring income over feed costs is recommended rather than price per unit of diet DM to fully assess economic benefits of reduced-starch diets. Based on these meta-analysis reviews of literature (Ferraretto et al., 2013; Fredin, 2015) to reduce dietary starch for peak and mid-lactation dairy cows may not be feasible and individual scenarios for each farm must be carefully evaluated.

Starch Digestibility in Corn Grain and Silages

Starch represents approximately 50 and 75%, respectively, of the energy value of corn silage and corn grain (calculated from NRC, 2001). Compared with other starch sources (i.e., barley and wheat), corn has lower ruminal and total tract starch digestibility (**TTSD**; Ferraretto et al., 2013).

A better understanding of factors affecting starch availability and digestion could lead to the formulation of more efficient and cheaper rations with lower starch levels and aid to prevent ruminal acidosis, which is typical in high-starch diets. In addition, focus on ruminal starch digestibility is desired as it alters efficiency of energy usage and increases ruminal microbial synthesis when dietary ruminal degradable protein levels are adequate (Firkins et al., 2006). Greater microbial protein synthesis explains the greater milk protein concentration per unit of rumen-digestible starch concentration (Ferraretto et al., 2013). An increase in starch digestion may lead to better nutrient utilization and decreased feed costs. Detailed descriptions about factors influencing starch utilization in corn silage and grain will be discussed in this section.

Starch digestibility of whole-plant corn silage (**WPCS**), high-moisture corn (**HMC**), and dry ground corn (**DGC**) may be affected by several factors. First, the starch endosperm is protected by the pericarp which, if intact, is highly resistant to microbial attachment (McAllister et al., 1994), thereby breakage of the seed coat is obligatory. Diets containing HMC with mean particle size (**MPS**) below 2 mm had greater total TTSD compared with HMC with MPS greater than 2 mm (95.2 to 89.5%; Ferraretto et al., 2013). Likewise, increased MPS reduced TTSD in DGC-based diets (77.7 to 93.3% for 4 mm and 1 mm respectively; Ferraretto et al., 2013). This is related to the increased surface area exposed for bacterial and enzymatic digestion with finer particles (Huntington, 1997). Greater starch digestibility and corresponding milk production by dairy cows is achieved when corn silage is harvested using a kernel processor with roll gap settings between 1 to 3 mm (Ferraretto and Shaver, 2012).

Reduced kernel particle size improves starch digestibility by increasing the surface area exposed to ruminal microbes. However, even the exposed endosperm is not fully digestible due to existence of a starch-protein matrix formed by the chemical bonds of zein proteins with starch granules (Kotarski et al., 1992; McAllister et al., 1993). Thus, the next step would be to liberate starch from its protein matrices. As corn matures, starch not only becomes more vitreous but more bonds are formed with zein proteins. This starch-protein matrix reduces starch digestibility. Ruminal in vitro starch digestibility was greater when HMC was harvested at lower DM content (Ferraretto et al., 2014). Furthermore, reduced TTSD were observed in diets containing WPCS above 40% DM in the meta-analysis review by Ferraretto and Shaver (2012). This may be related to an increase in the proportion of vitreous endosperm

in the kernel associated with greater maturity (Correa et al., 2002; Ngonyamo-Majee et al., 2009). Alternatively, a reduction in the extent of fermentation for drier WPCS (Der Bedrosian et al., 2012) may attenuate the breakdown of zein proteins during fermentation (Hoffman et al., 2011). Goodrich et al. (1975) harvested HMC with 67% DM and oven-dried corn to 73 and 79% DM to study the effects of moisture content on fermentation of HMC and observed a decrease in acetate and lactate concentrations and a corresponding increase in pH as DM content of HMC increased. Lower lactate and acetate concentrations are likely related to a reduced bacterial growth due to limited water availability (Muck, 1988). Goodrich et al. (1975) also observed reduced ruminal in vitro gas production as DM content increased, suggesting reduced starch digestibility for HMC at greater DM contents. Combining these results suggest that proper maturity at harvest is required to maximize starch digestibility in WPCS and HMC.

Research trials on the effects of storage length on ruminal in vitro starch digestibility (**ivSD**) of WPCS were summarized by Kung et al. (2018). Interestingly, all the summarized trials had a spike in ivSD after 30 to 45 days of storage followed by a gradual increase in ivSD after additional storage time. These results indicate that perhaps ivSD continuously increases during storage. Proteolytic activity, either from microbial or plant proteases, occurs more extensively during the anaerobic fermentation process (Baron et al., 1986). The anaerobic phase is characterized by a drastic decrease in pH (Muck, 2010), which favors the activity of plant proteases specific to the endosperm of cereal grains (Simpson, 2001), even though the activity of plant proteases is typically reduced under low pH (Muck, 1988). Junges et al. (2015) evaluated the contribution of proteolytic sources on protein solubilization in rehydrated corn ensiled for 90 days. These authors reported that bacterial proteases are responsible for 60% of the increase in soluble CP concentration, followed by kernel enzymes (30%), and fungi and fermentation end-products (5% each).

This variance in starch digestibility within and among feeds suggests that the assessment of starch digestibility is essential for adequate diet formulation. Although the incubation of feeds in ruminal fluid for 7 hours is the standard assay used in the United States (either in vitro or in situ) to rank feedstuffs, more accurate predictions of starch digestibility would benefit various industry sectors. Perhaps a similar approach to the various pools of NDF digestion used by the Cornell Net Carbohydrate Protein System (**CNCPS**) model could be an option. Recently, Fernandes et al. (2018) analyzed rapidly and slowly degradable fractions and rate of disappearance of starch in several starchy feedstuffs. Fraction A ranged from 13.4 to 96.1% of starch, whereas rate of disappearance varied from 2.1 to 11.5% per hour. Although the validation was only performed for mature corn grain, Fernandes et al. (2018) suggested that 0, 3 (or 6), and 48 hours of incubation could be feasible to evaluate digestibility and rank feedstuffs. Perhaps in combination with laboratory assays, the on-farm assessment of starch digestibility may be a great option.

Fredin et al. (2014) reported a strong relationship between fecal starch measurements and TTSD. These results suggest that additional measurements, such as starch content of the diet or marker concentrations of the feces or diet, are unnecessary. Furthermore, Fredin et al. (2014) reported high accuracy of near infrared reflectance spectroscopy to predict fecal starch, which allows for more rapid and inexpensive analysis. Although benefits of greater starch digestibility on milk production is well known,

it is very difficult to reliably estimate its economic impact. The exercise presented and discussed in this article is an attempt to provide some numbers to dairy producers and their nutritionists as a starting point.

To accomplish our goal, a hypothetical scenario was created and 5 values of fecal starch were arbitrarily chosen and used to predict TTSD using the equation of Fredin et al. (2014; Table 1). Subsequently, the amount of corn that would need to be supplemented in order to obtain the same amount of digestible starch as if TTSD was 100% was estimated using the following assumptions: dietary starch was 25% of DM and consumption of DM was 25 kg/day. Consequently, it was assumed that cows were eating 6.25 kg/day of starch. Based on TTSD, values of starch loss in the manure was calculated and ranged from 0 to 1.56 kg. If one consider that corn grain has 70% starch and 70% ruminal in vitro starch digestibility, for each kg of corn supplemented only 0.49 kg of digestible starch is provided. Thus, by dividing starch loss by 0.49, we reached the amount of corn necessary to fulfill for undigested starch. Last, US$130/ton (US$0.13/kg) was used to calculate corn grain costs. Values used in the present exercise is not representative of the entire American dairy industry, but it is a good indication of potential economic loss related to low starch digestibility. Thus, it is recommended that dairy farmers and their nutritionists perform similar calculations based on their own scenarios and goals.

Fecal starch does not indicate digestibility of specific feedstuffs but of total diets, and it can be used as a valuable tool to monitor specific groups over time by collecting samples from at least 10% of animals in the group. If fecal starch levels are above 3%, it is advised the evaluation of specific starchy feedstuffs to elucidate the problem. In addition, re-evaluation of fecal starch values are recommended after 2 or 3 weeks of dietary or management adjustments.

Conclusions

- Starch digestibility affects milk and milk components production;
- Several strategies may increase starch digestibility of individual ingredients; particularly mean particle size, maturity at harvest, and hybrid endosperm type;
- Reduction in dietary starch reduces price per unit of DM but analysis of income over feed cost is advised; and
- Combine fecal starch and milk analysis to optimize nutritional management.

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Table 1. Economic estimates of corn supplemented to fulfill undigested starch.

Fecal starch, % of DM	0	5	10	15	20
TTSD[1], % of starch	100	93.75	87.50	81.25	75.00
Starch intake[2], kg/cow/day	6.25	6.25	6.25	6.25	6.25
Starch loss[3], kg/cow/day	0	0.39	0.78	1.17	1.56
Corn grain supplementation[4], kg/cow/day	0	0.80	1.59	2.39	3.18
Corn grain cost[5], US$/cow/day	0.00	0.10	0.21	0.31	0.41

[1]Predicted from equation of Fredin et al. (2014); TTSD = 100 – (1.25 x fecal starch).
[2]Starch intake = (25 kg DMI x 25% starch) / 100
[3]Starch loss = starch intake – [(starch intake x TTSD) / 100]
[4]Corn grain supplementation = starch loss / 0.49
[5]Corn grain cost = corn grain supplementation x 0.13. Corn grain cost obtained from values reported by FeedVal 2012 on March, 2018.

Prevention, Assessment, and Mitigation of Mycotoxicosis in Dairy Cattle

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Summary

Mold growth is an inevitable consequence of feed production, as a result their harmful metabolites "The Mycotoxins" are commonly found in livestock diets. In the last 40 years, great advances in the field of mycotoxins have increased our knowledge on the detrimental effects of these toxins on animal production. Climate change and agronomic practices play an important role in the unpredictability of mycotoxin contamination of feedstuffs. The primary classes of mycotoxins are aflatoxins, zearalenone (**ZEA**), trichothecenes, fumonisins, ochratoxins (**OTA**) and the ergot alkaloids. Due to the high variety of feedstuff utilized in dairy operations and the high production stress typically associated with modern dairying, mycotoxins are important anti-nutritional factors in dairy nutrition programs. In order to maximize dairy performance and health, mycotoxin analysis and prevention strategies must be part of the all dairy nutritional and health programs.

Introduction

Dairy profitability is highly dependent on proper nutrition and health. It is therefore imperative that dairy owners, manager, nutritionists, and veterinarians consider the negative role of anti-nutritional compounds naturally present in feedstuffs commonly utilized to feed these animals. Among these compounds "the mycotoxins", which are toxic secondary metabolites produced by fungi (molds), should be closely monitored and minimized. There are hundreds of mycotoxins known, but few have been extensively researched and even fewer have good methods of analysis that are commercially available. The primary classes of mycotoxins are aflatoxins of which aflatoxin B1 (**AFB1**) is the most prevalent, zearalenone (**ZEA**), trichothecenes - primarily deoxynivalenol (**DON**) and T-2 toxin (T-2) - fumonisins, ochratoxins (**OTA**) and the ergot alkaloids.

A practical definition of a mycotoxin is a fungal metabolite that causes an undesirable effect when animals or humans are exposed. Usually, exposure is through consumption of contaminated feedstuffs or foods. Mycotoxicoses are diseases caused by exposure to foods or feeds contaminated with mycotoxins (Nelson et al., 1993). Mycotoxins exhibit a variety of biological effects in animals: liver and kidney toxicity, central nervous system effects and estrogenic effects, to name a few. Some mycotoxins, i.e., aflatoxin, fumonisin and ochratoxin, are carcinogenic.

Molds, Plants, and Climate Interactions

The primary mycotoxin-producing fungal genera are *Aspergillus*, *Fusarium* and *Penicillium*. Many species of these fungi

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produce mycotoxins in feedstuffs. Molds can grow and mycotoxins can be produced pre-harvest or during storage, transport, processing or feeding. Mold growth and mycotoxin production are related to plant stress caused by weather extremes, to insect damage, to inadequate storage practices and to faulty feeding conditions. In general, environmental conditions — heat, water and insect damage — cause stress and predispose plants in the field or feed in transit or storage to mold growth and mycotoxin contamination (Coulumbe, 1993). Computer models to predict mycotoxin concentrations in corn prior to harvest are based on temperature, rainfall and insect pressure (Dowd, 2004) and similarly for DON in wheat (Prandini et al., 2009). Molds grow over a temperature range of 10 to 40°C (50 to 104°F), a pH range of 4 to 8, aw (water activity) above 0.7 and moisture content >13 to 15%. Most molds are aerobic, and therefore, high-moisture concentrations that exclude adequate oxygen can prevent mold growth. However, in practical situations, molds will grow in wet feeds, such as silage or wet byproducts, when oxygen is available.

Worldwide, approximately 25% of crops are affected by mycotoxins annually (CAST, 1989), which could lead to billions of dollars of losses. The annual economic cost of mycotoxins to the U.S. agricultural economy is estimated to average $1.4 billion (CAST, 2003). Economic losses are due to effects on livestock productivity, crop losses and the costs of regulatory programs directed toward mycotoxins. The implications of mycotoxins on agricultural trade have been reviewed (Dohlman, 2003).

Occurrence and concentrations of mycotoxins are variable by year and associated with variation in weather conditions and plant stresses known to affect mycotoxin formation (Coulumbe, 1993). In the 2009 to 10 crop year, several regions of the U.S. experienced higher concentrations and incidence of mycotoxins primarily due to a wet and delayed harvest season. These weather/climate trends have been more and more frequent in recent years. Climate change and agronomic practices play a critical role in the plant/mold interactions necessary for mycotoxin outbreaks. A recent study by a group of subject matter experts (Wu et al., 2011) hypothesized that climate change (and the overall temperature increase) would play a significant role in increasing aflatoxin and fumonisin contamination in maize, while DON concentrations would see a reduction related to the ambient temperature/mold relationship. However, these researchers postulated that DON concentrations in maize could also increase in relation to climate change related cropping practices and other agronomic changes. One of the most significant and potentially detrimental changes could be the trend to reduce or even eliminate tilling practices. Mansfield et al. (2005) looked at the effect of tilling on DON content in maize and concluded that although tillage type (no-till vs. moldboard till) had no effect on DON incidence, no tilling resulted in significantly higher DON concentrations than moldboard tilling.

Although mycotoxins occur frequently in a variety of feedstuffs and are routinely fed to animals, it is less frequent that mycotoxins occur at concentrations high enough to cause immediate and dramatic losses in animal health and performance. However, mycotoxins at low levels interact with other stressors to cause subclinical losses in performance, increases in incidence of disease and reduced reproductive performance. To the animal producer, these subclinical losses are of greater economic importance than losses from acute effects and even more difficult to diagnose.

Mycotoxicosis

The study of mycotoxins began in early 1960's with the outbreak of Turkey-X disease in the U.K. This outbreak was linked to peanut meal imported from Brazil (Sargeant et al., 1961). Because of an intensive multidisciplinary research effort, a blue-fluorescent toxin was isolated and mycelia of *A. flavus* were observed. *A. flavus* was shown to produce the same toxic compound(s) found in the toxic peanut meal. The toxin was characterized chemically and biologically and was given the trivial name aflatoxin. Aflatoxin was shown to be very toxic and carcinogenic in some of the test animal species used, and it resulted in a toxic metabolite in milk of dairy cows (Allcroft and Carnaghan, 1962; 1963).

The discovery of aflatoxin and elucidation of some of its effects led to research on other livestock health and production problems linked with moldy feedstuffs. This research led to the discovery of additional mycotoxins produced by other fungi. In dairy cattle, swine and poultry, mycotoxin contamination of feeds affects growth, milk production, egg production, reproduction and immunity (Diekman and Green, 1992). Mycotoxins have also been involved in outbreaks of human diseases (CAST, 1989).

Animals experiencing a mycotoxicosis may exhibit a few or many of a variety of symptoms, including: digestive disorders, reduced feed consumption, unthriftiness, rough hair coat or abnormal feathering, undernourished appearance, low production, poor production efficiency, impaired reproduction and/or a mixed infectious disease profile. Mycotoxins can increase incidence of disease and reduce production efficiency. Some of the symptoms observed with a mycotoxicosis may therefore be secondary, resulting from an opportunistic disease, present because of mycotoxin-induced immune suppression. Immunotoxic effects of mycotoxins have been reviewed (Bondy and Pestka, 2000; Oswald et al., 2005). The progression and diversity of symptoms in a mycotoxicosis can be confusing, making diagnosis difficult (Schiefer, 1990). Diagnosis is further complicated by limited research, lack of feed analyses, nonspecific symptoms, few definitive biomarkers and interactions with other stress factors.

With few exceptions, a definitive diagnosis of a mycotoxicosis cannot be made directly from symptoms, specific tissue damage or even feed analyses. However, experience with mycotoxin- affected herds increases the probability of recognizing a mycotoxicosis. A process of elimination of other factors, coupled with feed analyses and responses to treatments can help identify a mycotoxicosis. More definitive diagnoses can be made for specific mycotoxins by detecting aflatoxin in milk or for fumonisin by induced changes in sphingolipid concentrations (Riley and Pestka, 2005). Regardless of the difficulty of diagnosis, mycotoxins should be considered as a possible cause of production and health problems when appropriate symptoms exist and problems are not attributable to other typical causes (Schiefer, 1990).

Safe Levels of Mycotoxins

Some of the same factors that make diagnosis difficult also contribute to the difficulty of establishing levels of safety. These include lack of research, sensitivity differences of animal species, imprecision in sampling and analysis, the large number of potential mycotoxins, interactions among mycotoxins and interactions with stress factors (Schaeffer and Hamilton, 1991). Field toxicities appear to be more severe than predicted from laboratory research.

Naturally contaminated feeds are more toxic than feeds with the same level of a pure mycotoxin supplemented into the diet. Aflatoxin produced from culture was more toxic to dairy cattle than pure aflatoxin added to diets (Applebaum et al., 1982). In swine, Foster et al. (1986) demonstrated that a diet containing pure added DON was less toxic than diets with similar concentrations of DON supplied from naturally contaminated feeds. Smith and MacDonald (1991) have suggested that fusaric acid, produced by many species of *Fusarium*, occurs along with DON to produce more severe symptoms. Lillehoj and Ceigler (1975) gave an example where penicillic acid and citrinin were innocuous in laboratory animals when administered alone but were 100% lethal when given in combination. These studies strongly suggest the presence of other unidentified mycotoxins in naturally contaminated feeds and that mycotoxin interactions are extremely important. It is well documented that several mycotoxins may be found in the same feed (Hagler et al., 1984). Abbas et al. (1989) demonstrated *Fusarium* species isolated from Minnesota corn produced multiple mycotoxins. Because animals are fed a blend of feedstuffs and because molds produce an array of mycotoxins, many mycotoxin interactions are possible. Speijers and Speijers (2004) discussed the combined toxicity of mycotoxins; and therefore, suggest daily tolerable intake limits for groups of mycotoxins.

Mycotoxin interactions with other factors also make it difficult to determine safe levels of individual mycotoxins. Animals under environmental or production stress may show the more pronounced symptoms. For example, there is a clear temperature interaction with fescue (ergot) toxicity, such that more pronounced symptoms are expressed during heat stress (Bacon, 1995). Jones et al. (1982) demonstrated that productivity losses in commercial broiler operations occurred when aflatoxin concentrations were below concern levels determined by controlled research in laboratory situations. The researchers hypothesized that general production stress had a significant contribution to the animal's susceptibility to the low concentrations of the toxins. The known dietary factors that interact with mycotoxins include nutrients such as fat, protein, fiber, vitamins and minerals (Brucato et al., 1986; Galvano et al., 2001). Thus, many factors and interactions make it difficult to relate field observations to those from controlled research. Mycotoxin effects vary by species and are also moderated by factors such as sex, age, duration of exposure and stresses of the environment and production.

Overall health and immune status also affect the animal's capability to cope with a specific concentration of a toxin or a combination of toxins. This is primarily due to the many mycotoxins with immunosuppressive properties and their interaction with animal health (Schiefer, 1990). Diagnosis therefore is quite difficult since disease outbreaks may be secondary, resulting from an opportunistic disease, due to a mycotoxin-induced immune suppression. Immunotoxic effects of mycotoxins are reviewed (Oswald et al., 2005; Bondy and Pestka, 2008).

Mycotoxins in Forages

One of the primary differences in exposure between ruminants and monogastrics is related to the role of forages as a source of mycotoxin exposure. Mycotoxins found in forages result in exposure of herbivores to a broad array of multiple mycotoxins. Many different mycotoxins have been found to occur in forages either in the field or in storage as hay or silage (Lacey, 1991). Some mycotoxicoses in cattle resulting from contaminated forages (Lacey,

1991; Gotlieb, 1997; Scudamore and Livesay, 1998) and byproduct feeds (Lillehoj et al., 1991) have been reviewed. Mold grows in hay stored too wet or with damp spots. The limiting factors for mold growth in silage are pH and oxygen. Silages stored too dry or insufficiently packed and covered can allow air infiltration, resulting in growth of yeast, depletion of silage acids, an increase in pH, and thus, conditions conducive for mold growth and deterioration of the silage. The occurrence, prevention and remediation of mycotoxin producing fungi in silage has been recently reviewed by Wambacq et al. (2016).

The most important pasture-induced toxicosis in the U.S. is tall-fescue toxicosis caused by endophytic alkaloids (Bacon, 1995). Other forage toxicoses of fungal origin include ergotism, perennial ryegrass staggers, slobbers syndrome, a hemorrhagic disease associated with dicoumarol produced in fungal-infected sweet clover and sweet vernal grass and syndromes of unthriftiness and impaired reproduction associated with Fusarium (Cheeke, 1995).

In Pennsylvania, Mansfield and Kuldau (2007) found multiple mycotoxigenic molds, including Aspergillus, Fusarium, Penicillium and Alternaria, in corn silage samples at harvest and after ensiling, suggesting the possible presence of multiple mycotoxins. El-Shanwany et al. (2005) isolated 43 fungal species belonging to 17 genera from 40 silage samples collected in Egypt. The most prevalent genera were Aspergillus and Penicillium followed by Fusarium and Gibberella. Mycotoxins were found in 206 of 233 grass or corn silage samples collected in Germany during 1997-1998 (Schneweis et al., 2000). Penicillium was the dominant genus followed by Mucoraceae, Monascus and Aspergillus. Penicillium is a major silage mold and may be a greater silage problem because it grows at a lower pH than do other molds.

Mansfield et al., (2008) investigated the presence of four Penicillium-produced mycotoxins (roquefortine C, MPA, patulin and cyclopiazonic acid) in fresh and ensiled corn silage in Pennsylvania. The four mycotoxins were often found to co-contaminate freshly harvested corn and were generally found in greater frequencies and concentrations after ensiling. Auerbach et al. (1998) found P. roquefortii in 89% of visibly moldy forage samples and 85% of samples without visible mold. Surveys of grass and corn silages in Europe have found an occurrence of P. roquefortii in as many as 40% of samples (Auerbach, 2003). Penicillium-produced mycotoxins in silages, such as roquefortine C, MPA and PR toxins, have been associated with herd health problems (Auerbach et al., 1998; Seglar et al., 1999; Sumarah et al., 2005). Data from Boysen et al. (2000), Seglar et al. (1999) and Sumarah et al. (2005) point to the possibility that PR toxin is a silage mycotoxin of potential concern. Seglar et al. (1999) suggested that PR toxin is a good marker for silages associated with dairy herds with health problems.

Mycotoxin Testing

The accurate determination of mycotoxin concentrations in grain and feeds depends on accuracy from sampling to analytical techniques. A statistically valid sample must be drawn from the lot, which is not simple because mycotoxins are distributed unevenly in grains and other feedstuffs. Most of the error in a single analysis is due to sampling — as much as 90% of the error is associated with the taking of the initial sample (Whittaker, 2003). Once collected, samples should be handled to prevent further mold growth. Wet samples may be frozen or dried before shipment, and transit time should be minimized.

The second-largest source of error is inaccurate grinding and subsampling of the original sample. Finally, the subsample is extracted, the extract purified using one of several techniques, and then the toxin is measured. Toxin determination may be by thin-layer chromatography plates, high-performance liquid chromatography, gas-liquid chromatography, enzyme-linked immunosorbent assays, spectrophotometer or by other techniques. New technologies are progressing rapidly.

Mold spore counts may not be very useful and are only a gross indication of the potential for toxicity, but mold identification can be useful to suggest which mycotoxins may be present. Blacklighting for bright-greenish-yellow flourescence (**BGYF**) is often used as a screening technique for aflatoxin in corn, but it is very inaccurate. Newer and better methods should be used.

Generally, laboratories provide analysis for only a limited number of mycotoxins, perhaps including aflatoxin, OTA, DON, ZEA, fumonisin and T-2 toxin. Laboratory analysis may be directed toward detection of high levels of mycotoxins associated with acute toxicity and serious animal disease rather than low levels associated with chronic effects, such as production losses, impaired immunity and significant economic losses. Therefore, minimum detection limits set by a laboratory may inhibit the diagnosis of a chronic mycotoxicosis.

Analytical techniques for mycotoxins are improving, costs are decreasing and several commercial laboratories are available that provide screens for an array of mycotoxins. The Federal Grain Inspection Service (USDA-GIPSA) provides a list on the internet of approved mycotoxin tests for grains and provides excellent background materials for the feed industry (at www.usda.gov/gipsa/pubs/mycobook.pdf). Laboratory methods can be found in "Official Methods of Analysis of AOAC International". Krska et al. (2008) provided an update on mycotoxin analysis focusing on recent developments including multi-mycotoxin methods and quick tests. Maragos and Busman (2010) reviewed the rapid and advanced tools for mycotoxin analysis.

Because analytical methods can be either qualitative or quantitative, done by inexpensive kits or by sophisticated analytical instruments and can be quick or fairly time consuming, it may be difficult to determine and select the right method for the right need (Scudamore, 2005).

Conclusions

More information is needed about why mycotoxins occur, when to expect them, how to prevent their occurrence and how to deal with their presence. More data are needed about animal toxicity and about interactions with other mycotoxins, nutrients and stress factors, such as disease organisms or environmental stress and about the role of mycotoxins in immunosuppression. Improved screening techniques are needed for monitoring mycotoxin occurrence, including the detection of multiple toxins, diagnosing toxicities and prevention and treatment (CAST, 2003).

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Vitamin Supplementation for Lactating Dairy Cows: Industry Perspective

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Summary

In the current 2001 7th revised Dairy NRC, supplemental vitamin requirements for lactating dairy cows are listed for vitamin A, Vitamin D, and Vitamin E (75,000; 20,000; and 545 IU per head per day, respectively). In addition, production responses to 20 mg supplemental biotin and 50 grams of rumen-protected choline were discussed in detail. Since that time, most research advances have been made in transition cows, with vitamin requirements for lactating cows in "set it and forget it" mode, especially with cows entering the lactation in good health status. However, supply disruptions in late 2017/early 2018 brought vitamin discussions and supplementation strategies to the forefront. Technological advances include: herd status auditing, new product forms, and new understanding of modes of action beyond classical deficiency signs.

Introduction

In late 2017, several factors resulted in an unprecedented drop in global vitamin supply and subsequent rise in prices. This was coupled with shortages and outages in some specialty markets, such as certain vitamin forms for liquid feeds. In the case of vitamin A (retinyl acetate) and vitamin D3, prices reached 3 to 10 times greater than previous typical levels and local availabilities were widely affected (Figure 1). These price increases and shortages resulted in many discussions and strategies at all levels of the ruminant feed supply chain. Should cost of a nutrient be a factor in determining biological adequacy? It certainly was in early 2018, as nutritionists and producers scrambled to re-evaluate vitamin supplementation strategies, based on actual or perceived shortages and as an attempt to control input costs.

Vitamin A and Beta-carotene

Vitamin A is needed for eyesight, growth, reproduction, and maintenance of epithelial tissues. The activity of vitamin A is measured in retinol equivalents (1 IU of vitamin A equals 0.3 μg of all-*trans* retinol), and the most prevalent supplemental form is the retinyl acetate ester, usually encapsulated in a "cross-linked" gelatin beadlet for feed storage stability. Signs of vitamin A deficiency include: abortion, retained placenta, reduced immune function, and calf morbidity and mortality (NRC, 2001). As reviewed by Weiss (2018a), adjustments in the NRC vitamin A requirement should be made (upward) for ration forage comprising less than 60% of the ration, and for milk production > 75 lb, or for storage losses, which can amount to 9%/month in high-stress premixes containing inorganic trace minerals and choline (Shurson, 2011). Dietary beta-carotene (**BC**) is the major precursor of vitamin A with an activity of 400 IU per milligram for ruminants. Dietary BC is

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absorbed with fat and converted to retinol by intestinal enzymes. In ruminants, intact BC is also absorbed and stored directly, without immediate conversion to retinol. Guernsey and Jersey cattle convert less BC to retinol in the enterocyte, resulting in higher circulating levels and more excretion in milk than in Holstein cattle.

BC also functions separately from vitamin A as an antioxidant and can directly enhance immunity with possible reproductive and mammary benefits (Chew, 1993). The National Research Council (NRC, 2001) concluded that, although available data were insufficient to establish a BC requirement for dairy cattle, additional dietary vitamin A should be considered with low forage diets, high corn silage diets, diets with low quality forages, and situations with high pathogen loads or reduced immunocompetence.

Beta-carotene status

Responses to BC supplementation have been inconsistent in part due to the wide variation in serum BC status (Weiss, 1998; deOndarza et al, 2009). Most BC is found in vegetative plants and concentrations decrease with plant maturity. Most grains and fermented feeds contain minimal levels of BC because of heat damage and breakdown during storage (Pickworth et al., 2012). A serum BC level of 3.0 μg/ml has been suggested as the level at which supplementation is beneficial (Frye et al., 1991). A large proportion of serum samples from the 1996 NAHMS study of U.S. dairy herds (NAHMS, 1996) contained less than 3.0 μg/ml BC. LeBlanc et al. (2004) found mean serum BC concentration of 1828 samples from peripartum (+/- 1 wk) Holstein cows from 20 Canadian herds to be 1.12 μg/ml (SD = 0.78). Stage of lactation greatly affects serum BC levels (Kawashima, 2009a), with the lowest occurring immediately pre-calving (Figure 2).

Although the mode of action is not well understood (improved antioxidant/immune status?), some studies have found supplemental BC to positively effect milk yield. Heat-stressed cows supplemented with 400 mg BC increased cumulative milk yield by 11% (Arechiga et al., 1998). Oldham et al. (1991) supplemented 300 mg BC and increased milk yield by 6.4% with this difference approaching significance. However, others have not seen production responses with supplemental BC (Bindas et al., 1984; Rakes et al., 1985; Wang et al., 1988b).

Immune function

Chew et al. (1982) reported that cows with lower plasma vitamin A, BC, and total retinol equivalents had more mastitis. Chew (1983) supplemented 300 mg BC and 53 KIU vitamin A, or 80 KIU vitamin A, or 53 KIU vitamin A, or no supplement from 30 days before calving to 70 DIM. In this study, BC had a positive effect on immune response. Rakes et al. (1985) supplemented 300 mg BC and numerically lowered SCC content of milk, and Wang et al. (1988b) required fewer clinical mastitis treatments in cows supplemented with 300 mg BC.

Other researchers have not found indications that BC improved immune function. Oldham et al. (1991) did not reduce the incidence of mastitis with supplemental BC. Bindas et al. (1984) found that supplementing 600 mg of BC per day had no effect on SCC. LeBlanc et al. (2004) could not relate serum BC concentrations with either retained placenta or mastitis. However, they did find that when there was a 100 ng/ml increase in serum retinol concentration during the last week prior to calving, there was a 60% reduction in clinical mastitis in early lactation.

Reproduction

Dietary BC levels have been linked to fertility as evidenced by higher concentrations of BC in the ovary, particularly the corpus luteum (Chew et al., 1984). Schweigert (2003) postulated that BC is converted to retinol specifically in the uterus and ovaries. Graves-Hoagland et al. (1988) found plasma BC to be positively related to progesterone production by corpus luteum cells. Cows that ovulated during the first follicular wave postpartum had a higher mean plasma BC concentration than anovulatory cows three weeks prepartum (Kawashima et al., 2009a). In a follow-up study, Kawashima et al (2009b) supplemented BC during the close-up period (500 mg/day or 2000 mg/day in two different experiments) and increased the number of ovulating cows at the first follicular wave postpartum. Pregnancy rate at 120 days postpartum in heat-stressed cows supplemented with 400 mg BC/day for > 90 days was increased (35.4 vs. 21.1%; Arechiga et al., 1998). Rakes et al. (1985) found that supplementing 300 mg of BC for the first 100 DIM reduced days to first estrus and reduced cervix diameters at 21 and 28 DIM ($P < 0.05$). Lotthammer (1978, 1979) found that supplemental BC improved conception rates, uterine involution, and ovulation and reduced incidence of cystic ovaries and early embryonic death. Others have seen no positive reproductive responses to BC supplementation in dairy cattle (Bindas et al., 1984; Marcek et al., 1985; Wang et al., 1988a; Wang et al., 1988b) possibly due to season or initial BC status (Weiss, 1998). Greenburg et al. (1986) concluded that BC did not improve reproduction in beef heifers.

Colostrum quality, Beta-carotene, and calf issues

Calves are born with minimal vitamin A liver stores, making ingestion of colostrum with high vitamin A and BC concentrations imperative, as both have proven to be important for proper immune function. Kehoe et al. (2007) found that the BC concentration of colostrum from cows sampled across Pennsylvania varied from 0.1 to 3.4 μg/g. Torsein et al. (2011) found that calves born with serum levels below 0.25 ug/ml (up to 40% of the calves in high-mortality herds) were 5.3 times more likely to die than calves with higher serum BC levels.

Supplementing 1 g/day of BC increased BC concentration in colostrum compared to control (3.1 vs 1.44 mg/L, respectively; Kaewlamun et al., 2011; Table 1). Concentration of colostral BC was also increased in cows supplemented with 800 mg BC during the close-up period (Prom et al., 2016). The number of calves with detectable BC concentrations was higher for calves receiving maternal colostrum from dams supplemented with BC, compared to calves born from control fed dams. Recently, Aragona et al. (2017a) fed 700 mg BC/cow/day for 4 wk prepartum to determine effects on colostrum quality and calf performance. Colostral IgG concentration increased (82.7 vs 57.6 g/L for BC and control fed cows, respectively), although colostrum yield was reduced in BC-supplemented cows. Calves born from cows supplemented with BC gained 0.44 g/g DMI compared to calves born from cows not supplemented with BC that gained 0.32 g/g DMI ($P = 0.03$).

Evaluating Beta-carotene status and targeting supplementation

Because the actual BC content of diets varies and BC status was usually unknown in previous research, it can be difficult to evaluate BC supplementation strategies. Mean serum BC can now be assessed on the farm using the iEx™ system, a single step denaturation and BC extraction into organic

solvent followed by BC measurement using iCheck® (BioAnalyt GmbH, Germany), a portable spectrophotometer (Schweigert et al., 2007). Routine BC measurements can be used to evaluate herd status and to recommend specific supplementation strategies in the field.

Vitamin D

Vitamin D requirements listed in NRC 2001 are listed as 18,000 to 25,000 IU/hd/day; levels adequate to prevent rickets and to assist in preventing milk fever. Hymoller et al. (2010) measured vitamin D synthesis in the skin of cows exposed to 5.4 hr of Danish sun/day, finding that exposure alone was adequate to support 20 to 25 ng 25-OH-D3/ml of serum, or just below adequacy. Since that time, researchers have focused on extra-rachitic responses, such as immunity (Lippolis et al., 2011) and gene expression (Viera-Neto et al., 2017). Nelson et al. (2016) surveyed 702 serum samples from US dairy herds, finding that most herds supplementing 30 to 50 KIU D3 maintained serum levels of 25-OH-D3 above the desired 30 ng/ml cutoff, with a mean observation of 69 ng/ml. However, one herd supplementing at 20 KIU saw 22% of the samples below 30 ng/ml.

25-OH-D3 approved for bovines

The 25-hydroxylated form of D3 (HyD®) was approved for bovine feeding in the US in October, 2018. Research with monogastrics in the US and with dairy cows in Europe and New Zealand has shown advantages in supporting skeletal health and peripartum calcium metabolism, along with many vitamin D-dependent reactions in immune tissue, muscle cell differentiation, and other extra-rachitic modes of action. Recent studies demonstrate that 25-OH-D3 positively influences calcium nutrition during different stages of the production cycle. The combination of 25-OH-D3 and diets with negative DCAD have been shown to reduce the nadir of Ca in plasma immediately post calving (Wilkens et al., 2012) and to reduce the incidence of diseases linked with sub-clinical hypocalcemia (Martinez et al., 2018ab). There have also been investigations into the use of 25-OH-D3 during lactation, with studies demonstrating an increase in absorption efficiency of both Ca and P, as well as a reduction in bone degradation and increase in bone formation (McGrath et al., 2012; Oehlschlager et al., 2014). Furthermore, a recent study also demonstrated a link between 25-OH-D3 and energy metabolism (Rodney et al., 2017). Viera-Neto et al. (2017) fed either 1 or 3 mg of D3 or 25-OH-D3 to pregnant, lactating Holstein cows. On day 21, researchers challenged cows with an intramammary *Streptococcus uberis* dose, and then measured rectal temperatures, mastitis severity, and several gene markers in milk somatic cells for 96 hours post-challenge. The severity of mastitis was decreased at 60 and 72 h post-challenge for 3 mg 25-OH-D3 vs. 1 mg D3 (Figure 3). These effects were explained in part by increasing availability of 25-OH-D3 for synthesis of 1,25-OH-D3 by 1α-hydroxylases in immune cells of infected glands.

Vitamin E

In transition cows, LeBlanc et al. (2004) found that, compared to healthy cows, cows that later contracted mastitis had lower serum alpha-tocopherol, retinol, and BC levels in the late dry period. Further, Goff et al. (2002) found that the metabolic draw of colostrum synthesis lowered serum status of all 3 vitamins versus mastectomized cows. Researchers at the University of Florida found that 2000 IU supplemental vitamin E had beneficial effects on milk production in heat-stressed multiparous cows, but no beneficial effects in first-lactation heifers (Staples et al., 2016). European researchers (Pottier et al., 2006) investigated

the effect of very high (12,000 IU/hd/day) levels of vitamin E upon experimentally-induced milk fat depression. In their study, cows were fed 1.86 kg/day of extruded linseed plus 190 g/hd/day of linseed oil. The vitamin E treatment elevated milk from 3.29 to 3.88 % and decreased *trans*-10 C18:1 by 47%.

B Vitamins

With a few exceptions, B vitamins are thought to be in adequate supply from microbial synthesis. However, several researchers have challenged whether microbial supply has kept pace with increasing production in modern dairy cows (Shaver and Bal, 2000), or whether rumen-protected forms might do a better job of reaching the small intestine. Weiss (2017) pointed out that since 1990, the average Holstein synthesizes about 33% more milk and components today, but has only increased DMI by 15% - a possible imbalance between supply and need. Our understanding of B vitamin synthesis, rumen destruction, and kinetics has advanced (Castagnino et al., 2017), and several encapsulated combination B vitamin products have been tested with good results in North America (Sacadura et al., 2008; Morrison et al., 2018).

Biotin

As one of the exceptions, use of supplemental biotin (usually 20 mg/hd/day) is widespread in the US and Canada. In its unprotected (straight vitamin) form, biotin bypasses most rumen destruction and enters the bloodstream as-is (Zimmerly and Weiss, 2001). Two recent meta-analyses were published in 2011: Chen et al. (2011) found from their review that milk yield was increased by 1.66 kg when biotin was included, and Lean and Rabiee (2011) found a milk yield increase of 1.29 kg in their analysis. In addition, Lean and Rabiee (2011) evaluated effects on hoof health, concluding that although studies were lacking in consistency of hoof measurements, overall biotin had a consistently positive effect on hoof health.

Niacin

Schwab et al. (2005) analyzed niacin lactation studies with dairy cows, finding that 12 grams supplemental increased 3.5 of FCM by 0.5 kg/day in a meta-analysis. Cost of supplemental niacin in mid-2019 would be about 10 cents/hd/day, so economic returns would be marginally positive. Morey et al. (2011) investigated a rumen-protected niacin source with early lactation cows, finding that 9.6 g niacin in protected form reduced NEFA. Aragona et al. (2017b) supplemented 16, 32, or 48 g of niacin to prefresh cows from -28 days to calving, and measured colostrum IgG concentration and yield, and calf performance. They found that supplemental niacin resulted in a linear increase in colostral IgG concentration up to the highest niacin level, and that calves born to dams receiving 32 g/day of supplemental niacin had ADG greater than the controls and other treatment levels.

Rumen-Protected Choline

Choline is usually considered in the water-soluble vitamin category, although requirements are in gram amounts rather than milligrams. Unprotected dietary choline is rapidly degraded in the rumen, but serves important functions in fat transport when absorbed. Rumen protected choline has been widely researched in transition and early lactation. Weiss (2018b) summarized 6 studies where 50 g of rumen protected choline was fed, noting a significant milk increase of 2.3 kg for the first 2 months of the lactation.

Conclusions

Research has shown that current recommended supplemental levels of vitamins A, D, and E are adequate to support excellent health and production in lactating dairy cows. Along with biotin, rumen-protected choline, and BC, these 6 vitamins are those most widely supplemented for dairy cows. Advances have been made in vitamin status auditing with several cowside assays available for serum retinol, alpha-tocopherol and BC, which can be used to tailor recommendations to ration and herd status. Our understanding of vitamin adequacy includes scientific bases for more than classical deficiency symptoms, including immune status, colostrum production, calf health, or storage losses. Vitamins not considered essential for adequacy but which are widely used for economic benefits also include biotin and rumen-protected choline. Future advances will include a better understanding of vitamin responses, the role of antioxidants in disease prevention, and rumen-protected forms of vitamins possibly marginal or limiting in high-producing dairy cows.

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Table 1. Colostrum responses to beta-carotene supplementation.

Study	Beta-carotene (mg/day)	IgG, mg/ml	BRIX%	Colostrum BC, mg/L	Calf Serum BC, ug/ml	Calf Serum IgG, mg/L
Kaewlamun et al. (2011)	1000/14 days			↑215% ($P < 0.01$)		
Prom et al. (2014)	800/21 days	↑3.4% (NS[1])	↑2.0% (NS)	↑239% ($P < 0.01$)	↑566% ($P < 0.05$)	--
Aragona et al. (2017a)	700/28 days	↑ 43% ($P < 0.05$)	--	--	NS	↑41% ($P < 0.01$)

[1]NS = Not significant.

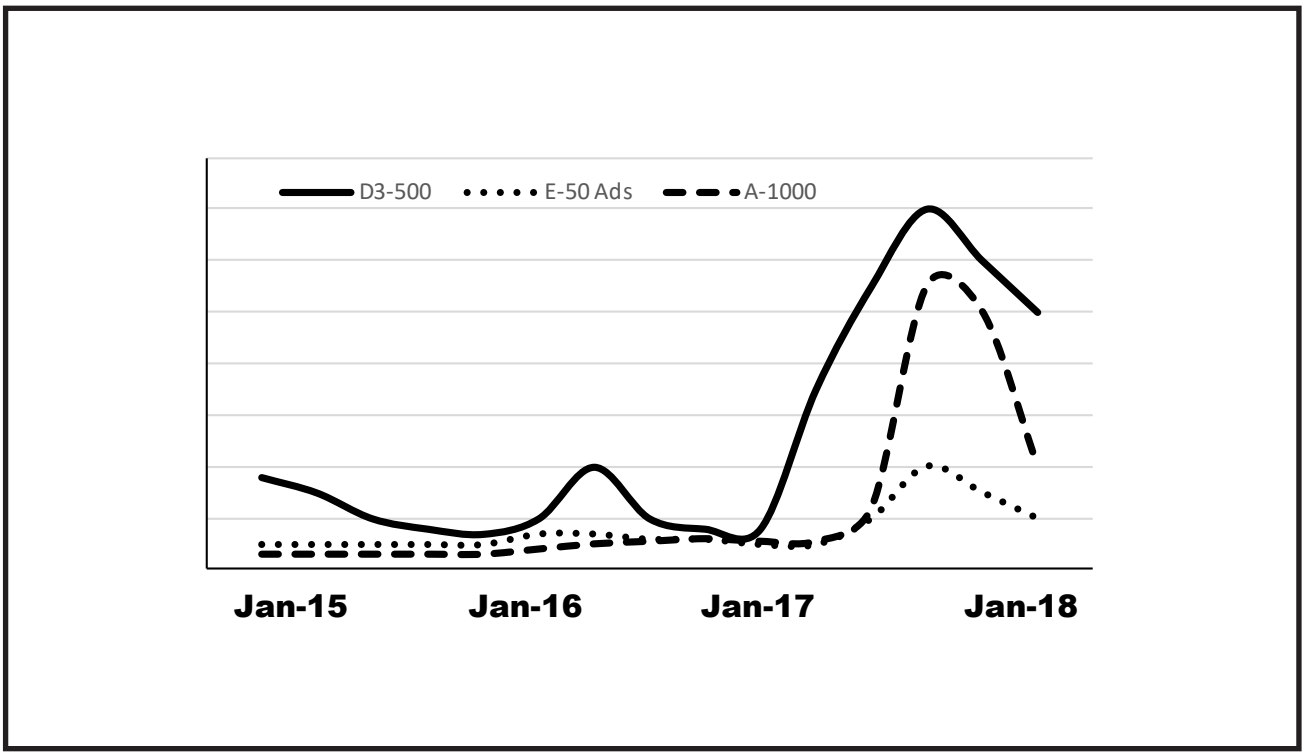

Figure 1. Vitamin prices in North America, 2015-2018 (DSM, 2019, personal communication).

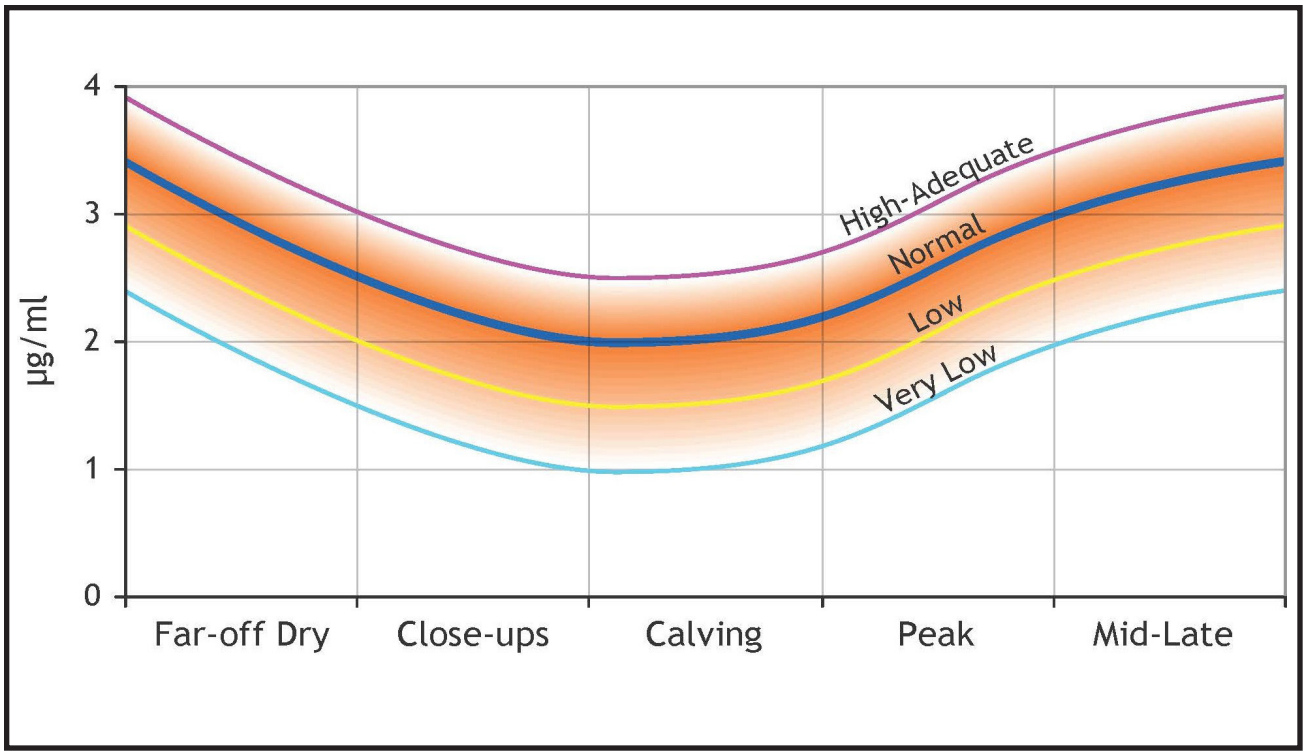

Figure 2. Herd whole blood β-carotene means from North American dairy herds, 2018 (DSM).

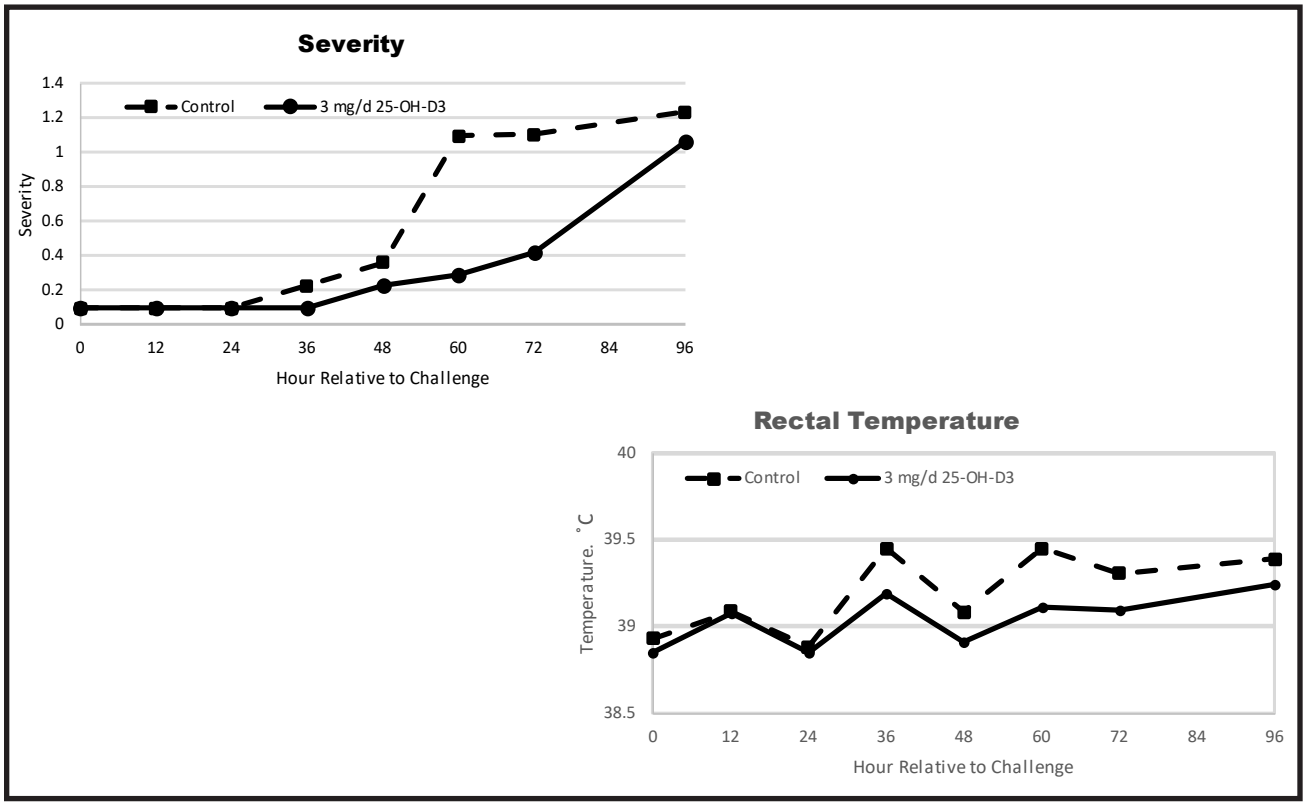

Figure 3. Effects of Vitamin D source on mastitis severity. 30 cows fed 1 mg vitamin D3 (Cholecalciferol) or 3 mg 25-hydroxyvitamin D3. Challenged with intramammary *Streptococcus uberis* at 21 day of treatment. (Viera-Neto et al., 2017).

Designing the Group Maternity Pen: Insights from the Cow's Perspective

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Abstract

Designing maternity pens for dairy cows is met with many challenges, including space constraints, the need to monitor the calving process, and accomodating the cows' maternal behavior. Historically, the maternity pen has been designed for ease and conveniance of farm management to keep pens clean and easily monitor the calving process. This focus on human convenience for managing the maternity pen may not be ideal for the cow. Although there may not be a perfect maternity pen design, these facilites should accommodate the cow's natural behaviors as she approaches calving. New research has provided insights into the behaviors of cows as calving approaches, which can help improve management and housing for cows before giving birth.

Introduction

The transition period, defined as the 3 weeks before to the 3 weeks after calving, is a critical time for cows in which they are at a high risk of disease. It has been estimated that between 30 to 50% of cows experience metabolic (e.g. ketosis and hypocalcemia) or infectious disease (e.g. metritis and mastitis) during the transition period (see LeBlanc, 2010 for a review). These diseases are an animal welfare concern for dairy cows and have economic repercussions for producers in the form of treatment, increased culling, and milk loss (Esposito et al., 2014). Up to this point, a majority of transition cow research has focused on nutrition and management strategies (reviewed by LeBlanc et al., 2006; Sepúlveda-Varas et al., 2013). It has been suggested that a better understanding of maternal behavior in the periparturient period may provide insight into the high incidence of disease during the transition period (Sepúlveda-Varas et al., 2013). This presentation will describe recent research that has focused on developing a stronger understanding of the cow's innate behaviors before calving (see Proudfoot, 2019 for a detailed review).

Management of Transition Dairy Cows

Management and grouping of transition dairy cows is largely based on farm size and nutritional strategy (Overton and Waldron, 2004). Cows are moved into the close-up pen (an area where the cow starts her close-up period, approximately 3 weeks before calving) to facilitate feeding a diet that is specifically formulated to support the cow as she prepares to give birth (Overton and Waldron, 2004). In the US, 64.3% of calvings occur in group maternity pens while 31.1% of calvings occur in individual calving pens (USDA, 2014). For some herds, cows enter group maternity pens (the maternity pen is any pen where a cow gives birth to her calf), at the start of their close-up period and stay

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until after calving, while others herds move cows out the close-up and into individual maternity pens when calving is imminent (Cook, 2019). Generally, maternity pens are located in high traffic areas to increase monitoring capabilities of farm workers to make sure a cow's labor is progressing normally. However, management strategies for close-up and maternity areas that are based on diet, grouping strategies, and easy monitoring may not be a desirable calving pen for the cow. An understanding of maternal behaviors and the motivations of cows at calving is needed to help us design more ideal close-up and maternity pens.

Behavior Around Parturition

Behavioral changes in preparation for calving

As calving approaches, cows begin to express a suite of maternal behaviors to ensure calf survival, which begins with the delivery of a live calf. In a natural setting, these behaviors include restlessness and seeking isolation to find a desirable calving site to ensure a successful delivery (reviewed by Rørvang et al., 2018b). In wild ungulates, following the birth of the calf, calf survival is dependent on the formation of the cow-calf bond because the calf relies on the damn for nutrition and protection from predators (Leuthold, 1977). Although in modern dairy production, calves are removed from the dam following birth and post-calving, pre-calving maternal behavior has not been eliminated and facilities should be designed to accommodate these behaviors.

At the onset of labor, dairy and beef cows on pasture seek isolation away from the herd to find a secluded place to calve with visual cover (Lidfors et al., 1994). In theory, isolation seeking in ungulates may serve as an anti-predatory strategy (Leuthold, 1977) and reduce disturbances from other cows around calving to facilitate formation of the cow-calf bond. However, in modern dairy facilities, there is less room for cows to be secluded at calving in both group and individual pens than in pasture environments. Group pens contain more than one cow housed at variable stocking densities, which may make it difficult for a pre-parturient cow to separate from penmates because of a lack of space. In addition, other cows may be attracted to the odor and pheromones emitted during labor that may attract them to the laboring cow (Jensen and Rørvang, 2018). Other cows may also spend time licking alien calves in group pens (Edwards, 1983), creating more disturbances for cows during labor. In individual maternity pens, cows are separated from the rest of the group to give birth. However, they are sometimes designed with space constraints and located in high traffic areas of the barn where the calving process can be easily monitored. Depending on the presence of cows in maternity pens and human activity in the barn, it may be difficult for indoor-housed dairy cows to perform innate isolation seeking behavior at calving.

Isolation seeking behavior in indoor facilities

Recent research suggests that indoor-housed cows have retained the motivation to hide during labor when housed individually (Proudfoot et al., 2014a,b). For example, when given the choice between an open bedded pack area and a 'sheltered' area to seclude themselves, 81% of cows calved within the hide during the day (Proudfoot et al., 2014a). In a follow-up study, researchers found that when housed in an individual maternity pen, 79% of cows sought a secluded 'corner' to give birth if available (Proudfoot et al., 2014b; Figure 1). The results from these studies indicate that cows have retained the motivation to hide at calving in individual calving pens and may be more motivated to hide during the daytime.

Although cows are motivated to hide at calving, the amount of coverage provided by a hide at calving does not appear to be an important factor to cows when selecting a calving site. For example, researchers provided cows with 3 hide options in individual maternity pens of varying coverage from the group pen: and tall and narrow (1.8 x 1.5m), low and wide (1 x 2.5m), tall and wide (1.8 x 2.5m) (Rørvang et al., 2017). The authors expected cows to prefer the most secluded environment; however, cows with normal duration of labor generally had no preference for hide shape. Comparatively, cows with prolonged labors (an average of 159 minutes of stage II labor) sought the most secluded calving space. Limited research has been performed to determine the motivation of cows in indoor group maternity pens to isolate themselves at calving. Preliminary data from our group's most recent work in this area will be presented.

Management and Housing Cows During Parturition

Maternity pens should ideally create an environment where the calf has a successful start to life and the cow has a successful start to her lactation. The environment should be clean and dry, and facilitate natural calving behaviors of the cow. Cows go through hormonal and behavioral changes at calving and it is important to understand the needs of the calving cow in both systems.

Managing the cow using individual maternity pens

Managing cows in individual maternity pens has both its advantages and challenges as compared to group calving pens. Individual pens may be easier to clean and are often located in high traffic areas where it is easier to monitor cows during calving. Individual maternity pens also reduce disturbances from other cows during calving (Edwards, 1983). However, as herd animals, it may be stressful for cows to be kept in social isolation in unfamiliar surroundings (Rushen et al., 1999). Additionally, cows kept in individual maternity pens for more than 3 days are at a higher risk of ketosis and displaced abomasum (Nordlund et al., 2006). Due in part to these findings, cows are kept in individual pens for a minimal amount of time. To avoid keeping cows in individual pens for too long, the practice of cows being moved into calving pens "just in time" when signs of calving are clear, including the presence of the amniotic sac or feet are visible outside the vulva, is sometimes used.

Previous research has explored the appropriate time to move cows from group pens into individual maternity pens. Proudfoot et al. (2013) found that cows moved into individual maternity pens before labor (on average 74 hours before calving) and during early stage I labor (on average 11 hours before calving) had normal duration of stage II labor, comparatively, cows moved during late stage I labor (on average 2 hours before calving) had a longer than normal duration of labor by approximately 30 minutes. This increase in labor length suggests the normal labor process was disrupted when cows were moved during labor. A longer duration of stage II labor has been associated with stillbirths (Gundelach et al., 2009) and dystocia (Schuenemann et al., 2011), thus, farms that use individual maternity pens should move cows when signs of early labor are visible. One major challenge of using individual calving pens is identifying cows in labor and moving them at the appropriate time. If close-up pens are not regularly monitored for cows in labor, the likelihood of cows calving in unwanted areas, such as the freestall, is greatly increased.

Management of cows in group maternity pens

Calving in group maternity pens allows cows to stay in a familiar environment and doesn't disrupt the progression of labor. However, cows may encounter more social challenges when calving in group pens, and depending on the stocking density of group pens, it may be difficult for cows to isolate at calving. Current recommendations for stocking density in group maternity pens is based on anecdotal evidence and is highly variable ranging from 9.3 m^2 to 18.6 m^2 per cow (Cook and Norlund, 2004; Graves, 2006). Space is a common constraint for producers when designing maternity pens. However, insufficient space in group maternity pens may limit a cow's ability to perform motivated calving behaviors.

Previous studies which have explored stocking density in the pre-calving period have focused on stocking density at the feedbunk. Overstocking at the feedbunk during the close-up period increases agonistic behaviors between cows (Proudfoot et al., 2009; Huzzey et al., 2012) and is may be especially problematic for cows of a lower social status (Huzzey et al., 2012). However, there is evidence that cows prioritize lying over eating when one resource is limited (Munksgaard et al., 2005), as such, lying space may also be important in pre-calving environments. Thus, more research assessing the appropriate stocking density of group maternity pens is still needed.

Research assessing the impact of group housing on maternal behavior, including isolation-seeking, of dairy cows is also limited. Findings for cow motivation to use manmade hides at calving are highly variable. Jensen and Rørvang (2018) created cubicle hides located on the walls of a group maternity pen that had equal dimensions but either a narrow (1.5 m) or wide opening (3 m) to the group pen. Only 10% of cows used a hide at calving, while the remainder calved in the group pen. However, cows spent more time in a secluded area with a wide opening before and after calving. These findings suggest that secluded areas may be important to cows in the time period around calving and not only during the labor process.

In another study, group housed cows were motivated to seclude at calving but were unwilling to work for access to secluded areas (Rørvang et al., 2018a). A group maternity pen was designed with cubicle hides bordering the outside of the group pen and cows were able to enter the cubicles at any time. Each hide had a gate that was either permanently tied open or cows had to push to open and was closed behind the cow, prohibiting more than one cow occupying the hide at a time. Approximately 50% of the cows in the study moved from the group pen into a hide to give birth. However, cows were more likely to calve in the hide if the gate was permanently tied open. The findings suggest that cows may be motivated to hide at calving but are not willing to work to gain access to this space.

To date, the separated area for cows to seek isolation during labor used dimensions of that were similar in design (e.g., a cubicle of L-shape). This type of design may not be optimal for cows seeking seclusion at calving. It is possible that cows view hides as a resource, thus dominant cows perform resource guarding behavior. If there is competition for the hide, cows may be less likely to use the hide at calving because they are unwilling to work for access to resources at calving (Rørvang et al., 2018a). More research is needed to explore the optimal design of a secluded area in a group maternity pen.

Conclusions and Recommendations

Maternity pens should be ideally be located in quiet areas of the barn where there is minimal activity. Adequate space allowance should be provided in indoor maternity pens (individual or group) to facilitate isolation behaviors at calving and to improve cleanliness of the area. Cows should ideally be provided the opportunity to hide at calving using manmade resources, although the appropriate design of these hides may vary depending on maternity pen type (individual or group). Secluded areas for cows can be created using many resources (e.g., hay bales, plywood, shade cloth, curtains, etc.) with the end goal of creating a space where a cow can feel isolated from penmates or caretakers working in the barn.

Farms that use "just in time" calving should create clear protocols on the appropriate time to move cows to individual maternity pens. Cows should be moved into individual maternity pens when signs of early labor are visible (e.g. raised tail, restless behavior, and relaxed pelvic ligaments) to avoid increasing the duration of stage II labor. However, this practice requires consistent monitoring of close-up pens and when mismanaged can result in cows calving in unwanted areas which has negative impacts on both the cow and calf.

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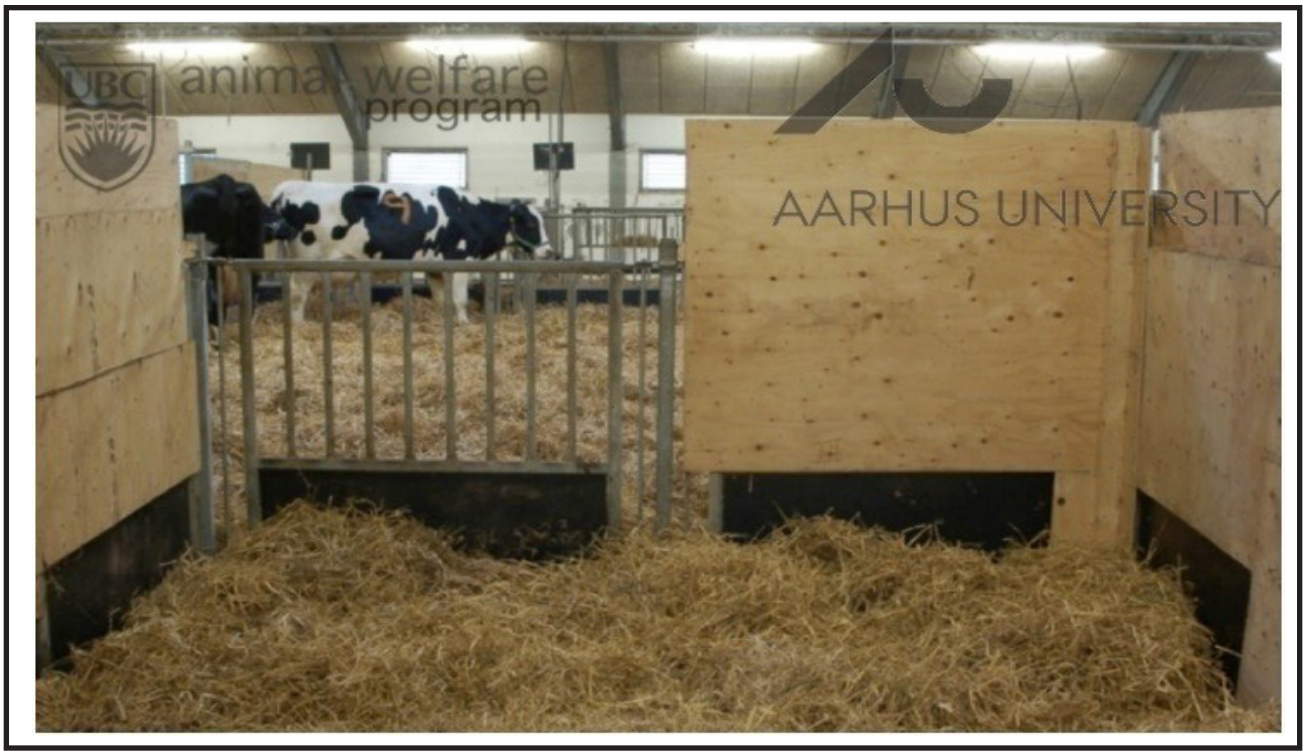

Figure 1. Design of a partially covered individual maternity pen. Covered areas were created with plywood.

Lipid Mobilization and Inflammation During the Transition Period

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Abstract

The transition period of dairy cows is characterized by changes in the lipid mobilization process in adipose tissues (**AT**) that include enhanced lipolysis and reduced lipogenesis. This imbalance leads to a net release of non-esterified fatty acids (**NEFA**) into the AT environment and circulation. Increasing the availability of these energy dense molecules is a mechanism of metabolic adaptation that is necessary to fulfill energy deficits driven by fetal growth and the onset of lactation. However, intense lipolysis and limited rates of lipogenesis lead to a considerable reduction in AT mass during the first 3 weeks after parturition. At the same time, lipolysis induces a remodeling process in AT that is characterized by a moderate inflammatory response with infiltration of macrophages. In cows that transition well into peak lactation, lipolysis decreases and AT inflammation resolves as lactation progresses. Nevertheless, if lipolysis dysregulation occurs and lipolysis rate does not decrease, AT inflammation becomes chronic and leads to poor lactation performance, reproductive failure, and increased risk for culling. This article summarizes the process of lipid mobilization in transition dairy cows, elaborates on the concept of AT remodeling and inflammation, and discusses how these biological processes affect transition cow health and lactation performance.

Introduction

Lipid mobilization is a bioenergetic process that includes lipogenesis and lipolysis. The AT, as the major body of energy reserve in mammals, is specialized in the storage and release of fatty acids through lipid mobilization. AT lipogenesis comprises the assembly of triglycerides through a stepwise addition of fatty acids catalyzed by glycerol-3-phosphate acyltransferase, 1-acylglycerol-3-phosphate acyltransferase, lipins, and diacylglycerol acyltransferase (Takeuchi and Reue, 2009). During lipolysis, AT's adipose triglyceride lipase, hormone-sensitive lipase, and monoacylglycerol lipase breakdown the triglyceride molecule into glycerol and NEFA [reviewed by Arner and Langin (2014)]. Released NEFA are either re-esterified to triglycerides through lipogenesis or exported into the bloodstream where they are transported by albumin and Fetuin-A for use in other tissues as fuel or secreted in milkfat (Strieder-Barboza et al., 2018). During the transition period, the net release of NEFA from AT into circulation is the result of reduced lipogenesis and enhanced lipolysis within adipocytes (De Koster et al., 2018). Normally, lipolysis decreases and lipogenesis replenishes adipocytes' triglyceride stores as lactation progresses. However, when AT exhibits an impaired response to the anti-lipolytic effects of insulin, lipolysis becomes intense and protracted, and lipogenesis is

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drastically reduced. Cows that exhibit high lipolysis rates around parturition are at a higher risk for inflammatory and metabolic diseases and have impaired lactation and reproductive performance. Among the mechanisms driving these deleterious effects, there are alterations in the inflammatory responses within the AT that lead to dysregulation of metabolic and immune functions in AT and systemically.

Adipose Tissue Remodeling in Transition Dairy Cows is an Inflammatory Process Induced by Lipolysis

The consequences of enhanced lipolysis and reduced lipogenesis in AT of transition cows go beyond the release of NEFA into circulation. Body weight loss during the first 3 weeks after calving is largely explained by a 25 to 35% reduction in the total AT mass and is driven by lipid mobilization (Akter et al., 2011). Excessive lipolysis also induces a remodeling process in AT that is characterized by an inflammatory response that includes infiltration of macrophages and changes in the structure and composition of its extracellular matrix (Contreras et al., 2017b).

Macrophages are the most abundant immune cell type in the AT of ruminants, accounting for 5 to 10% of its stromal vascular cells [i.e. non-adipocytes; Ampem et al. (2016)]. In transition dairy cows and in mid-lactation cows in negative energy balance induced by feed restriction protocols, lipolysis enhances the migration of macrophages into the adipose organ (Contreras et al., 2015; Contreras et al., 2016; Vailati-Riboni et al., 2017; Newman et al., 2019). During excessive lipolysis induced by clinical diseases, such as displaced abomasum and ketosis, AT macrophage populations increase to more than 20% of the cells in the stromal vascular fraction or 2% of the total number of cells in AT (Contreras et al., 2015; Häussler et al., 2017). The role of AT macrophages during lipolysis is to contain and eliminate the highly cytotoxic products of triglycerides breakdown that include NEFA, diglycerides, and monoglycerides (Lee et al., 2013).

The phenotype of AT macrophages is broadly classified as classical (**M1**), which characterizes those cells that are active in pro-inflammatory responses, and alternative (**M2**), which include macrophages that promote inflammation resolution. At any given time, AT macrophages are a combination of M1, M2, and intermediate phenotypes. In transition cows with pronounced negative energy balance, including those with displaced abomasum and ketosis, AT macrophages are predominantly M1 and accumulate in aggregates within omental and subcutaneous depots (Contreras et al., 2015; Newman et al., 2019). In mid-lactation cows exhibiting moderate lipolysis induced by a short 4 day feed restriction protocol, macrophage infiltration into the same AT depots occurs, but without variations in their inflammatory phenotype (Contreras et al., 2016).

In transition cows, AT remodeling may be a major mechanism driving AT specific insulin resistance as described by Zachut and et al. (2013). In their study, cows that lost more body condition during early lactation exhibited a pronounced reduction in the phosphorylation of key components of the insulin signaling pathways, including IRS-1 and AKT, compared to cows with low lipolysis and minimal weight loss. The mechanism linking AT remodeling and AT insulin resistance is the inflammatory responses of AT macrophages that once activated potent blockers of insulin signaling, including IL1-β, IL-6, resistin, and TNF-α (Martinez-Santibanez and Lumeng, 2014).

Lipolysis Products as Modulators of Inflammation and Metabolic Function

Fatty acids released during lipolysis are potent modulators of the activity of macrophages and other immune cells. AT macrophages exposed to saturated FA rapidly acquire an M1 like inflammatory phenotype through the activation of Toll-Like Receptors (**TLR**) 1, 2, 4, and 6 (Suganami et al., 2007; Grant and Stephens, 2015; Velloso et al., 2015). Saturated FA, such as lauric, myristic, and palmitic, strongly activate TLR4 and enhance the secretion of monocyte chemotactic protein-1 (**MCP-1**) (Han et al., 2010). Importantly, these saturated FA are preferentially mobilized from AT during the transition period (Douglas et al., 2007; Contreras et al., 2017a).

Polyunsaturated fatty acids released during lipolysis modulate immune function and inflammation through their oxidation products (oxylipids). For example, linoleic acid is oxidized by 15-lipoxygenase (**LOX**) and by other non-enzymatic reactions to produce hydroxyl-octadecadienoic acids (**HODE**). 13-HODE, a product of lipooxigenases and cyclooxigenases, promotes M2 polarization during lipolysis and acts as a PPAR gamma ligand that promotes adipogenesis and lipogenesis (Lee et al., 2016). In contrast, 9-HODE, promotes M1 polarization and could enhance macrophage infiltration into AT (Vangaveti et al., 2010).

In dairy cows, linoleic acid is the most abundant polyunsaturated fatty acid in plasma and in AT and it is preferentially mobilized by lipolysis during the transition period (Contreras et al., 2010). The dynamics of the plasma and AT contents of its derived oxylipids are linked with lipolysis intensity. In healthy transition cows, plasma 13-HODE increases at 1 week after parturition from its levels at 1 week before calving. In contrast, 9-HODE, an indicator of oxidative stress, remains unchanged. In AT, 9-HODE tends to increase after parturition and 13-HODE is higher than at either 1 or 4 weeks before calving. AT content of 13-HODE is positively associated with plasma beta hydroxybutyrate concentrations (Contreras et al., 2017a). It is expected that as lipolysis rate and oxidative stress status increase there will be an accumulation of 9-HODE within AT. Although more research is needed to characterize the dynamics of 9- and 13-HODE in AT and systemically, in the future, HODEs could be used as lipolysis intensity markers and disease risk or lactation performance predictors in transition dairy cows.

Lipolysis and Immune Function

Excessive lipolysis impairs the efficacy of the inflammatory responses of both the innate and the adaptive immune system cells [reviewed by Contreras et al. (2018)]. For example, cows challenged with Strep. uberis intramammary and with high lipolysis rates induced by feed restriction, exhibit an increased number of immature polymorphonuclear cells in circulation that have lower phagocytic activity compared with cows in positive energy balance (Moyes et al., 2009). In transition cows, high lipolysis rates are associated with reduced chemotactic activity and impaired phagocytosis in neutrophils (Nonnecke et al., 2003). The same population of cells has limited oxidative burst when circulating NEFA are above 500 μ*M* and its viability is drastically reduced when NEFA concentrations reach >1 m*M* (Ster et al., 2012). The inflammatory response of macrophages and polymorphonuclear cells are also affected by excessive lipolyisis during the transition period. Exposure to high NEFA concentrations reduces the mitogenic capacity of these immune cell populations and limits their secretion of IFN-γ and IgM (Ster et al., 2012). Excessive lipolysis also affects the function of cells of

the adaptive immune system. High NEFA concentrations are associated with increments of the B lymphocyte populations and reduction in the numbers of γδ T lymphocytes. Decreased numbers of γδ T lymphocytes are observed in cattle with deficient immune responses in epithelial tissues (Pollock and Welsh, 2002). In summary, lipolytic products, such as NEFA, impair the inflammatory responses of innate and adaptive immune cells and reduce their capacity to clear pathogens, leading to increased disease susceptibility in transition dairy cows.

Adipokines Modulate Systemic Immunity and Metabolism

AT modulates the immune and metabolic functions of dairy cattle through the secretion of adipocyte-derived peptides (i.e., adipokines). Of these adipokines, only adiponectin, leptin, and resistin are well characterized in transition dairy cows. Recent studies demonstrate that the synthesis of these adipokines is modulated by AT remodeling and inflammation and their secretion may be associated with changes in immune and metabolic functions around parturition.

Adiponectin enhances insulin sensitivity in adipocytes, hepatocytes, and muscle cells. At the same time, this adipokine promotes fatty acid β-oxidation in liver and the skeletal muscle. In transition dairy cows, plasma adiponectin concentrations are reduced during the first week after parturition compared to levels observed during the dry period and peak lactation (Kabara et al., 2014). In addition to metabolic effects, adiponectin modulates the inflammatory responses of human and bovine macrophages by reducing their expression and secretion of tumor necrosis factor (**TNF**) alpha and other pro-inflammatory cytokines (Kabara et al., 2014). Adiponectin is also an important modulator of adaptive immunity as it is required for dendritic cell activation and T-cell polarization (Jung et al., 2012). Excessive AT inflammation reduces the secretion of adiponectin by adipocytes, thus impairing the use of NEFA as energy substrate in liver and skeletal muscle.

Leptin modulates the inflammatory responses locally and systemically. Hypoleptinemia impairs the efficacy of T cell immune responses by reducing their capacity for pathogen clearance. Leptin is also necessary for adequate maturation and inflammatory responses in dendritic cells. In macrophages and polymorphonuclear cells, leptin signaling is required for phagocytosis in response to toll-like-receptor activation (Naylor and Petri Jr, 2016). Similar to adiponectin, leptin reaches its nadir during the first week after calving, while the highest plasma concentrations are observed early during the dry period (Chilliard et al., 2005). The effect of AT remodeling on leptin secretion during the transition period is currently unknown.

Resistin is another adipokine with the capacity to systemically modulate immune and inflammatory responses. In dairy cows, plasma resistin peaks during the first week after calving and returns to prepartum levels by 5 weeks in milk (Reverchon et al., 2014). In humans and rodents, resistin expression in adipocytes is stimulated by IL-6, hyperglycemia, and growth hormone. Resistin impairs insulin signaling in adipocytes and is characterized as a pro-inflammatory adipokine (AL-Suhaimi and Shehzad, 2013). It is unknown if AT remodeling and inflammation during the transition period can enhance resistin secretion, but this possibility should be considered as a mechanism for AT specific insulin resistance in dairy cows.

Modulating Lipid Mobilization in the Transition Period

Reduced lipogenesis and increased lipolysis are homeorhetic adaptations to negative energy balance that maintain energy availability for milk production. Although, the process of lipid mobilization is affected by physiological, nutritional, and genetic, management factors, there are different management, nutritional, and pharmacological tools that can be used to limit lipolysis and could potentially promote lipogenesis [reviewed in (Contreras et al., 2018)]

Maximizing dry matter intake (**DMI**) during the transition period reduces lipolysis and promotes lipogenesis. At the same time, it is necessary to limit the sudden drop in feed intake commonly observed during the final weeks of the dry period (Grummer et al., 2004). In addition to maintaining DMI, prepartum diets should be balanced to meet but not exceed energy requirements. This is usually accomplished by feeding high levels of fiber (Allen and Piantoni, 2014). It is important to note that overfeeding energy during the last weeks of gestation enhances lipolysis postpartum and increases the risk of fatty liver (Douglas et al., 2006). Cows that gain excessive body condition score (**BCS**) during the dry period have larger adipocytes that are more sensitive to lipolytic stimuli postpartum (De Koster et al., 2016). An additional feeding strategy is to boost the production of ruminal propionate postpartum by feeding high amounts of moderately fermentable starch (van Knegsel et al., 2007). This nutritional intervention limits AT lipolysis by enhancing insulin secretion (McCarthy et al., 2015).

To complement ration balancing strategies, the inclusion of nutritional supplements that limit lipid mobilization in the diet of transition cows can be considered. Feeding niacin (as nicotinic acid) reduces AT lipolysis by limiting the activity of hormone sensitive lipase (Kenez et al., 2014). However, niacin supplementation has shown inconsistent results (Schwab et al., 2005; Havlin et al., 2016). This may be related to timing of niacin supplementation. When fed only post-partum, niacin does not have FFA-lowering effects (Havlin et al., 2016). However, supplementing niacin throughout the entire transition period was shown to effectively reduce AT lipolysis (Schwab et al., 2005).

Methyl donors are also nutritional supplements that when fed to transition cows limit lipid mobilization. Among these, choline and methionine are reported to reduce lipolysis in AT when fed alone (Cooke et al., 2007; Li et al., 2016) or combined (Sun et al., 2016). Chromium supplementation may promote lipogenesis in AT by enhancing the activity of the insulin receptor in adipocytes (Vincent, 2004). Nevertheless, reports on the pro-lipogenic activity of chromium are inconsistent with some studies demonstrating a NEFA lowering effect (Hayirli et al., 2001; Yasui et al., 2014) and others showing no changes in plasma lipids (McNamara and Valdez, 2005; Smith et al., 2008). Currently, the pool of available pharmacological and nutritional interventions that reduce lipolysis or enhance lipogenesis is still very limited. Exploring new drug targets that enhance insulin sensitivity and block the lipolytic response in adipocytes will facilitate the management of transition dairy cows.

Evaluating Adipose Tissue Function in Dairy Cattle

Transition cow management programs often include routine measures of clinical and production parameters that can directly or indirectly evaluate adipose tissue function. BCS is a good measure of subcutaneous adiposity and the dynamics of BCS changes around

parturition subjectively describes lipolysis rates. Alternatively, the use of image biomarkers obtained during ultrasound examination of adipose tissues provides an objective evaluation of BCS, avoiding the variability associated with subjective visual measurements. Subcutaneous adipose tissue depth is strongly correlated with BCS evaluation when measured by trained personnel and is highly sensitive and specific in predicting plasma NEFA values at close-up and calving in dairy cattle (Strieder-Barboza et al., 2015). If using subjective BCS assessment, mature cows should approach calving with a BCS of 3.0 to 3.5 and heifers with 3.25 to 3.75 as excessively thin or over-conditioned cows are more susceptible to disease.

Currently, the most common direct measure of lipolysis is plasma NEFA. In preventive herd medicine, pre and post calving plasma NEFA values are used as early lactation disease predictors. Similar to plasma NEFA, post-partum plasma β-hydroxybutyrate indicate negative energy balance and predict disease risk in early lactation (Ospina et al., 2013). Lipolysis can also be evaluated at the group or individual animal level using the milk fat to milk protein percentage ratio. Milk fat increases as plasma NEFA rise. Cows with milk fat to milk protein ratio values higher than 2 during the first week after calving are at a higher risk for developing retained fetal membranes, DA, clinical endometritis, and being culled before the end of lactation (Toni et al., 2011).

Novel biomarkers of AT function are being explored. Low concentrations of the NEFA transporters albumin and fetuin-A are associated with low lipogenic activity in AT (Strieder-Barboza et al., 2018) and may indicate higher risk for developing fatty liver. HODEs and other oxylipids that are markers of inflammation in AT may provide disease risk information regarding AT function but still require large epidemiological studies to be validated. It is necessary to mention that single biomarkers do not provide enough information to support management decisions during the transition period. However, when multiple biomarkers are analyzed together and combined with health, production, nutritional, and environmental data, biomarkers become essential for identifying metabolic problems related to extended periods of intense lipolysis (Contreras et al., 2017b). The mechanisms for this cause effect relationship may include lipolysis induced AT remodeling processes, alterations in the expression of adipokines, and the development of insulin resistance.

Conclusions

Lipid mobilization modulates the inflammatory responses within AT and systemically. Lipolysis, a key component of the lipid mobilization proces, is a major trigger of inflammation within AT by driving macrophage infiltration into AT and inducing changes in the structure of the adipose organ. Systemically, lipolysis products, including NEFA and oxylipids, are inflammatory mediators that modulate immune and metabolic functions. Reducing the intensity and duraton of the periparturient lipolytic surge by management and nutritional interventions may ensure a rapid return to positive energy balance and ensure a productive lactation.

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Quantifying Protein Mobilization in Transition Dairy Cows

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Abstract

Cows are able to mobilize adipose, protein, and glycogen stores to meet energy and amino acid requirements that increase in late gestation and early lactation. While we are able to use body condition score (**BCS**) change and measurements of non-esterified fatty acids (**NEFA**) and ketones to quantify adipose tissue mobilization, less is understood about protein mobilization. Two primary techniques have been used to quantify protein mobilization: 1. ultrasound images to measure muscle depth at different locations and 2. measuring quantities of waste products from muscle degradation like 3-methylhistidine in blood or urine. These methods combine to give us more insight on when and to what extent animals are mobilizing muscle in order to meet the demands of late gestation and early lactation.

We observed that cows start to mobilize muscle prior to calving and that even 60 days into lactation they are still mobilizing muscle. BCS is not a good indicator of muscle depth or whole body muscle mass; currently there is no visual way to assess for muscle quantity in animals. However, muscle depth was a predictor of muscle mobilization. Cows that had more muscle depth prior to calving ended up mobilizing more muscle through the transition period. On average, cows mobilized 19% of their muscle depth at the longissimus dorsi from before calving to 60 days post calving. However, the amount of muscle that a cow had prior to calving was a strong indicator of how much muscle would be mobilized through transition ($R^2 = 0.68$; $P < 0.0001$); cows with more muscle prior to calving, mobilize more muscle through early lactation. Amino acids derived from muscle can be used to support milk production through the production of milk protein and lactose; therefore, optimizing muscle mobilization may be beneficial to support milk production in early lactation as long as doing so does not negatively impact health.

Introduction

During late gestation, the cow is mobilizing skeletal muscle in order to meet amino acid and potentially glucose requirements of the fetus in addition to provide amino acids for body maintenance and colostrogenesis. Komaragiri and Erdman (1997) estimated that empty body protein prior to calving represented between 12 and 13% of body weight (**BW**) for an animal at optimal body condition. In their study, cows prior to calving had approximately 95 kg of protein and mobilized in excess of 20 kg of protein from prior to calving to 5 weeks postpartum. At the same time, empty body fat represented 19 to 24% of BW and was dependent on BCS. Cows were capable of mobilizing in excess of 80 kg of adipose from prior to calving to 12 weeks postpartum. Although adipose

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tissue mobilization represents a considerably larger pool, protein mobilization from primarily skeletal muscle represents a considerable pool of amino acids for the transition dairy cow to utilize. Understanding the extent and implications of protein mobilization around parturition will allow us to develop feeding strategies to optimize tissue mobilization in order to maintain both health and production.

Amino Acids Mobilized from Skeletal Muscle

Skeletal muscle that is mobilized prior to parturition can be used for a number of outcomes, including milk protein synthesis, direct oxidation, or gluconeogenesis (Kuhla et al., 2011). Because of the mismatch between amino acids in milk and amino acids in muscle, as well as preferential use of specific amino acids for gluconeogenesis, there may be an imbalance of amino acids in circulation. Alanine and glutamine are the amino acids used in the greatest quantity for gluconeogenesis in the dairy cow (Drackley et al., 2001). Moreover, compared to milk, skeletal muscle is lower in branch chain amino acids; therefore, skeletal muscle may be mobilized in excess in order to meet the amino acid requirements for milk protein synthesis in early lactation. Evidence of an imbalance in plasma amino acid profile in early lactation is observed by a decrease in essential amino acids in plasma and an increase in non-essential amino acids in plasma around parturition (Kuhla et al., 2011).

Individual amino acids have shown to impact feed intake and impact insulin signaling. In early lactation, dairy cattle tissues that is insulin sensitive are more insulin resistant and there are relatively low levels of circulating insulin (De Koster and Opsomer, 2013). However, leucine has been shown to increase insulin secretion in laboratory animals and increase α-amylase production by the pancreas in dairy cattle (Liu et al., 2015; Sadri et al., 2017), showing that individual amino acids likely have an impact on feed regulation and also tissue mobilization due to their effects on insulin. While it is not clearly defined, it is reasonable to believe that amino acids, indirectly through their conversion to ketones and glucose or directly through amino acid signaling, impact feed intake.

Ultrasound Imaging to Quantify Tissue Mobilization

Tissue mobilization during the transition period traditionally equates to mobilization of adipose tissue. Both commercial farms and research trials routinely measure NEFA and β-hydroxybutyrate (**BHBA**) as indicators of how much adipose tissue is being mobilized. Additionally, change in BCS has been used a proxy for subcutaneous adipose tissue mobilization with recommendations to minimize the extent of body condition loss through calving (Garnsworthy, 2006; Roche et al., 2009). It is well established that excess NEFA leads to accumulation of adipose in the liver and reduces the capacity of the liver to synthesize glucose (Drackley, 1999). However, less is known about the extent and implications of muscle mobilization during the transition period.

Researchers are using ultrasound scans of the longissimus dorsi muscle and back fat thickness to quantify muscle depth and back fat thickness prior to calving through peak lactation (van der Drift et al., 2012; Boerman, unpublished). van der Drift (2012) found that even at the same BCS, cows had differences in muscle depth and back fat thickness and speculated that maybe more important was the ratio of fat to muscle to determine which tissue would be mobilized. In research conducted in our lab, ultrasound images were taken from

at 7 time points from 35 days before expected calving to 60 DIM. We observed that cows started mobilizing muscle and adipose prior to calving and that 60 DIM represented the smallest quantity of both muscle depth and back fat thickness. At approximately 21 days prior to calving, the muscle depth of cows averaged 4.5 cm, with a range of 2 to 6.5 cm. By 60 DIM, average muscle depth was 3.4 cm with a range of 1.9 to 4.7 cm. Muscle depth was not related to BCS and therefore, cannot be predicted by visual observation of the cow.

Cows with the most muscle depth prior to calving mobilized the most muscle when calculating muscle depth mobilization from 21 days prior to expected calving to 60 DIM (R^2 = 0.68; $P < 0.0001$; Figure 1). On average, cows mobilized 19% of their muscle depth from before calving to 60 days of lactation; however, some cows actually gained muscle depth during early lactation, whereas other cows mobilized nearly 50% of their muscle depth. There is considerable variation in protein mobilization among cows, and the extent of mobilization appears to be related to the amount of muscle depth.

Back fat thickness depth has previously been used to estimate total amount of subcutaneous fat. Although, we know that livestock do not uniformly deposit subcutaneous fat uniformly, Schroder and Staufenbiel (2006) estimated that 1 mm of back fat thickness equates to approximately 5 kg of adipose tissue. Cows with more back fat prior to calving mobilized more back fat (from 21 days prior to expected calving to 60 DIM; $R^2 = 0.86$; $P <$ 0.0001; Figure 2). Interestingly, animals were all fed the same diet during late lactation and the dry period that met or exceeded both protein and energy requirements; however, there was not a strong relationship between muscle depth and back fat thickness (Figure 3). Together, these data show that cows mobilize the tissue that they have excess of and that there is little relationship between muscle depth and back fat thickness.

Metabolites that Predict Muscle Mobilization

Creatinine and 3-methylhistidine can be used to determine proteolysis around parturition. Creatinine is a waste product produced by muscle at a relatively constant rate and can be used as an indicator of total muscle mass. 3-methylhistidine is used to estimate protein mobilization because it is a product of actin and myosin degradation and is not used for protein synthesis (Chibisa et al., 2008). Concentrations of 3-methylhistidine are excreted at a rate relative to muscle breakdown. At times when muscle catabolism exceeds anabolism, increases in 3-methylhistidine are observed. When analyzing for 3-methylhistidine, it is important to be able to separate out 1-methylhistidine from 3-methylhistidine (Houweling et al., 2012). The former may be difficult to separate out from 3-methylhistidine, but it is not thought to be related to protein mobilization. Being unable to separate out methylhistidine products would result in elevated and inaccurate numbers for 3-methyl histidine and would not accurately represent protein mobilization.

In order to correct for differences in muscle mass between cows and between stage of gestation or lactation, using the ratio of 3-methylhistidine to creatinine allows the comparison of protein mobilization per amount of muscle mass. Muscle mobilization measured as 3-methylhistidine and the ratio of 3-methylhistidine:creatinine were both elevated in the first 2 weeks prior to calving compared to 4 weeks prior to calving and 7 weeks post calving (Pires et al., 2013). Work done in our laboratory observed that cows have elevated 3-methylhistidine concentrations in the week prior to calving and continue

to have elevated 3-methylhistidine and 3-methylhistidine:creatinine concentrations in the 3 weeks post calving relative to day of parturition. Indicating that mobilization of protein occurs prior to calving and continues for at least 3 weeks post calving.

What are the Implications for Mobilized Tissue?

While most cows mobilize more adipose tissue to meet energy requirements around calving, previous research and preliminary data from our lab shows that muscle is mobilized in large and varying quantities. Unlike adipose tissue that can accumulate in the liver, reduce gluconeogenesis, and reduce feed intake, less is known about the potential negative health effects of mobilizing large amounts of muscle. In order to maintain structural soundness, animals will have to maintain a certain amount of skeletal muscle. Although, it is energetically expensive to mobilize and then re-accrete tissue (NRC, 2001), utilizing muscle as an energy source in early lactation is an adaptation to the onset of lactation. Studies have shown that animals that lose more weight in early lactation have reduced reproductive performance (Buckley et al., 2003; Zachut and Moallem, 2017. However, these studies did not try to quantify weight loss as either muscle or adipose. Comparing high weight loss and low weight loss groups of cows, cows that mobilized more body tissue resulted in more days open and lower conception rates (Zachut and Moallem, 2017). More mechanistic approaches are being used to determine if there are relationships between fertility and the gene expression of enzymes related to the conversion of amino acids to glucose (Moran et al., 2016). Data suggest that cows with lower fertility began using body reserves for glucose production earlier than cows with higher fertility, potentially indicating that cows that mobilize more muscle will have reduced fertility. Before making recommendations to try to increase muscle mobilization, we need to have a better understanding of the potential negative impacts on reproduction.

Conclusions

Protein is mobilized from dairy cows prior to parturition to meet fetal amino acid requirements and for milk protein synthesis during colostrogenesis. Post-calving, protein continues to be mobilized for milk protein synthesis and for the production of ketones and glucose. While less is known about skeletal muscle mobilization than adipose tissue mobilization, ultrasound imaging and measuring proteolysis products (i.e., 3-methylhistidine) provides an indication of the extent and timing of protein mobilization. Preliminary work done in our lab indicates that there is a strong relationship between the amount of muscle and the extent that an animal will mobilize muscle. Similarly, there is also a strong relationship between the amount of back fat and the extent of back fat that will be mobilized through late gestation and early lactation. There is not a strong relationship between muscle depth and back fat thickness, indicating that cows mobilize the tissue that they have in excess. Although there are energetic considerations for muscle accretion, there may also be benefits to mobilizing muscle to meet glucose demands in early lactation rather than relying on adipose tissue mobilization. Certainly more work to better understand mechanisms that regulate tissue mobilization, as well as understanding of nutritional strategies that will influence muscle mobilization, are needed.

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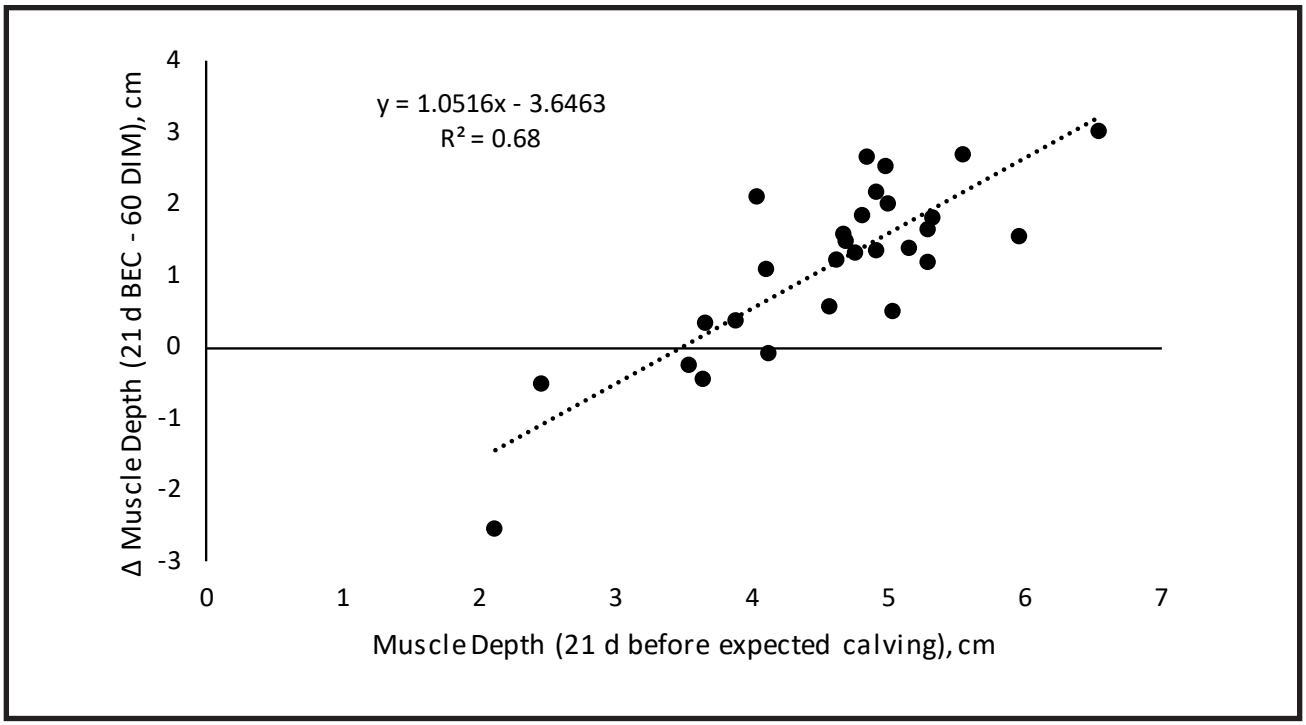

Figure 1. Muscle depth measured at the longismuss dorsi muscle at 21 days before expected calving by muscle mobilization measured from 21 days before expected calving (BEC) to 60 DIM. If values on the y axis are positive, it indicates that cows mobilized muscle from 21 d before expected calving to 60 days in milk.

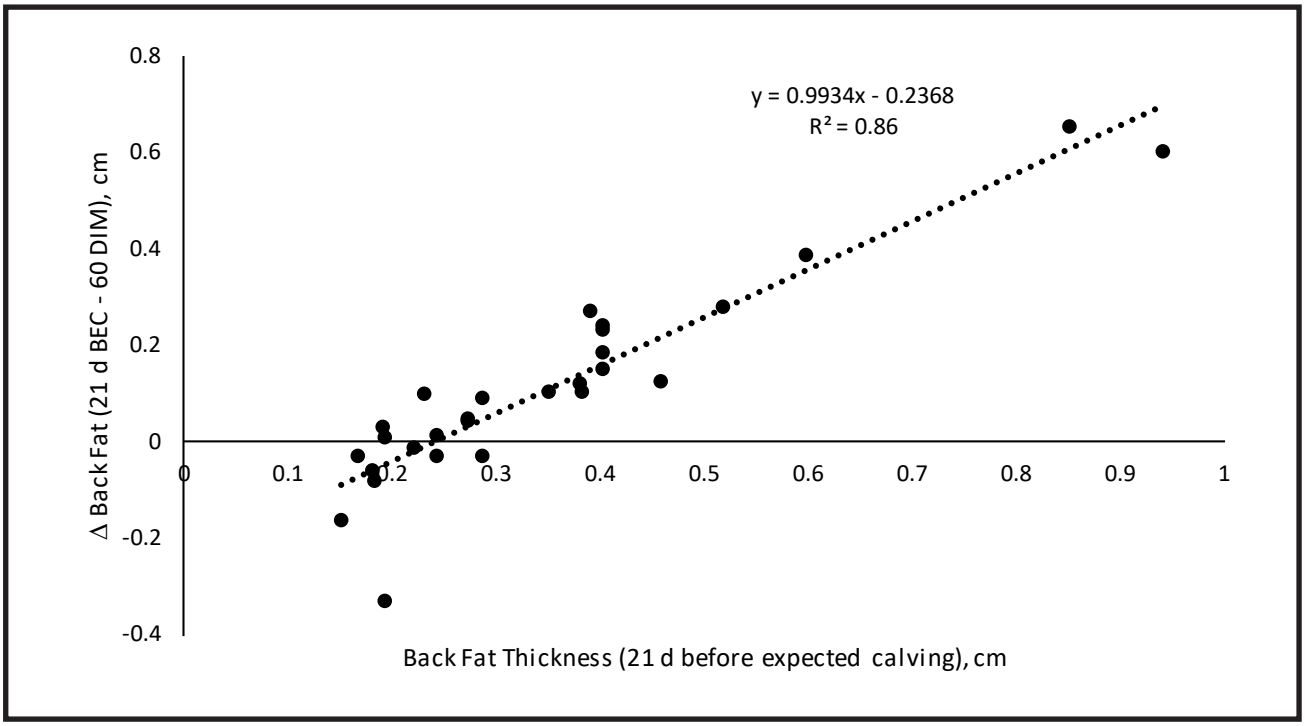

Figure 2. Back fat thickness measured above the longismuss dorsi muscle at 21 days before expected calving (BEC) by back fat mobilization measured from 21 days before expected calving to 60 DIM. If values on the y axis are positive, it indicates that cows mobilized back fat from 21 days before expected calving to 60 days in milk.

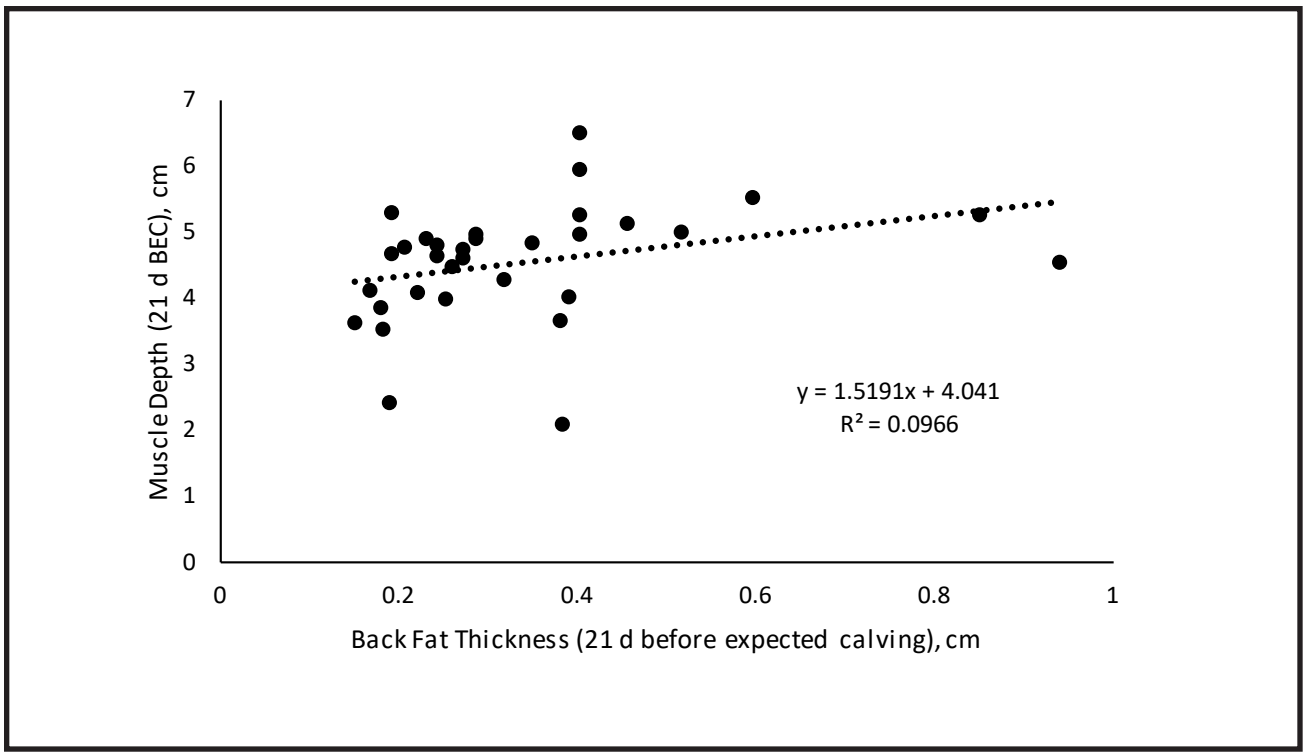

Figure 3. Relationship between muscle depth and back fat thickness measured from ultrasound images taken 21 days before expected calving (BEC) at the longissimus dorsi muscle.

Feeding Blends of Fatty Acids for Transition Cows

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Introduction

Recently, the effects of individual fatty acids (**FA**) on digestibility, metabolism, and production responses of dairy cows has received attention. In fresh cows, the high metabolic demand of lactation and reduced DMI during the immediate postpartum period result in a state of negative energy balance. Approaches to increasing energy intake of postpartum cows include increasing starch content of the diet and supplementing FA to increase the energy density of the diet. However, feeding high starch diets that promote greater ruminal propionate production during early lactation could be hypophagic and therefore further reduce DMI and increase the risk of ruminal acidosis and displaced abomasum (Allen and Piantoni, 2013). Some authors suggest that caution should be exercised when using supplemental FA to increase the caloric density of diets in early lactation dairy cows, since a high lipid load may affect the endocrine system, feed intake, and increases the risk for metabolic disorders (Kuhla et al., 2016). However, just as we recognize that not all protein sources are the same, it is important to remember that not all FA or FA supplements are the same. We will briefly review the biological processes and quantitative changes during the metabolism of FA, the digestibility of these FA, and their overall impact on performance. Our emphasis in the current paper is on recent research supplementing palmitic (C16:0), stearic (C18:0), and oleic (C18:1) acids on feed intake, nutrient digestibility, and milk production.

Effect of Fatty Acids on NDF Digestibility

Changes in intake and digestibility of other nutrients, such as NDF, due to FA supplementation may affect positively or negatively the digestible energy value of any FA supplement. Weld and Armentano (2017) performed a meta-analysis to evaluate the effects of FA supplementation on DMI and NDF digestibilities of dairy cows. Addition of vegetable oil decreased NDF digestibility by 2.1 percentage units but did not affect DMI. Feeding saturated prilled supplements (combinations of C16:0 and C18:0) did not affect DMI, but increased NDF digestibility by 0.22 percentage units. Overall, the authors concluded that the addition of a fat supplement, in which the FA are 16-carbon or greater in length, has minimal effects on NDF digestibility, but the effect of C16:0-enriched supplements were not evaluated.

We recently utilized a random regression model to analyze available individual cow data from 6 studies whereby C16:0-enriched supplements were fed to dairy cows (de Souza et al., 2016). We observed that NDF digestibility was positively impacted by total C16:0 intake (Figure 1A) and DMI was not affected. This suggests that that the increase in NDF digestibility when C16:0-enriched supplements

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are fed to dairy cows is not explained through a decrease in DMI. Additionally, when comparing combinations of C16:0, C18:0, and C18:1 in supplemental fat, we observed that feeding supplements containing C16:0 or C16:0 and C18:1 increased NDF digestibility compared with a supplement containing C16:0 and C18:0 (de Souza et al., 2018a).

With early lactation cows, Piantoni et al. (2015b) fed a saturated fat supplement (~ 40% C16:0 and 40% C18:0) and observed that fat supplementation increased NDF digestibility by 3.9% units in the low forage diet (20% fNDF) but had no effect in the high forage diet (26% fNDF). When evaluating the effects of timing of C16:0 supplementation (**PA**) on performance of early lactation dairy cows (de Souza et al., 2019), we observed that C16:0 supplementation consistently increased NDF digestibility ~ 5% units over the 10 weeks of treatment compared with control (Figure 1B).

Effects of C16:0, C18:0, and C18:1 on Fatty Acid Digestibility

Our recent FA digestibility research has utilized and focused on C16:0, C18:0, and C18:1. Of particular importance, Boerman et al. (2017) fed increasing levels of a C18:0-enriched supplement (93% C18:0) to mid-lactation dairy cows and observed no positive effect on production responses, which was likely associated with the pronounced decrease in total FA digestibility as FA intake increased (Figure 2A). Similarly, Rico et al. (2017) fed increasing levels of a C16:0-enriched supplement (87% C16:0) to mid-lactation dairy cows and even though a positive effect was observed on production response up to 1.5% diet DM, a decrease in total FA digestibility with increasing FA intake was observed (Figure 2B). However, considering that the range in FA intake was similar across both studies, the decrease in total FA digestibility was more pronounced when there was increased intake/rumen outflow of C18:0 rather than C16:0. This is supported by our meta-analysis, in which a negative relationship between the total flow and digestibility of FA was observed, with the decrease in total FA digestibility driven by the digestibility of C18:0 because of the negative relationship between duodenal flow and digestibility of C18:0 (Boerman et al., 2015). The exact mechanisms for these differences in digestibility are not understood; however, potential causes include the lower solubility of C18:0 compared to C16:0, which would be more dependent of emulsification for absorption (Drackey, 2000). Additionally, results have shown that C18:1 has greater digestibility than C16:0 and C18:0 (Boerman et al., 2015). Freeman (1969) examined the amphiphilic properties of polar lipid solutes and found that C18:1 had a positive effect on the micellar solubility of C18:0. To further understand what factors influence FA digestibility, we utilized a random regression model to analyze available individual cow data from 5 studies that fed a C16:0-enriched supplement to dairy cows. We observed that total FA digestibility was negatively impacted by total FA intake, but positively influenced by the intake of C18:1 (unpublished results). This is supported by a recent study in which abomasal infusion of C18:1 increased FA digestibility without negatively affecting feed intake (Prom et al., 2018). Finally, we recently evaluated the effects of varying the ratio of dietary C16:0, C18:0, and C18:1 in basal diets containing soyhulls or whole cottonseed on FA digestibility. We observed that feeding a supplement containing C16:0 and C18:1 increased FA digestibility compared with a supplement containing C16:0, a mixture C16:0 and C18:0, and a non-fat control diet. The supplement containing a mixture C16:0 and C18:0 reduced FA digestibility compared with the other treatments (de Souza et al., 2018a). This is displayed in Figure 3 by using a Lucas

test to estimate the apparent digestibility of the supplemental FA blends. The slopes (i.e., digestibility of the supplemental FA blends) in soyhulls based diets were 0.64, 0.55 and 0.75 and in cottonseed diets were 0.70, 0.56 and 0.81 for supplements containing C16:0, a mixture C16:0 and C18:0, and a mixture of C16:0 and C18:1, respectively. This supports the concept that a combination of 16-carbon and unsaturated 18-carbon FA may improve FA digestibility, but reasons for this need to be determined.

In fresh cows, there is scarce information about the effects of supplemental FA on FA digestibility. We recently conducted a study to evaluate the effects of timing of C16:0 supplementation on nutrient digestibility of early lactation dairy cows (de Souza et al., 2019). We observed a treatment by time interaction for C16:0 supplementation during the fresh period (1 – 24 DIM); although C16:0 reduced total FA digestibility compared with control, the magnitude of difference reduced over time (Figure 4). Interestingly, we also observed an interaction between time of supplementation and C16:0 supplementation during the peak period (25 – 67 DIM), due to C16:0 only reducing FA digestibility in cows that received the control diet in the fresh period. This may suggest an adaptive mechanism in the intestine when C16:0 is fed long-term. In a recent study, increasing C18:1 in the FA supplement blends during early lactation increased digestibility, resulting in increased energy intake (de Souza et al., unpublished). Understanding the mechanisms responsible for this effect deserves future attention, as does the impact of other supplemental FA during early post-partum on FA digestibility and nutrient digestibility.

Effects of C16:0, C18:0, and C18:1 on Production Responses

We have recently carried out a series of studies examining the effect of individual saturated FA on production and metabolic responses of lactating cows. In a dose response study with mid lactation cows, feeding a C18:0-enriched supplement (93% C18:0) increased DMI but had no effect on the yields of milk or milk components when compared to a non-FA supplemented control diet, which was probably associated with the decrease in FA digestibility (Figure 2A, Boerman et al., 2017). Our results, and those of others, indicate that C16:0 supplementation has the potential to increase yields of energy corrected milk (**ECM**) and milk fat as well as the conversion of feed to milk, independent of production level when it was included in the diet for soyhulls or C18:0 (Rico et al., 2014; de Souza et al., 2018a). We recently utilized a random regression model to analyze available individual cow data from 10 studies whereby C16:0-enriched supplements were fed to post peak dairy cows (de Souza et al., 2016). We observed that energy partitioning toward milk was increased linearly with C16:0 intake, as a result of a linear increase in milk fat yield and ECM with increasing intake of C16:0.

When we compared combinations of C16:0, C18:0, and C18:1 in FA supplements, a supplement containing more C16:0 increased energy partitioning toward milk due to the greater milk fat yield response compared with the other treatments (de Souza et al., 2018a). In contrast, a FA supplement containing C16:0 and C18:1 increased energy allocated to body reserves compared with other treatments. The FA supplement containing a combination of C16:0 and C18:0 reduced nutrient digestibility, which most likely explains the lower production responses observed compared with the other treatments. Interestingly, in a follow up study, we compared different ratios of C16:0 and C18:1 in FA supplements fed to post-peak cows and observed that supplements with more C16:0 favored energy partitioning to milk in cows producing less than 45 kg/day, while

supplements with more C18:1 favored energy partitioning to milk in cows producing greater than 60 kg/day (de Souza and Lock, 2017). Also, regardless of production level, supplements with more C18:1 increased BW change. This may suggest that C16:0 and C18:1 are able to alter energy partitioning between the mammary gland and adipose tissue, which may allow for different FA supplements to be fed in specific situations according to the metabolic priority and needs of dairy cows. Further research is needed to confirm these results in cows at different stages of lactation or other physiological conditions.

In early lactation cows, Beam and Butler (1998) fed a saturated FA supplement (~ 40% C16:0 and 40% C18:0) and observed that FA supplementation decreased DMI and did not affect yields of milk and ECM in the first 4 weeks after calving. Piantoni et al. (2015b) fed a similar saturated FA supplement (~ 40% C16:0 and 40% C18:0) and observed that FA supplementation during the immediate postpartum (1 to 29 DIM) favored energy partitioning to body reserves rather than milk yield, especially in the lower forage diet. The high forage diet with supplemental FA increased DMI and tended to decrease body condition score (**BCS**) loss compared with the same diet without FA supplementation. Also, regardless of forage level, feeding supplemental FA increased DMI, decreased BCS loss, but tended to decrease milk yield. When cows were fed a common diet during the carryover period, the low forage diet with FA supplementation fed during the immediate postpartum continued to decrease milk yield and maintained higher BCS compared with the other treatments. On the other hand, Weiss and Pinos-Rodriguez (2009) fed a similar saturated FA supplement (~ 40% C16:0 and 40% C18:0) to early-lactation cows (21 to 126 DIM) and observed that when high-forage diets were supplemented with FA, the increased NEL intake went toward body energy reserves as measured by higher BCS with no change in milk yield. However, when low-forage diets were supplemented with FA, milk yield increased (2.6 kg/day) with no change in BCS.

We evaluated the effects of timing of C16:0 supplementation on performance of early lactation dairy cows (de Souza and Lock, 2019). During the fresh period (1 to 24 DIM), we did not observe treatment differences for DMI or milk yield (Figure 5A), but compared with control, C16:0 increased the yield of ECM by 4.70 kg/day consistently over time (Figure 5B). However, C16:0 reduced BW by 21 kg (Figure 6), and BCS by 0.09 units and tended to increase BW loss by 0.76 kg/day compared with CON. Feeding C16:0 during the peak period (25 to 67 DIM) increased the yield of milk by 3.45 kg/day, ECM yield by 4.60 kg/day (Figure 5), and tended to reduce BW by 10 kg compared with control (Figure 6).

In a recent study, a non-FA supplemented control diet was compared with diets supplemented at 1.5% DM with FA supplements differing in the ratio of C16:0 and C18:1 (de Souza et al., 2018b). FA treatment diets were: 80:10 (80% C16:0 + 10% C18:1); 70:20 (70% C16:0 + 20% C18:1); and 60:30 (60% C16:0 + 30% C18:1). From days 25 to 60 postpartum, all cows were offered a common diet to evaluate carryover effects. During the fresh period, FA-supplemented diets increased milk yield, ECM, and milk fat yield (Figure 7). Increasing C18:1 in FA treatments decreased plasma NEFA and BW loss and tended to increase DMI and plasma insulin (Figure 7). Increasing C18:1 in FA treatments did not affect milk yield, ECM, and the yields of milk fat and protein. During the carryover period, cows that received FA-supplemented diets during the fresh period increased ECM and milk fat yields compared with the control treatment.

Interestingly, Greco et al. (2015) observed that decreasing the ratio of omega-6 to omega-3 FA in the diet of lactating dairy cows while maintaining similar dietary concentrations of total FA improved productive performance in early lactation. A dietary omega-6 to omega-3 ratio of approximately 4:1 increased DMI and production of milk and milk components compared with a 6:1 ratio. Approximately 1.3 kg of milk response could not be accounted for by differences in nutrient intake, which suggests that reducing the dietary FA ratio from 6:1 to 4:1 can influence nutrient partitioning to favor an increased proportion of the total net energy consumed allocated to milk synthesis. Further studies focusing on altering ratio of dietary FA are warrant, especially in early lactation cows.

Effects of Supplemental Fatty Acids on Reproduction

A recent meta-analysis of 17 studies reported a 27% increase in pregnancy rate in the first postpartum artificial insemination (**AI**) when dairy cows were fed fat supplements during the transition period (Rodney et al., 2015). In addition, the interval from calving to pregnancy was reduced. The inclusion of the very long chain omega-3 FA eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) in the form of fish meal, fish oil, or algae in the diet has been shown to improve either first-service or over- all pregnancy in 6 studies (Santos and Staples, 2017). A study conducted at the University of Florida (Silvestre et al., 2011) demonstrated that supplementation with Ca salts (1.5% of dietary DM) enriched in fish oil-derived FA starting at 30 DIM improved pregnancy rate/AI compared with Ca salts of palm FA (52.8 vs. 45.5%). Additionally, pregnancy loss between 32 and 60 days after AI was reduced by feeding Ca salts containing EPA and DHA (6.1 vs. 11.8%). Recently, Sinedino et al. (2017) observed that feeding 100 g/day of an algae product containing 10% of DM as DHA starting in the third week postpartum increased pregnancy rate by 39% and reduced days to pregnancy by 22 days (102 vs. 124 days). Therefore, polyunsaturated long-chain FA including omega-6 and omega-3 seem to be more effective at improving pregnancy in dairy cows than those containing mainly C16:0 and C18:1. Furthermore, a meta-analysis indicated that the probability of pregnancy increased by 26% and the days from calving to pregnancy decreased by 34 days when *trans*-10, *cis*-12 conjugated linoleic acid was fed as a Ca-salt product across 5 studies involving 221 early lactation dairy cows (de Veth et al., 2009). Feeding long-chain FA might improve reproduction in dairy cattle through several potential mechanisms, including reducing negative energy balance, changes in follicle development and improvements in oocyte quality, improved early embryo development, and reduced pregnancy loss. Since individual FA have a direct effect on several metabolic processes, research should focus on determining "ideal" combinations of FA for cows under specific physiological conditions and for specific purposes.

Conclusions

The addition of supplemental FA to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. Although, in general, FA supplementation has been shown to increase milk yield, milk fat yield, and improve reproduction performance, great variation has been reported in production performance for different FA supplements, and indeed, the same supplement across different diets and studies. Results are contradictory about the benefits of FA supplementation to early lactation dairy cows. We propose that this is a result of differences in FA profile of supplements used and the time at which FA supplementation starts. However,

our recent results suggest the use of specific supplemental FA and FA blends in the fresh period should be considered; however, further work is required to characterize the sources of variation in response to FA supplementation. Just as we recognize that not all protein sources are the same, it is important to remember that not all FA sources and FA supplements are the same. The key is to know what FA are present in the supplement, particularly FA chain length and their degree of unsaturation. Once this information is known, it is important to consider the possible effects of these FA on DMI, rumen metabolism, small intestine digestibility, milk component synthesis in the mammary gland, energy partitioning between the mammary gland and other tissues, body condition, and their effects on immune and reproductive functions. The extent of these simultaneous changes along with the goal of the nutritional strategy employed will ultimately determine the overall effect of the FA supplementation, and the associated decision regarding their inclusion in diets for lactating dairy cows.

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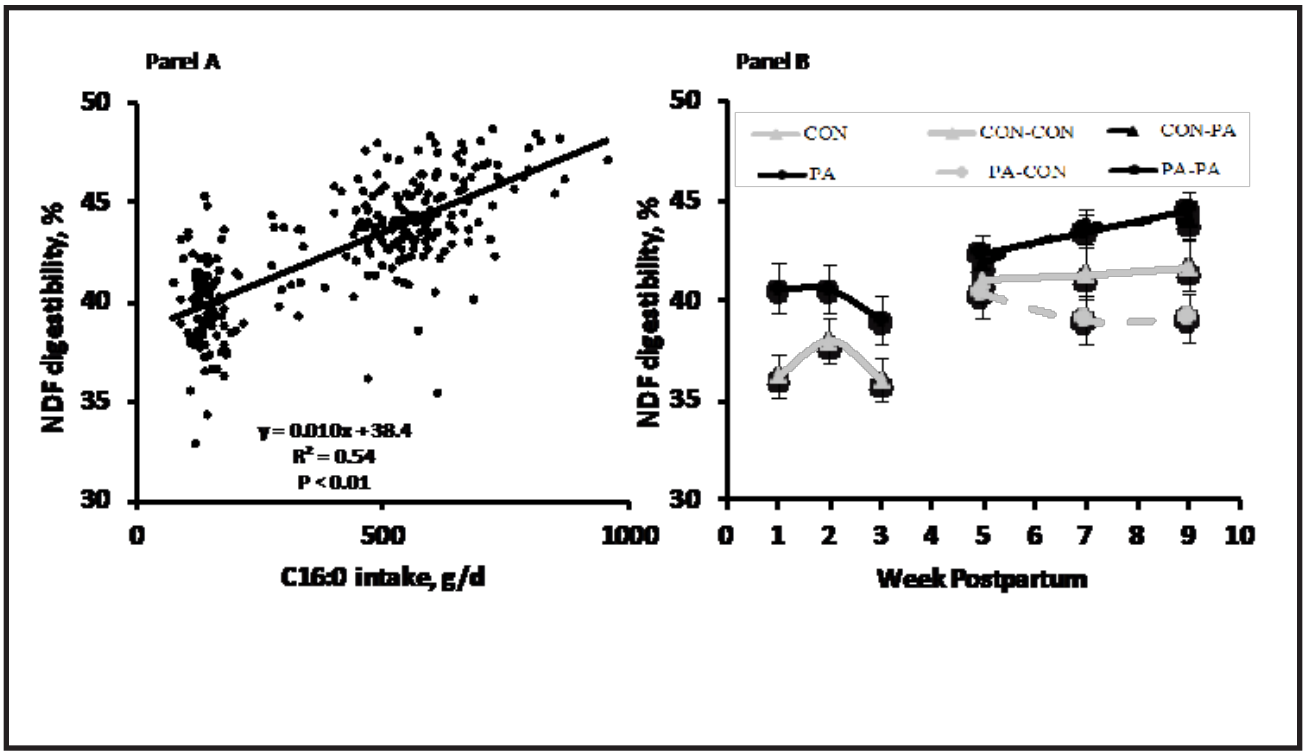

Figure 1. Panel A: Relationship between C16:0 intake and NDF digestibility of dairy cows fed C16:0-enriched FA supplements. Panel B: The effects of C16:0-enriched supplementation in early lactation cows on NDF digestibility. Results in Panel A represent a combined data set evaluated using a random regression model from 6 studies feeding C16:0-enriched supplements on NDF digestibility of post-peak cows (de Souza et al., 2016). Results in Panel B utilized 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period) or from 25 to 67 DIM (peak period). From de Souza et al. (2019).

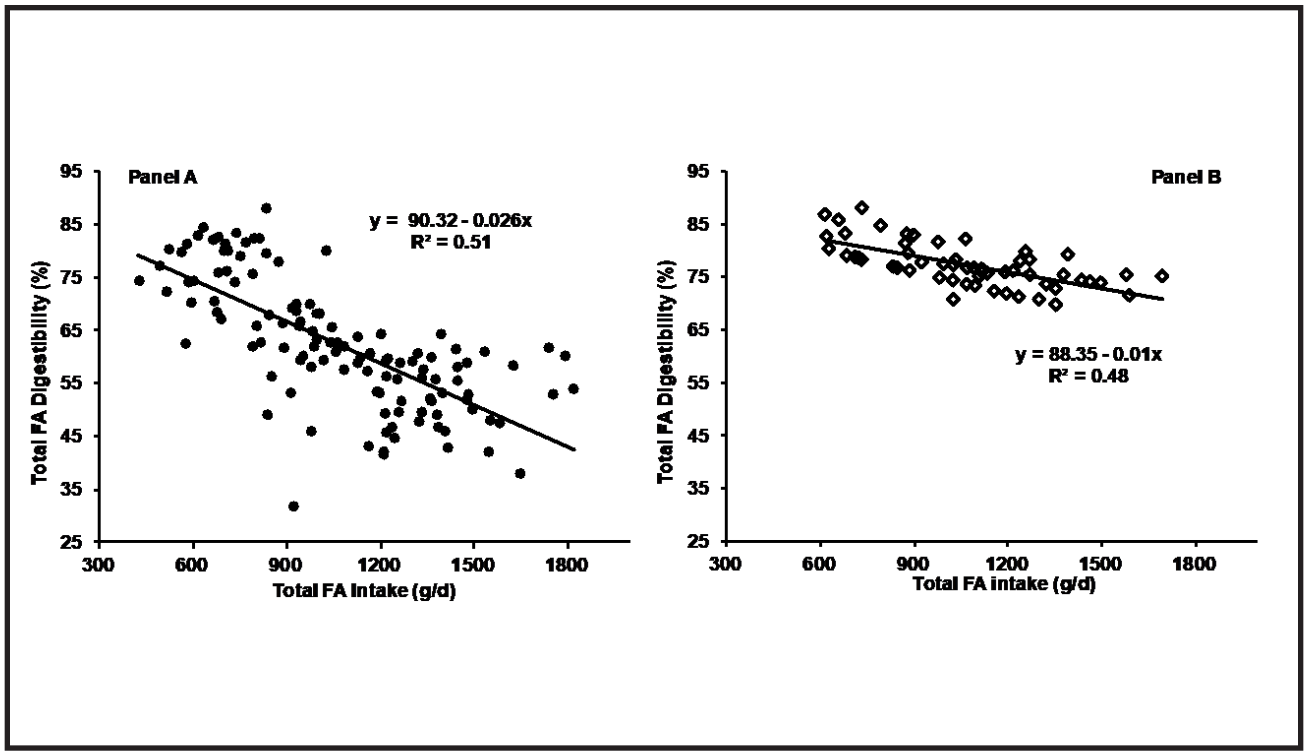

Figure 2. Relationship between total FA intake and apparent total-tract FA digestibility of dairy cows supplemented with either a C18:0-enriched supplement (Panel A) or a C16:0-enriched supplement (Panel B). Results in Panel A utilized 32 mid-lactation cows receiving diets with increasing levels (0 to 2.3% dry matter) of a C18:0-enriched supplement (93% C18:0) in a 4 X 4 Latin square design with 21-d periods (Boerman et al., 2017). Results in Panel B utilized 16 mid-lactation cows receiving diets with increasing levels (0 to 2.25% dry matter) of a C16:0-enriched supplement (87% C16:0) in a 4 X 4 Latin square design with 14-d periods (Rico et al., 2017).

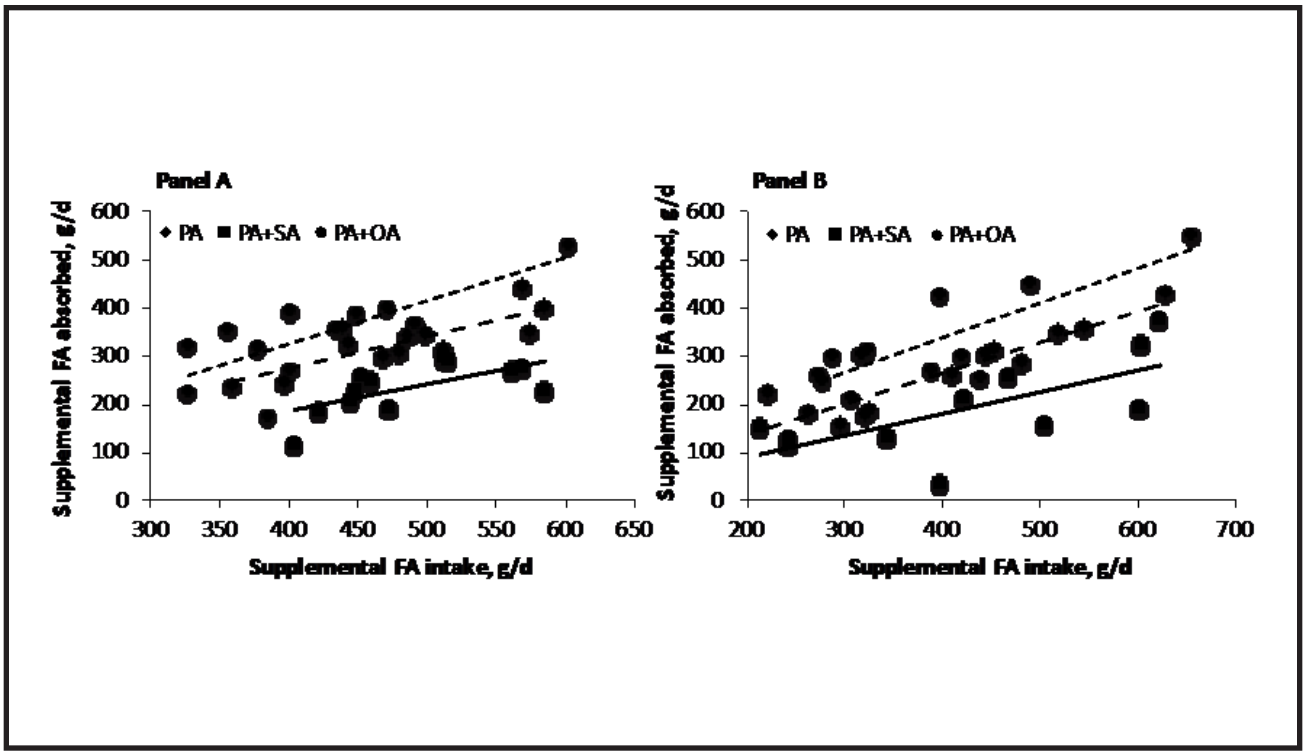

Figure 3. Lucas test to estimate total FA digestibility of supplemental FA treatments when cows received either a soyhulls basal diet (Panel A) or a cottonseed basal diet (Panel B) PA long-dashed line (1.5% of FA supplement blend to provide ~ 80% of C16:0); PA+SA solid line (1.5% of FA supplement blend to provide ~ 40% of C16:0 + 40% of C18:0); and PA+OA short-dashed line (1.5% of FA supplement blend to provide ~ 45% of C16:0 + 35% of C18:1). Digestibility of supplemental FA was estimated by regressing intake of supplemental FA on intake of digestible supplemental FA. The mean intakes of FA and digestible FA when cows were fed the control diet were subtracted from the actual intakes of total FA and digestible FA for each observation. From de Souza et al. (2018a).

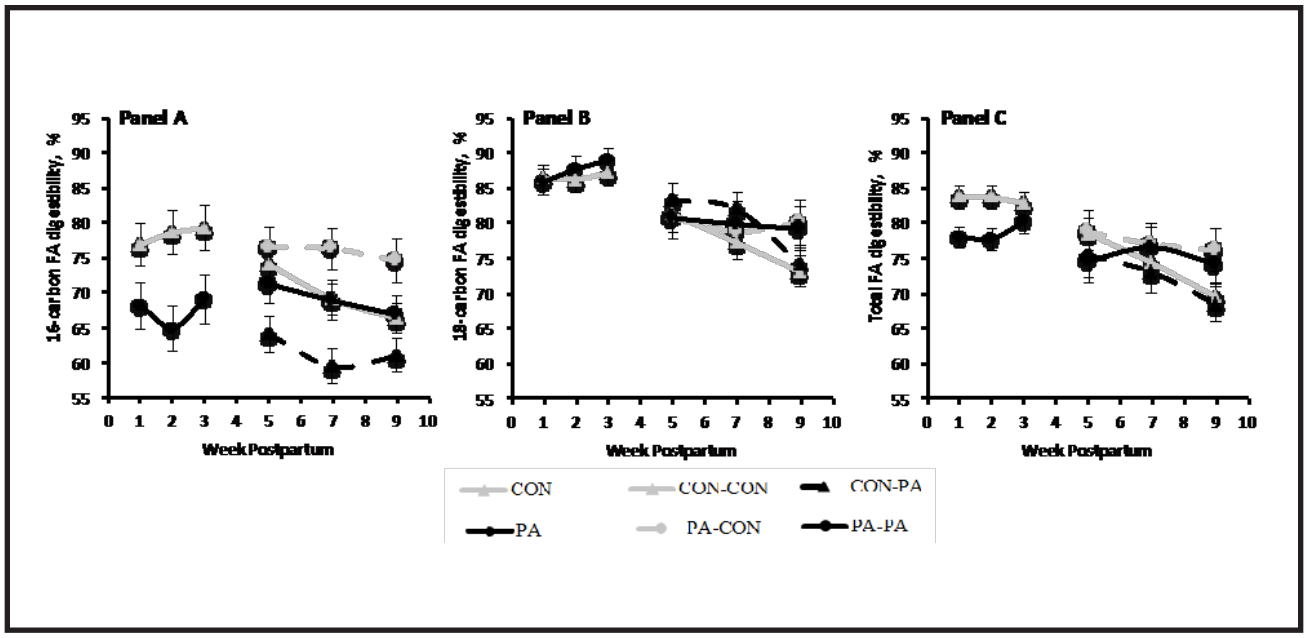

Figure 4. The effects of C16:0-enriched supplementation for early lactation cows on digestibility of 16-carbon (Panel A), 18-carbon (Panel B), and total FA (Panel C). Results utilized 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period FR) or from 25 to 67 DIM (peak period). From de Souza et al. (2019).

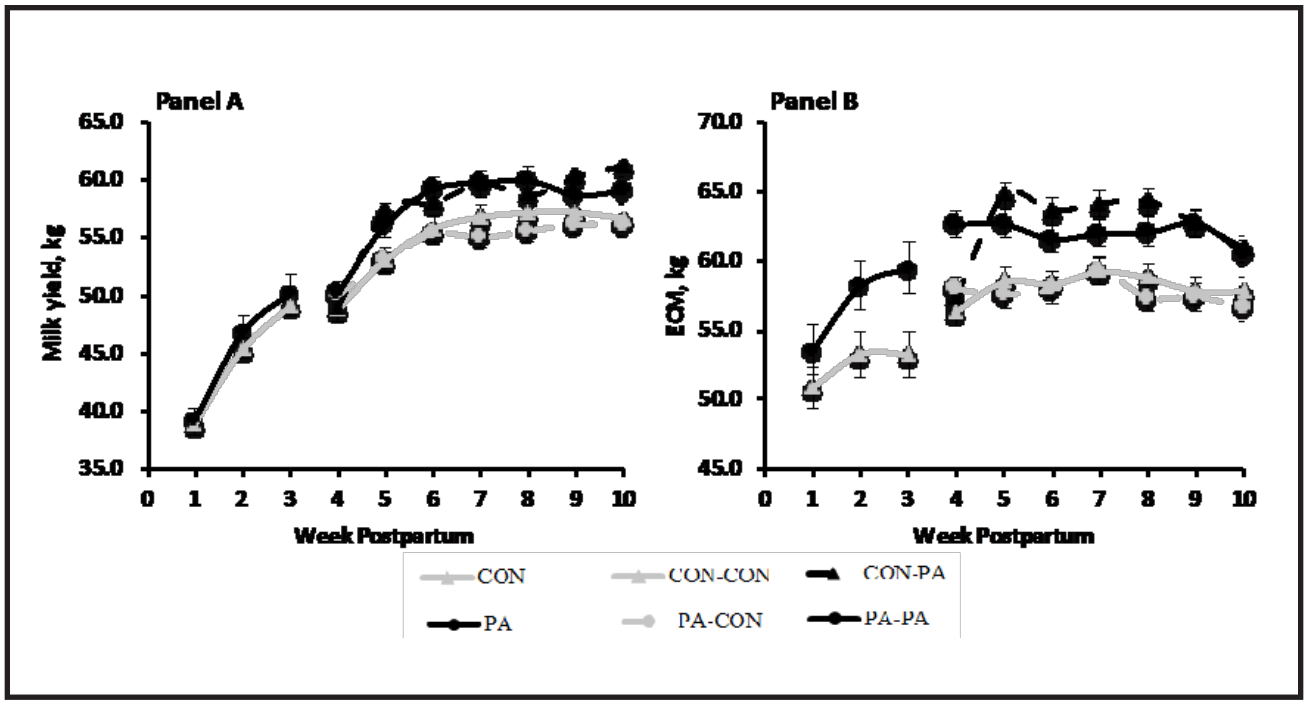

Figure 5. The effects of C16:0-enriched supplementation in early lactation cows on the yield of milk (Panel A) and ECM (Panel B). Results from 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period FR) or from 25 to 67 DIM (peak period). From de Souza and Lock (2019).

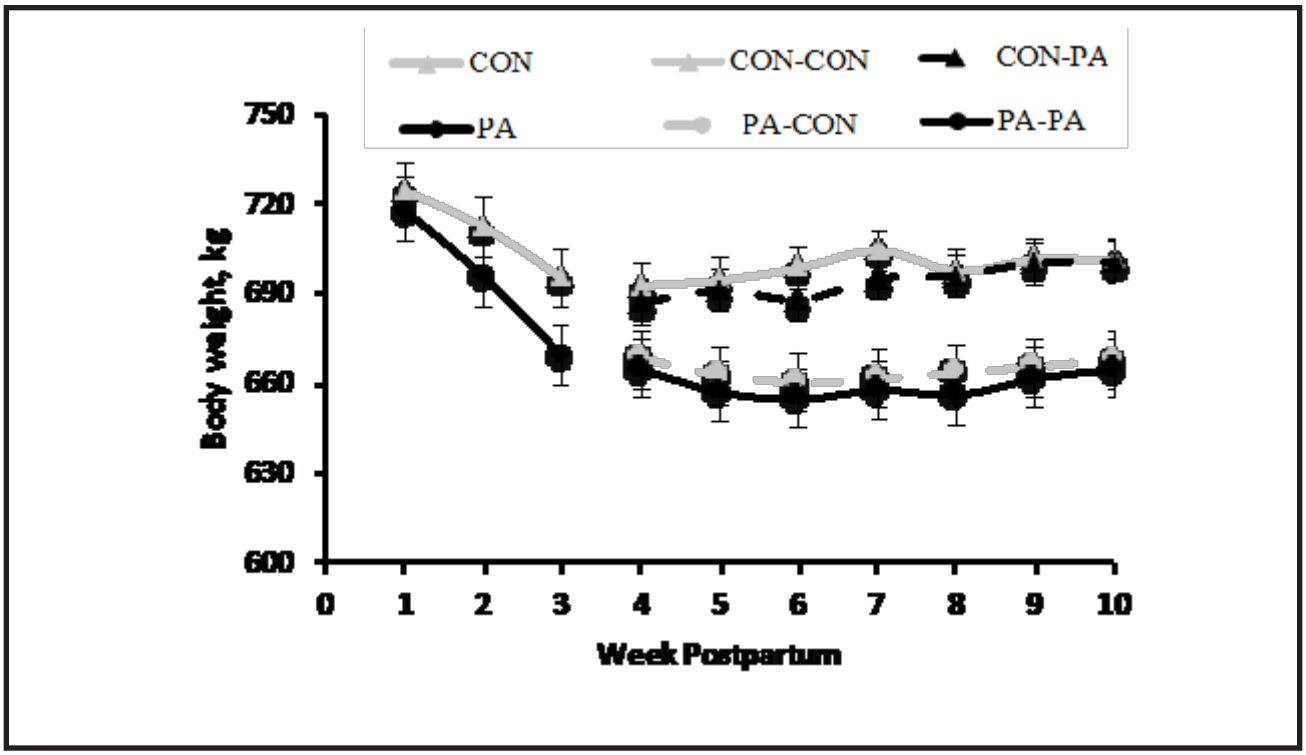

Figure 6. The effects of C16:0-enriched supplementation in early lactation cows on body weight. Results from 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period) or from 25 to 67 DIM (peak period). From de Souza and Lock (2019).

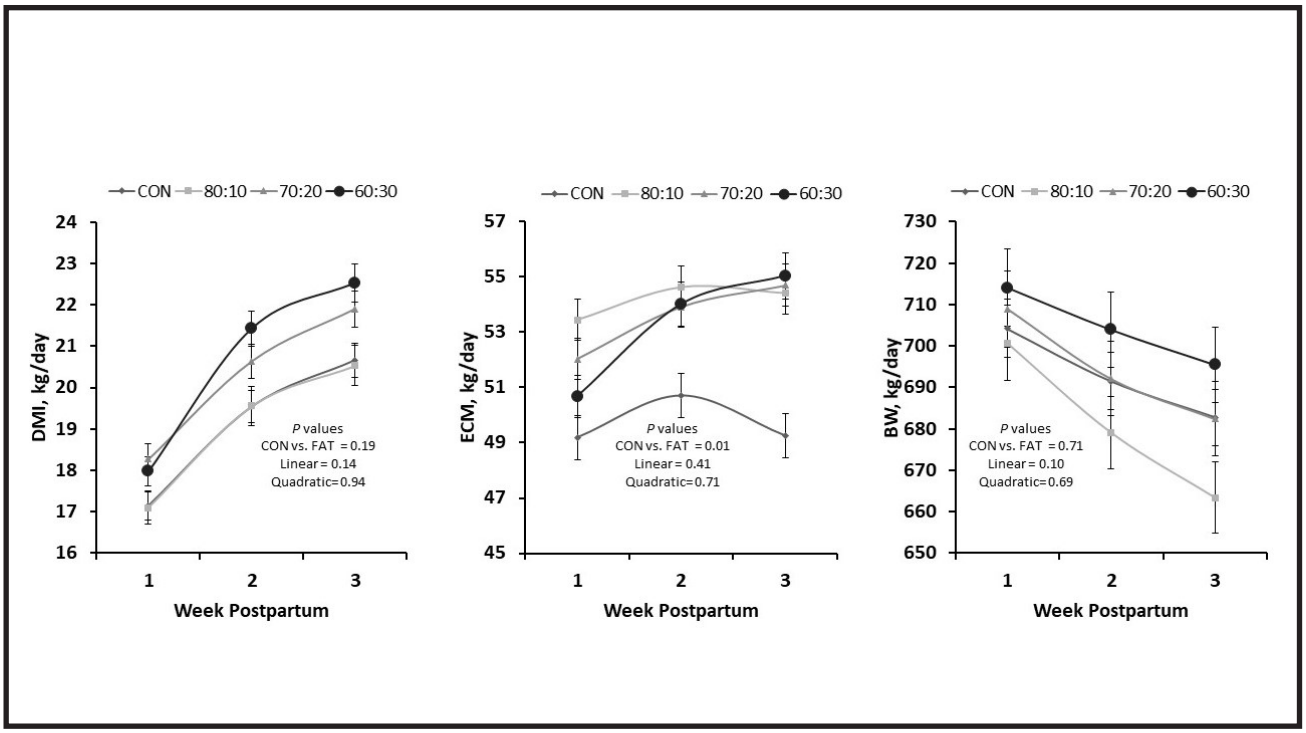

Figure 7. The effects of altering the C16:0 to C18:1 ratio of supplemented fats in early lactation cows on DMI, milk yield, and BW. Results from 52 early-lactation cows receiving the following diets: no supplemental fat (CON) and diets supplemented at 1.5% DM with FA supplements differing in the ratio of palmitic (C16:0) and oleic (C18:1) acids. FA treatment diets were: 80:10 (80% C16:0+10% C18:1); 70:20 (70% C16:0+20% C18:1); and 60:30 (60% C16:0+30% C18:1). From de Souza et al. (2018b).

Contemplating the Energetic Consequences of Bovine Mastitis

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Abstract

Mastitis is a common and expensive disease in the dairy industry that significantly reduces the quality and quantity of milk produced by the dairy cow. The reduction in the amount of milk produced is resultant of a multitude of factors, but the purpose of this article is to reflect upon how the activated immune system may consume specific substrates necessary for milk synthesis. The 3 key immune cells implicated in mastitis, the neutrophil, macrophage, and lymphocyte, have marked demands for substrates to carry out their immune related functions. For instance, both the neutrophil and macrophage phagocytose bacteria and kill the bacteria by the generation of reactive oxygen species. Generation of these reactive oxygen species is a largely glycolytic process. Additionally, amino acids are used by some lymphocytes for the synthesis and production of antibodies. Uniquely, in the instance of mastitis, these active immune cells are specially located in direct competition with the secretory mammary cells, which would compete for the substrates that would be used for milk synthesis.

Introduction

Mastitis remains the most common and expensive disease in the US and global dairy industries. The economic losses that result from mastitis are consequence of: 1) reduced milk production and quality, 2) increased labor, veterinary costs, and drug usage, 3) discarding abnormal milk and antibiotic laden milk, and 4) prematurely culling affected animals. Although the losses of mastitis are a consequence of many factors, the greatest financial loss is due to reduced milk production in affected animals (Blosser, 1979). Milk yield loss in response to mastitis has been recognized to occur for decades, but seldom is the question asked: *Why does this occur?* The answer to this question has many answers and many of which are interconnected and not independent of one another. The objective of this article is to consider and speculate how mastitis reduces milk production. Given the nutritional emphasis of the attendees of the Tri-State Dairy Nutrition Conference, a specific focus of this article will be the key substrates that are necessary for milk synthesis and contemplate what happens to these substrates during a mastitis event.

Factors Influencing Milk Production

The mechanisms that affect milk production should be briefly reviewed and appreciated if we are to build upon this concept and reflect how mastitis affects milk production. On the farm, numerous management and genetic decisions are dissected, scrutinized, and implemented with the goal of increasing milk production per cow. It is easy to be overwhelmed

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by the mountain of decisions that the dairy owner, herdsman, veterinarian, and nutritionist must make to achieve this goal. An incomplete listing of some of the key factors known to affect milk production include days in milk, nutrition status and energy balance, milking frequency, parity, breed, heat stress, metabolic diseases, and mastitis. Cutting through this thick fog of interweaving and associated elements, it can be simplified and recognized that only 2 factors actually determine a cow's milk production. Milk production is solely dictated by the number of milk secreting cells in the gland and the average rate at which these cells synthesize and secrete milk components. Yes, much simpler. All management practices used to improve milk yield affect one, or both of these central elements, and anything that would affect one or both of these key elements would ultimately affect milk production, for better or worse.

Mammary cell number

The idea that the number of mammary cells secreting milk would influence the amount of milk produced is nothing new and has been examined for decades. If differences in the number of cells in a lactating mammary gland were profound enough, differences in udder size would be observed. Make no mistake, this method of appraising an udder's productive capacity based on size is extremely unreliable and crude. It is being used here strictly for illustrative purposes. Perhaps a relevant and striking example would be the comparison of udders from beef and dairy cattle. In general, the udders of beef cows are smaller and far less productive than their dairy counterparts. Keys et al. (1989) made such a comparison and examined the udders of 10 Holstein and 10 Hereford heifers during their first gestation and at 49 days in milk. Animals were randomly selected for euthanasia at 150, 180, and 260 days of gestation and at 49 days in milk. At euthanasia, the udders were removed and examined. Researchers quantified the total amount of DNA in the collected udders. Total mammary DNA was used as a proxy for the number of cells in the gland because DNA is constant among cells and allows for comparisons to be made on the number of cells between glands. Overall, it was observed that the amount of total mammary DNA increased as gestation progressed, indicating growth of the udder for both the beef and dairy breeds. Even though both udders grew, the amount of total mammary DNA was starkly different between breeds. The Holstein udders had anywhere from 2 to 4.7 times the amount of total mammary DNA than the Hereford's during the sampled time points. Many studies have sought to define the relationship between mammary cell number and milk production and have been summarized by Davis (2017). Overall, the described relationship between milk yield and mammary cell number vary considerably from study to study. Some have defined a relationship as high as $r = 0.69$, assuming the udder is healthy (Davis, 2017), to a complete lack of a relationship (Knight, 2000). Needless to say, mammary cell number does not explain milk yield entirely but would indeed influence the amount of milk produced.

Mammary cell activity

The other part of the milk production equation is mammary cell activity. A profound example of how mammary cell activity affects milk yield can be inferred from a study by Capuco et al. (1997). In this study, researchers studied the dry period and sought to understand why it is so integral for the next lactation's performance. Two treatment groups were used. The first was a group of 13 multiparous cows that were dried-off 60 days prior to expected calving and represented the "typical" dry period. The other treatment included 13 multiparous cows that were not dried-off and continuously milked during this time. During the 60-day period,

cows were selected and euthanized in groups of 3 or 4 per treatment group at 53, 35, 20, and 7 days before expected calving. At euthanasia, the udder was removed and used for analysis. When the entire udder was ground and analyzed to measure the amount of DNA as a means to gauge the number of cells in the glands, there was no difference between these 2 treatments. This indicates that the traditional dry period does not affect the number of cells that would be in the gland at the next lactation rather than if the animal was milked continuously. This observation is peculiar as it is well documented that cows that have a dry period produce considerably more milk than cows that do not (Swanson, 1965; Schlamberger et al., 2010) or experience a dry period that is inadequate in length (Sanders, 1928). When Capuco et al. (1997) examined these mammary tissues further to understand changes within the gland, it was observed that there was more cell death and proliferation in the non-lactating cow mammary glands, indicating removal and replacement of cells. The researchers concluded that the dry period facilitates "turnover" and replacement of damaged and senescent secretory mammary epithelial cells. This turnover is expected to allow these cells to be more active during the next lactation and are thought to be the reason why having a dry period before the ensuing lactation results in greater milk production rather than continuous milking.

Mastitis

With a clearer understanding of what dictates milk production, let us come back to mastitis. *What is mastitis?* Mastitis is simply inflammation of the mammary gland: masto- from the Greek meaning breast and *-itis* from the Latin meaning inflammation. Inflammation in the bovine mammary gland can develop for many reasons, but the predominant reason is an intramammary infection (**IMI**). Most IMI are a result of bacteria entering the mammary gland via passage through the teat streak canal, proliferating, and establishing an infection. The inflammation that is present during an IMI originates solely from the bovine and is her response to the IMI. Almost counter intuitively, this inflammatory response is beneficial from a biological standpoint because it serves as a means to eliminate the pathogen while also removing any damaged cells and tissues in the mammary gland. This is important as successful removal of all these elements would not only clear the infection but also prevent tissue necrosis that would exacerbate the inflammatory cascade and cause further tissue damage.

Reflecting on this biological discussion of what is inflammation is of limited help because it is not all that definitive or measurable. This is why the dairy industry, in large, measures mammary inflammation by quantifying the number of cells in milk. These cells are more commonly referred to as somatic cells. The concentration of these cells in a milliliter of milk is referred to as the somatic cell count (**SCC**). Although simple, this measure provides great utility. An increase in the SCC is indicative of an increase in the number of immune cells in the mammary gland. This is because immune cells are recruited to the mammary gland to address an invading pathogen during an IMI. Logically, an increase in the number of immune cells in the gland would indicate that there is an active immune response occurring and inflammation is present. Indeed, quantifying the number of somatic cells in milk has occurred for over a century (Campbell, 1909; Prescott and Breed, 1910). A center point of this effort has been to understand the relationship between the SCC and the presence of bacteria in milk, indicating an IMI (Campbell, 1909; Cherrington et al., 1933). Many SCC thresholds have been presented and discussed over the years on what SCC value should be used as a cutoff to indicate an IMI.

This conversation becomes easily complexed when the nuances and intricacies of this concept are recognized. For instance, should the SCC cutoff be determined for a composite sample of all 4 quarters that has been collected throughout the entire milking, or should it instead be a foremilk sample collected from a single quarter? Each one would likely require its own cut-off. Additionally, mastitis pathogens differentially affect the SCC and using a single threshold may not apply for all pathogens; this could result in misclassification of a gland's infection status. With this brief acknowledgment of this complex system, there will not be a detailed discussion here but instead the reader is referred to Schepers et al. (1997), Jashari et al. (2016), and Petzer et al. (2017) where a more detailed discussion can be found. Instead, let us simply appreciate that SCC is used as a gauge for mammary inflammation, and a higher SCC would be grossly indicative of greater inflammation. Indeed, it is well described that there is a negative relationship between increasing SCC and milk production (Table 1).

Types of immune cells

The neutrophil, macrophage, and lymphocyte are the 3 core immune cells that comprise the SCC. Importantly, these cells have different functions when it comes to responding and clearing an IMI. The neutrophil is the primary immune cell that is initially recruited to an IMI and is part of the innate immune system. These cells are the "first responders" and seek to identify and neutralize pathogens while, at the same time, recruit other immune cells to the site of infection or inflammation. This is achieved by neutrophils producing chemical messages that "attract" and "communicate" with other immune cells. Neutrophils seek to neutralize/kill bacteria by either phagocytosis, producing and releasing cytotoxic granules into the immediate environment and/or forming extracellular nets to "tangle" and trap bacteria (Amulic et al., 2012). An example of a bovine neutrophil that has phagocytosed several staphylococci is depicted in Figure 1 (Panel A). Killing internalized bacteria is of paramount importance so that bacteria do not freely proliferate inside the cell. When the neutrophil "grabs" the bacteria, it releases some reactive oxygen species to begin killing the bacteria (Paape et al., 2002). Examples of a few reactive oxygen species would include hydrogen peroxide, superoxide, and hydroxyl radicals. The bacteria that are bound to the neutrophil's cell membrane are subsequently internalized by the pseudopodia of the neutrophil and are continuously subject to reactive oxygen species (Paape et al., 2002). Surrounding mammary tissues may be damaged by the reactive oxygen species during the process of binding and internalizing bacteria. The result of this can lead to further increases in the inflammatory status of that tissue by having neighboring mammalian cells produce and release more chemical to attract more immune cells. Neutrophils are short lived as the typical half-life of a neutrophil in blood is 8.9 hours and only remain in mammary tissues for 1 to 2 days after migrating from the blood (Paape et al., 2002). Because of the neutrophils' short life, continuous recruitment into the gland is necessary to maintain a sustained immune response.

Macrophages are also part of the innate immune system, but they have different functions than the neutrophil. An example macrophage is depicted in Figure 1 (panel B), and it can be easily appreciated that these cells are rather large. Macrophages are primarily regarded as tissue resident immune cells that serve as sentinels to detect pathogens while also assisting in "directing" any initiated immune response. Macrophages are not short lived like neutrophils but can persist in tissues for months (van Furth, 1968). Similar to neutrophils, they can phagocytose bacteria and also produce

chemical messages to attract other immune cells to infected/inflamed tissues. Importantly with macrophages' phagocytosis of bacteria, macrophages can present bacterial contents and parts to other immune cells to stimulate an adaptive immune response. This allows an adaptive immune response to be generated for the specific infectious agent.

Grossly stated, B and T cells comprise the lymphocytes and are part of the adaptive immune system; an example lymphocyte from a mammary gland is presented in Figure 1 (panel B). These cells are specifically recruited for precise tasks after careful generation and selection. For the B cell, a primary purpose is to produce antibodies that assist with the immune response. These antibodies are made of amino acids and are designed to either opsonize bacteria, which labels the bacteria for phagocytosis, or they may be used to thoroughly coat the invading pathogens so the pathogen cannot bind to mammary tissues. Together, these mechanisms contribute to removing the pathogen from the udder. T cells, on the other hand, do not synthesize and secrete antibodies; instead, they perform several other functions. T cells can help direct the immune response by regulating the production of chemical messages that would influence how many immune cells might be recruited to the site of inflammation. The T cells can also help stimulate and activate B cells. This is achieved by the B and T cell interacting and directing how the B cell should develop. Additionally, T cells can identify bacterial infected cells and direct them to undergo controlled cellular death to contain the infection's spread.

Metabolic Demands of the Immune System

While a large number of various immune cells and their respective functions have been reviewed, it is most important to recognize that all the cellular processes associated with these functions and mechanisms consume energy, some to a great magnitude. For instance, neutrophils and macrophages that phagocytose bacteria require energy and substrates for not only "chasing down" and ingesting the bacteria but also producing the reactive oxygen species necessary for killing the bacteria. For the neutrophil, glucose is a significant metabolite that is used for energy during these processes. As discussed by Paape et al. (2002), glycogen granules are present in the cytoplasm of the neutrophil and comprise 20% of the cell's dry matter components. This is significant given glycogen is merely repeat glucose molecules. Glycogen can be broken-down via glycogenolysis and the resulting individual glucose monomers can be used for the generation of ATP via glycolysis. Indeed, the neutrophil is largely categorized as a glycolytic cell (Kramer et al., 2014) and is recognized to uptake glucose from the surrounding environment, as well as use the intracellular glycogen stores during phagocytosis (Borregaard and Herlin, 1982). This is important as it is largely recognized that glucose is not overly abundant in the lactating ruminant and a large proportion of this glucose is used in the synthase of lactose. Lactose is regarded as the chief osmoregulator of milk and considerably influences milk yield. Indeed, milk yield is dramatically reduced when lactose synthesis is impeded (Stacey et al., 1995). It is, therefore, logical to expect that if glucose were instead utilized by the immune system rather than lactose synthesis, milk production would be reduced.

Systemic immune response

Kvidera et al. (2017) recently investigated the effects of the activation of the immune system on glucose utilization at the whole animal level. Researchers utilized 18 lactating dairy cows and divided them amongst

3 treatment groups. The 3 treatment groups were a control group receiving no treatment, a lipopolysaccharide (**LPS**) treatment group that received a single intravenous bolus of LPS, and the third was another LPS treatment group that received continuous glucose administration to maintain blood glucose concentration. As to be expected, LPS administration elicited an increase in the concentration of various acute phase proteins in the blood, signifying that an immune response was generated. Blood glucose levels spiked immediately after the LPS bolus infusion, and then sharply decreased to their lowest point at approximately 3 hours after LPS administration. The LPS cows receiving glucose infusion had their blood glucose concentrations "rescued" to pre-infusion baseline levels by 4 hours post LPS challenge; these blood glucose levels were similar to the control cows for the remainder of the 12-hour study. In contrast, LPS cows that did not receive glucose remained hypoglycemic after the initial spike and were consistently lower that the other treatments. Kvidera et al. (2017) concluded that the LPS induced immune system activation consumed a considerable amount of glucose because of the immune system's activation. Overall, the researchers estimated that during their 12-hour experiment, the immune system consumed greater than a kilogram of glucose. The authors explicitly emphasize the fact that this calculation is significantly underestimated.

Localized immune response

Let us turn back to mastitis and appreciate that during a mastitis event, there is an activated immune response at the local level of the mammary gland. This activated immune response includes the previously discussed neutrophils, macrophages, lymphocytes, and all their associated cellular functions. An example of mammary tissues from an uninfected and *Staphylococcus aureus* infected bovine mammary gland are presented in Figure 2 (panels A and B). The stark increase in the number of immune cells that can be present in inflamed tissues is striking as immune cells can be observed in both the luminal space and tissues of the mammary gland. The result of this localization is that the active immune cells are placed in the same locale as mammary cells seeking to uptake glucose for lactose and milk synthesis. With the increased understanding of glucose utilization of the immune system as demonstrated by Kvidera et al. (2017), I would expect a similar phenomenon to occur, but at the localized level of the mammary gland. Because the neutrophil is recognized to be central to the initial immune response during mastitis and comprises the largest percentage of the SCC, I would expect these cells to utilize a significant amount of glucose in these mammary tissues. This would reduce the amount of glucose available for lactose synthesis.

Briefly mentioned earlier, amino acids play a significant role as a substrate required for the synthesis of antibodies in B cells, but no studies were identified that quantify the metabolic and amino acid requirements for bovine antibody synthesis. As such, no definitive statement can be made on how antibody synthesis at the local level of the mammary gland might affect milk protein synthesis. It is, however, well appreciated that a considerable presence of plasma cells (a type of B cell that produces antibodies) is found in bovine mammary gland tissues (Enger et al., 2018) and that certain types of plasma cells become more prevalent during an IMI (Nickerson and Heald, 1982). It could be speculated that if the demand of these activated plasma cells is significant enough to consume a large amount of amino acids, some of which being essential, milk protein synthesis would likely be reduced. The fact that the concentration of the key whey proteins, α-lactalbumin and β-lactalbumin, and total casein proteins are

reduced during subclinical mastitis may support this notion (Ishikawa et al., 1982; Pyorala, 2003).

Lastly, pictured in Figure 3 are mammary tissues that were collected from uninfected and *Staphylococcus aureus* infected mammary tissues. These tissues were examined in a previous study that sought to understand how mastitis affects the proliferation of the cells in the mammary gland (Enger et al., 2019). A key focus was to examine the epithelial cells that would be responsible for milk synthesis and determine if mastitis would affect the number of these cells that were proliferating. Interestingly, a greater number of cells in the stroma compartment of *Staphylococcus aureus* infected tissues were observed to be positive for proliferation when compared to tissues from uninfected mammary glands. The existence of these proliferating cells is associated with the fact that these tissues contained greater infiltration of immune cells. The majority of these proliferating cells were putatively classified as immune cells, more of the lymphocyte and macrophage nature given their nuclear shape. The significance of this observation suggest that immune cells are going to this location and then receiving signals to grow and divide. Cellular proliferation would require substrates from the surrounding environment. Admittedly, it cannot be determined here if these cells are proliferating in the mammary gland itself or traveling to other immune related tissues. Yet, the fact that these cells are positive for the proliferation marker indicates that these cells would indeed be growing and initiating specific cellular processes, which require energy, to divide.

Conclusion

It is well established that mastitis negatively affects milk production. The energetic/substrate demands of a mastitis event have been discussed and it can be appreciated that there are undeniably energy requirements for an activated immune system. In the instance of mastitis, the activation of the immune cells is focused at the local level of the mammary gland, which places these cells in direct competition with the mammary cells that would be synthesizing and secreting milk components. This competition is likely to redirect the same nutrients that would be used for milk secretion and synthesis to the activated immune cells in the gland. As such, it is important to recognize that nutrients being fed to the cow to support milk production may be instead being utilized by the immune system to address a preventable disease. This emphasizes the importance of preventing mastitis and limiting its prevalence and incidence as the consequences of mastitis are likely to negate any intended improvements in milk yield that are nutritionally driven.

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Table 1. Milk yield losses associated with milk SCC and SCC linear score.[1]

SCC (cells/mL)	SCC Linear Score	Predicted milk yield (lb/day)	Cumulative milk yield loss (lb/day)
12,500	0	64.2	0
25,000	1	62.9	1.3
50,000	2	61.6	2.6
100,000	3	60.3	3.9
200,000	4	59.2	5
400,000	5	57.6	6.6
800,000	6	55.9	8.3
1,600,000	7	54.1	10.1
3,200,000	8	51.9	12.3
6,400,000	9	49.5	14.7

[1]Table adapted from Akers and Nickerson (2011) who adapted and utilized data from Jones et al. (1984).

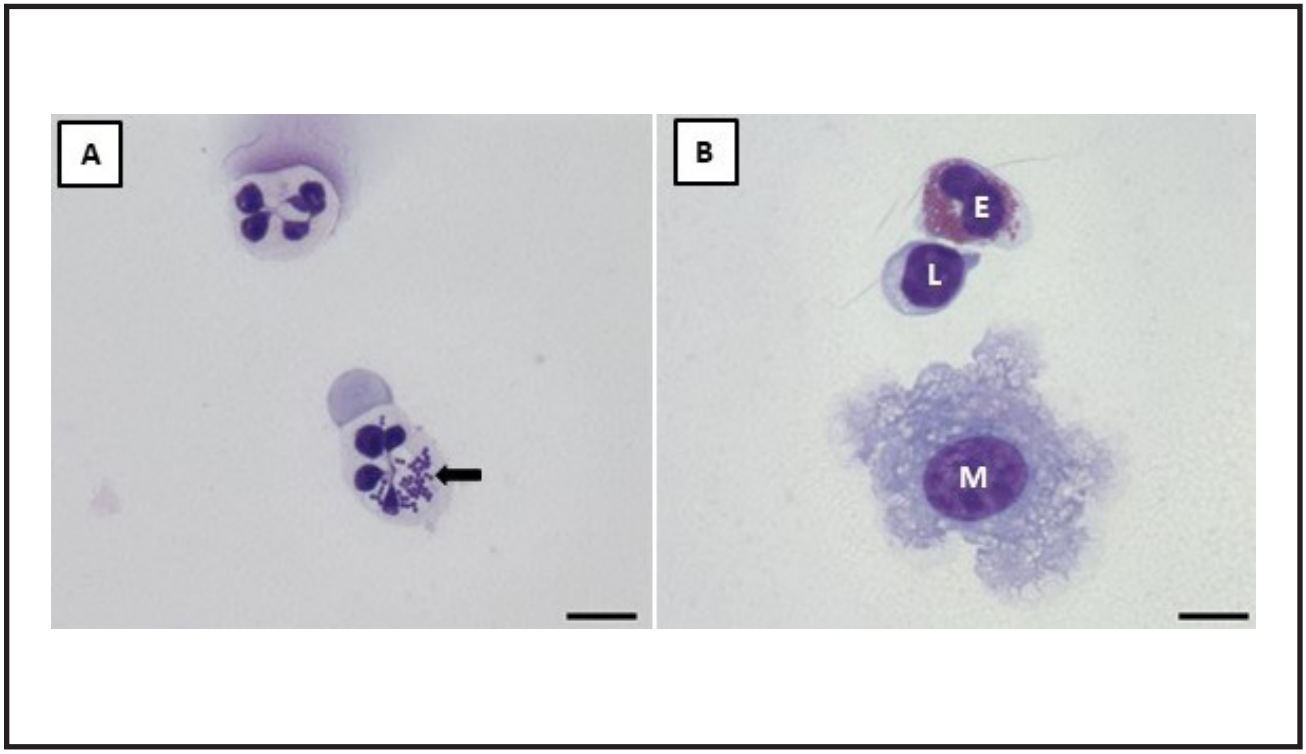

Figure 1. Somatic cells collected from bovine mammary glands stained with Wright–Giemsa stain are presented. Neutrophils (n = 2) are shown in panel A with the lower neutrophil containing intracellular *Staphylococcus aureus* (arrow). Panel B depicts a macrophage (M) a lymphocyte (L) and an eosinophil (E). Images are from Enger et al. (2018). Scale bars = 10 µm.

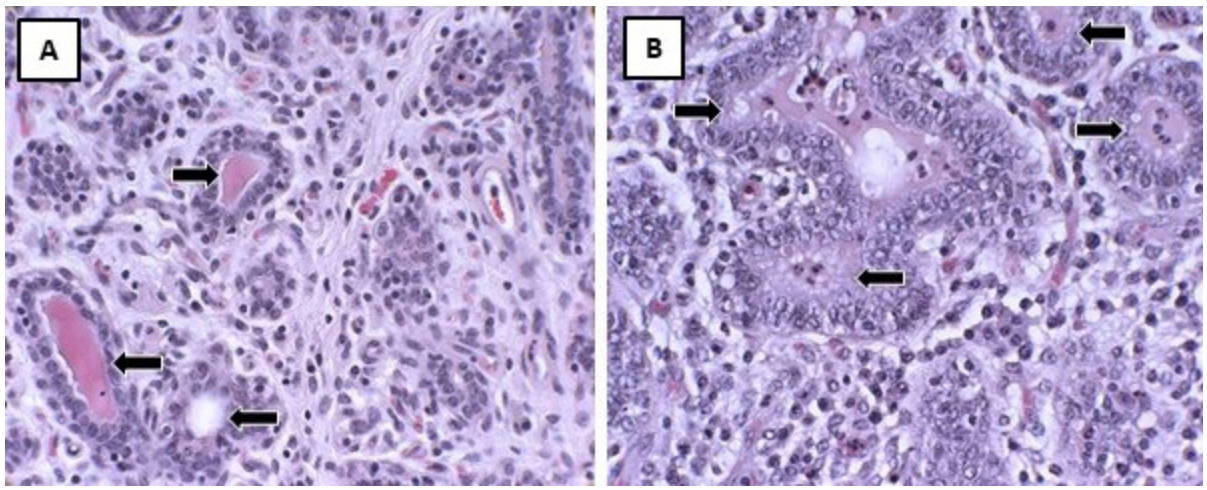

Figure 2. Bovine mammary tissues collected from an uninfected (panel A) and a *Staphylococcus aureus* infected mammary gland (panel B) are presented. No immune cells are present in the luminal space (arrows) of the uninfected mammary tissues but immune cells are abundant in the lumens of *Staphylococcus aureus* infected glands. A considerable increase in the number of immune cells in the stromal compartment of the *Staphylococcus aureus* gland compared to the uninfected gland is evident. Unpublished images from Enger et al. (2018).

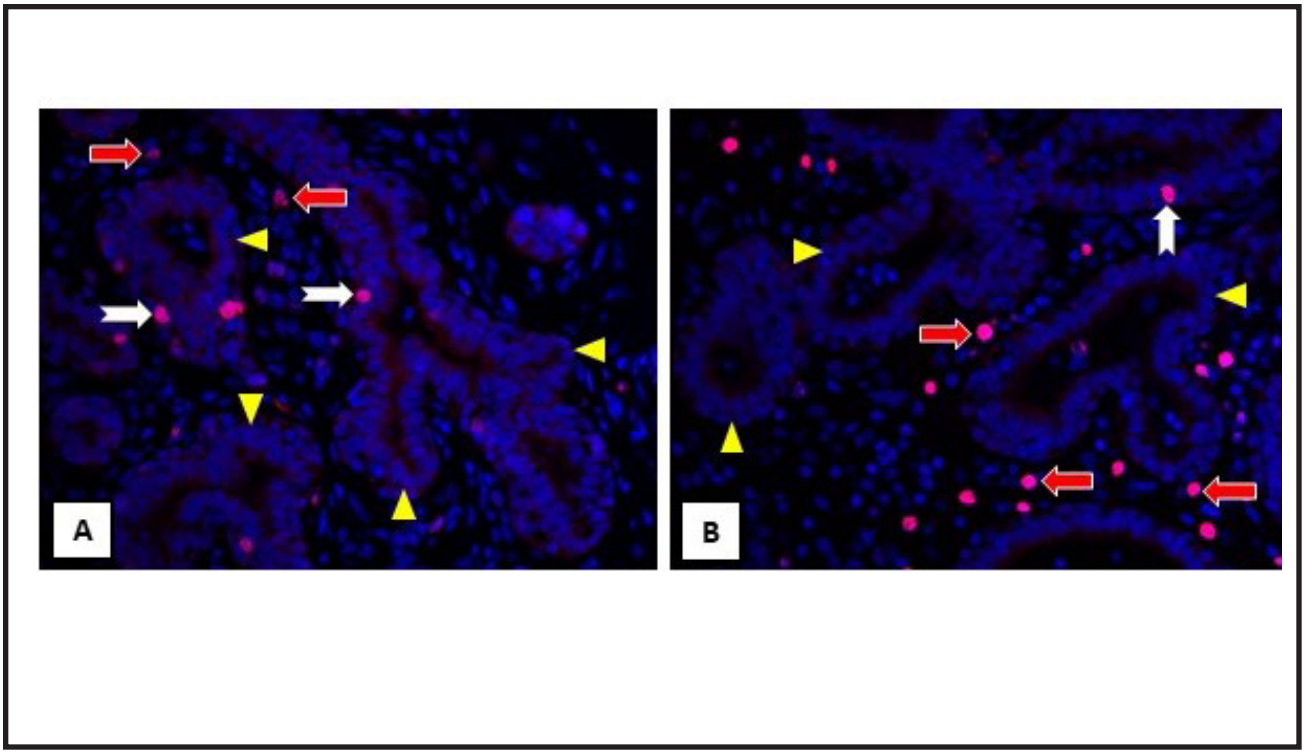

Figure 3. Florescent labeling of proliferating cells (Red) was conducted in uninfected (panel A) and a *Staphylococcus aureus* infected (panel B) mammary tissues. Blue objects are nuclei. Epithelial structures are identified with yellow triangles and proliferating cells in the epithelium are identified by notched white arrows. More proliferating cells were observed in the stromal compartment (red arrows) of *Staphylococcus aureus* infected mammary tissues than non-infected and these cells were putatively identified as being immune cells. Note that immune cells are abundant in the lumen of the *Staphylococcus aureus* mammary tissues, indicating a marked degree of immune cell infiltration of these tissues. Unpublished images from Enger et al. (2019)

Breeding Dairy Cattle for Improved Feed Efficiency: An Overview

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Abstract

The economic importance of selecting for improved feed efficiency has been clearly recognized by cattle producers. It has the potential to reduce costs considerably, minimize environmental impacts (e.g. reduce nutrient loss in manure and methane intensity) and improve the cattle industry profitability. Feed efficiency is a complex trait that describes units of product output per unit of feed input, with the units generally being mass, energy, protein, or economic value. The objective of this paper is to present, using layman's terms, a summarized overview of genetic selection for improved feed efficiency and international initiatives to implement genomic selection for feed efficiency, with a focus on dairy cattle. Various studies have indicated that feed efficiency, assessed based on alternative indicators, is heritable and genomic selection can be successfully implemented. However, selection based on genomic information still requires genotyping of selection candidates, as well as continued collection of phenotypic and genotypic records from genetically-representative individual animals (i.e. training population). Initiatives around the world have worked collaboratively to develop research and gather datasets for successful implementation of joint genomic evaluations.

Introduction

The global human population is expected to reach 9.8 billion people by 2050 (FAOSTAT, 2019), and consequently, a substantial increase in food demand is expected. In addition, the projected reduction in poverty and expansion of the middle class will reflect in a greater demand for larger amounts of high-quality meat and dairy products, produced under exemplary welfare conditions and leaving reduced environmental footprints. Therefore, there is an urgent need to develop strategies to optimize the efficiency of food production. The current worldwide cattle population has more than 1.5 billion animals, with over 105 million being raised in Canada and the United States (FAOSTAT, 2019). Feeding is currently one of the largest expenses in cattle production (Ho et al., 2013; Connor, 2015), and therefore, even a small improvement in nutrient utilization (i.e. better digestibility and/or greater nutrient absorption) can have major economic and environmental impacts worldwide. Among them, reductions in feeding costs will positively impact not only the farmer's profitability, but also the final prices of meat and dairy products available to consumers.

In order to optimize animal nutrition practices, there have been significant investments in research over the past decades. Consequently, the science of animal nutrition has evolved rapidly and resulted in major contributions

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to a better understanding of the nutritional physiology of cattle and its nutrient requirements, which has brought as outcome advances in diet formulation, supplementation, and techniques for food processing and storage (Eastridge, 2010; Coffey et al., 2016; Ondarza and Tricarico, 2017; Tedeschi et al., 2017). Despite the clear effectiveness of all these developments, the need for a more permanent and cumulative solution has been envisioned through genetic selection for a long time in various livestock species, including cattle (e.g., Stone et al., 1960; Koch et al., 1963; Freeman, 1967; Herd et al., 2003).

The economic importance of selecting for improved feed efficiency has been clearly recognized by cattle producers. Selecting animals for feed efficiency has the potential to reduce costs considerably, minimize environmental impacts (e.g. reduce nutrient loss in manure and methane intensity) and improve the cattle industry profitability (Richardson and Herd, 2004; Basarab et al., 2013). However, the inclusion of feed efficiency in selection indexes used in commercial breeding programs has been delayed for various reasons, among them: 1) the limited amount of phenotypic records for feed efficiency (and related variables) in commercial herds; 2) the differences in measurement protocols and data sources (e.g. different breeds, lactation stages, parity, diet, etc.); and 3) unclear definition of the breeding goal (based on indicator traits) (Berry and Crowley, 2013; Pryce et al., 2014; Connor, 2015; Hurley et al., 2016).

With the more recent advancements in genomic methods and technologies, selection for feed efficiency in cattle has become more feasible, as genomics can be used as a tool to transfer the knowledge generated in research farms to genetically-connected commercial populations (Connor, 2015). However, selection based on genomic information still requires genotyping of selection candidates, as well as continued collection of phenotypic and genotypic records from genetically-representative individual animals (i.e., training population). The objective of this paper is to present, using layman's terms, a summarized overview of genetic selection for improved feed efficiency and international initiatives to implement genomic selection for feed efficiency, with a focus on dairy cattle.

Definitions of Feed Efficiency and Indicator Traits

Dairy cattle breeding programs have been successful on improving traits of interest to the industry (as reviewed by Miglior et al., 2017). The first step to promote genetic progress in the right direction in any breeding program is to clearly define the breeding goals. In the case of feed efficiency, it has been broadly defined as animals that eat less with no compromise in performance or that produce more consuming the same amount of feed. In other words, feed efficiency describes units of product output per unit of feed input, with the units generally being mass, energy, protein, or economic value (Vandehaar et al., 2016). It is also of interest of cattle breeders to select animals that do not compromise other vital functions (e.g., reproduction, health, etc.) in order to achieve a greater feed efficiency.

Feed efficiency is a very complex trait, as feed intake and nutrient utilization are associated with various biological and physiological mechanisms that can be altered by the environment (e.g., diet composition, nutritional management practices) and other genetic effects (e.g., breed). For instance, variability in feed efficiency can arise due to variations in feed intake levels, digestion of feed (and the associated energy costs) and absorption of nutrients, metabolism (anabolism and catabolism associated with body composition), physiological stage, health

status, rumen microbial metabolism, activity, and thermoregulation (Herd et al., 2004; Herd and Arthur, 2007; Patience et al., 2015; Li et al., 2016).

Over time, a large number of indicator traits have been proposed and utilized to assess feed efficiency (Koch et al., 1963; Berry and Crowley, 2013; Pryce et al., 2014; Connor, 2015; Hurley et al., 2016; Ondarza and Tricarico, 2017). In 1963, Koch et al. suggested the use of "residual feed intake (**RFI**)" as an indicator of feed efficiency. In brief, RFI measures, through a regression model, the difference (residual) between the observed feed intake and expected feed intake (based on feeding requirements assessed according to metabolic body weight (**BW**) and level or quantity of product outcome). Additional energy sinks, such as energy required for certain activities and reproduction, can also be included in the calculations (Berry and Crowley, 2013; Pryce et al., 2014). RFI has been widely used in beef cattle (Berry and Crowley, 2013); however, more recently, it has also started to be studied in dairy cattle (Waghorn et al., 2012). In the case of dairy cattle, RFI is calculated by regressing dry matter intake (**DMI**) on various energy sinks of the animal, including parameters representative of milk yield and composition, metabolic BW, changes in BW and/or body condition score, and lactation stage (Connor, 2015; Byskov et al., 2017).

As described in Pryce et al. (2015), the practicality and costs of collecting individual feed intake on a large number of animals motivated the implementation of selection for improved feed efficiency based on indirect traits, such as production levels, BW (or predicted BW) and/or conformation traits. Some national breeding programs (e.g., Australia, New Zealand and USA) have incorporated this indirect measure of feed efficiency into their selection indexes (VanRaden et al., 2007; Veerkamp et al., 2013; Pryce et al., 2014; Pryce et al., 2015). One of the limitations with this approach is that the true variation in feed efficiency remains uncaptured (Pryce et al., 2015). Gibson (1986) presented a correlation between RFI and predicted feed efficiency (derived from BW and production) of 0.84, indicating the relevance to actually measure feed intake (as discussed in Pryce et al., 2014).

In the 1990's, there was a great interest from the industry to include feed efficiency as part of the dairy breeding objectives, which motivated various organizations to collect individual feed intake records for research and genetic evaluations, as described in various studies, such as Van Arendonk et al. (1991) and Veerkamp et al. (1994). The majority of these pioneer studies investigating feed efficiency in dairy cattle focused on individual feed intake recorded in lactating cows. In this context, Veerkamp et al. (2014) suggested selecting for reduced DMI predicted using actual DMI records in addition to selected yield and type traits.

Another group of indicators of feed efficiency are based on nutrient usage, such as energy and nitrogen efficiency, unfolding nutrient partitioning between milk production and other nutrient uses (Ondarza and Tricarico, 2017). Energy conversion efficiency is calculated as milk energy output divided by metabolizable energy intake. Similar to feed efficiency based on DMI, it does not account for mobilization of body reserves. To account for body reserve changes, "residual energy intake" has also been proposed as actual metabolizable energy intake minus the predicted energy requirement of the cow based on production, BW, changes in BW and/or body condition score, and gestational energy needs (Mantysaari et al., 2012).

As the costs to measure feed intake in individual cows are still high, alternatives have been investigated. A very promising option is to use predictor traits that can be measured in a large number of animals from an easily acquired sample, such as milk, blood, sensors, and automated recording systems. Some examples include: infrared thermography (Montanholi et al., 2010), plasma concentrations of IGF-1 (Moore et al., 2005), milk mid-infrared (**MIR**) spectrometry (O'Donovan et al., 2014; Wallen et al., 2018), and fatty acid composition (Kelly et al., 2010). Currently, the majority of Dairy Herd Improvement (**DHI/DHIA**) milk laboratories routinely quantify major milk components, such as fat or protein, using MIR spectrometry due to its efficiency and low cost compared to traditional chemical analysis. Thus, the MIR spectrometry may yield very useful source of information for genetic selection to improve feed efficiency.

Genetic Architecture of Feed Efficiency

Before including a trait in a genetic selection index, it is important to evaluate its heritability (h^2) in the population of interest, as well as its genetic correlation with other economically important traits. These genetic parameters give insights into the rate of genetic progress that can be achieved per generation and contribute to better designing the genetic evaluation systems. Studies in the literature have indicated that feed efficiency, assessed in different ways using indicator traits, is moderately heritable (Table 1). For example, Williams et al. (2011) reported that significant variation in RFI exists in dairy heifers and this could be an alternative to indirectly selecting dairy cows for improved feed efficiency, as it is easier to record feed intake in heifers (similar systems compared to beef cattle). Spurlock et al. (2012) estimated genetic parameters and made recommendations regarding traits related to energy balance, including DMI, BW, body condition score, energy-corrected milk production, and gross feed efficiency.

The h^2 estimates presented in Table 1 indicate that feed efficiency, measured using the different indicator traits, has a moderate genetic component, and therefore, can be improved through genetic selection. The wide range of h^2 estimates reported in the literature are likely related to the different populations used in each study, as genetic parameters (such as h^2 estimates) are population-specific. Thus, this suggests the importance of (re-)estimating specific genetic parameters for each population.

It is important to note that selection for improved feed efficiency might also impact other economically important traits, due to genetic correlations. Genetic correlations published for different indicator traits of feed efficiency and some production traits are summarized in Table 2.

Genomic Selection

As previously outlined, the costs and practicality of measuring individual feed intake (and related traits, such as BW) in a large number of animals with pedigree information has limited the implementation of genetic selection for feed efficiency. More recently, genomic selection has become widely available in the dairy cattle industry and has enabled selection of breeding candidates based on their predicted genetic merit for feed efficiency. This is because animals from research herds can be used as a training population to estimate the marker effects, which are then used to predict the breeding values for selection candidates based on their own genotype (Veerkamp et al., 2015). In brief, genomic selection refers to the use of genome-wide genetic markers to predict breeding values of selection candidates (Meuwissen et al., 2001).

In 2014, Gonzalez-Recio et al. described the implementation of heifer feed efficiency in the Australian selection index, using genomic selection and its impact in the industry. In 2015, the same research group (Pryce et al., 2015) defined and described the implementation of genetic evaluation for feed saved, as a new indicator of feed efficiency in dairy cows. "Feed saved" combines RFI with mature BW estimated using estimated breeding values (**EBV**) for predicting maintenance costs, so that feed requirements are quantified in a single breeding value. Since April 2015, feed saved has been included as part of the Australian national selection index.

The success of the use of genomics to select for improved feed efficiency can be measured based on the accuracy of genomic predictions, which depends on various factors, including trait heritability, size of the training population (number of individuals with both genotypes and phenotypic records), linkage disequilibrium, SNP chip panel used for genotyping, and effective population size. Among those factors, the number of animals used in the training population is still the main limiting factor to implement genomic selection for feed efficiency in the dairy industry (Berry and Crowley, 2013). Some alternatives have been investigated to increase the training population for feed efficiency, including the use of data from nutrition studies (Veerkamp et al., 2014; Tempelman et al., 2015) and combining data from different countries (de Haas et al., 2012; Pryce et al., 2012; Banos and Coffey, 2012; Berry et al., 2014; Tempelman et al., 2015) or breeds (Khansefid et al., 2014). It is worth noting that in the last few years, a collaboration group named "The global Dry Matter Initiative (**gDMI**)" has been created to combine feed intake records, which includes 10 research herds from 9 countries (de Haas et al., 2015). Other contributions to international genetic evaluations for feed intake in dairy cattle are presented in Berry et al. (2014). There are also other initiatives to combine data from all over the world for genomic predictions for feed efficiency, such as the Efficient Dairy Genome Project (http://genomedairy.ualberta.ca/), which will be mentioned in more details later.

In general, genomic predictions for feed efficiency have been performed based mainly on DMI and RFI, which is probably related to the greater availability of phenotypic records for these indicator traits. Some accuracies of genomic predictions for DMI and RFI that have been reported in the literature are summarized in Table 3. These results indicate that there is still room for improving the prediction of genomic breeding values. The refining of the statistical models used, as well as an increase in the training populations, will likely contribute to improve the observed accuracies.

Data Collection and International Efforts for Data Gathering

To genetically select animals for improved feed efficiency, at least pedigree information and individual phenotypic records associated with feed intake and production traits are required. The simplest way to record DMI is based on the amount of feed offered and refused by each cow per day, with the associated DM percentage. Other important variables to be recorded are milk production and composition, lactation stage, water intake, diet composition, BW and body condition score over the course of lactation, health/disease events, and reproductive performance traits. It is important to notice that even if not all these variables are used in the genetic/genomic evaluations, they might be useful in the future for research and also selection purposes. Furthermore, the costs to record these additional traits are low compared to the cost of individual feed intake recording (Veerkamp et al., 2015).

There are various automated systems available for feed intake recording, including Calan Broadbent (American Calan Inc. Northwood, NH), Gallagher Animal Management Systems (Hamilton, New Zealand), GrowSafe 4000 System (GrowSafe Systems, Ltd., Airdrie, AB, Canada), and the RIC system (i.e. Insentec; Hokofarm Group B.V., Marknesse, The Netherlands). These systems are mostly based on radio-frequency identification to track and record individual feed intake, as well as feeding behavior (e.g. number of visits per day and intake duration). As discussed by Connor (2015), the use of these systems in dairy cattle has been limited to research herds or growing heifers. The use of automated feed monitoring systems in larger groups of lactating cows is greatly hindered by the limited feeding capacity of the automated feed bunks, meaning that significantly fewer cows can be fed from a single bunk relative to growing cattle to accommodate substantially greater intakes of lactating cows (Connor, 2015).

It is well-established that the success and long-term sustainability of any livestock breeding program is largely dependent on the amount and quality of pedigree, phenotypic and genotypic data available for genetic and genomic evaluations. As feed efficiency is difficult and expensive to measure, a global effort to enlarge the training population for genomic evaluations is crucial and has the potential to greatly benefit all groups involved in the project. In addition to the gDMI Project mentioned before, the Efficient Dairy Genome Project (EDGP, www.genomedairy.ualberta.ca) is a large international research project led by Canadian institutions aiming to develop strategic research, tools, and the whole infrastructure to implement genetic and genomic evaluations for improved feed efficiency and reduced methane emissions in dairy cattle.

The EDGP database was developed in 2017 to allow data sharing among the international collaborators. Currently, the database contains records on feed intake of 5,289 cows and methane emissions on 1,337 cows from 8 research herds in 6 countries (Australia, Canada, Denmark, Switzerland, United Kingdom and United States). An international genetic evaluation seems possible due to the high level of relatedness of the Holstein population, the most common dairy breed with records for feed efficiency. Moreover, all collaborators are members of the International Committee for Animal Recording (ICAR, www.icar.org/), providing standardized information on production records.

Conclusions

Feed efficiency, assessed based on different indicators, is a heritable trait and can be improved through genetic and genomic selection. There is still a need to refine the breeding goal and identify indicator traits that can be easily and cheaply measured. Various groups around the world are collaboratively working to refine the methods used in the evaluations, as well as enlarging the datasets used for genomic evaluations.

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Table 1. Heritability (h^2) estimates for different indicator traits of feed efficiency in dairy cattle.

Trait	Paper	h^2 ± SE
Dry matter intake	Vallimont et al. (2010)	0.18 ± 0.06
	Williams et al. (2011)	0.17 ± 0.10
	Liinamo et al. (2012)	0.23 ± 0.12
	Tetens et al. (2014)	0.37 ± 0.04
	Shonka et al. (2015)	0.52 ± 0.13
	Bilal et al. (2016)	0.12 ± 0.01
	Byskov et al. (2017)	0.37 ± 0.06
	Lu et al. (2018)	0.23 ± 0.02
Energy intake	Köck et al. (2018)	0.07 ± 0.03 to 0.13 ± 0.02
Energy-corrected milk	Köck et al. (2018)	0.08 ± 0.03 to 0.12 ± 0.02
Residual Feed Intake	Hurley et al. (2017)	0.04 ± 0.08 to 0.11 ± 0.08
	Van Arendonk et al. (1991)	0.19 ± 0.12
	Krover et al. (1991)	0.22 ± 0.11
	Jensen et al. (1995)	0.36 ± 0.17
	Svendsen et al. (1993)	0.02 ± 0.08
	Vallimont et al. (2011)	0.01 ± 0.05
	Williams et al. (2011)	0.27 ± 0.12
	Byskov et al. (2017)	0.23 ± 0.05
	Lu et al. (2018)	0.16 ± 0.02

h^2 ± SE: heritability ± standard error.

Table 2. Genetic correlations (r_g) between different indicator traits of feed efficiency and production traits in dairy cattle.

Feed efficiency trait	Production trait	Paper	$r_g \pm SE$
Dry matter intake	Milk yield	Gonzalez-Recio et al. (2014)	0.10 ± 0.11
		Vallimont et al. (2010)	0.51 ± 0.32
	Fat yield	Gonzalez-Recio et al. (2014)	-0.03 ± 0.10
		Vallimont et al. (2010)	0.53 ± 0.34
	Protein yield	Gonzalez-Recio et al. (2014)	-0.11 ± 0.08
		Vallimont et al. (2010)	0.55 ± 0.37
	Somatic cell score	Vallimont et al. (2010)	-0.15 ± 0.28
	Body weight	Liinamo et al. (2012)	0.54 to 1.00
		Vallimont et al. (2010)	0.52 ± 0.35
	Body condition score	Gonzalez-Recio et al. (2014)	0.37 ± 0.32
		Liinamo et al. (2012)	0.11 to 0.45
		Vallimont et al. (2010)	0.37 ± 0.46
Residual Feed Intake	Milk yield	Veerkamp et al. (1994)	-0.11 to 0. 07
		Gonzalez-Recio et al. (2014)	0.07 ± 0.08
	Fat yield	Gonzalez-Recio et al. (2014)	0.02 ± 0.07
	Protein yield	Gonzalez-Recio et al. (2014)	0.03 ± 0.07
		Veerkamp et al. (1994)	-0.11 to -0.02
	Lactose	Veerkamp et al. (1994)	-0.19 to -0.05
	Body weight	Korver et al. (1991)	0.03
		Van Arendonk et al. (1991)	0.01
	Body condition score	Gonzalez-Recio et al. (2014)	0.71±0.32
		Veerkamp et al. (1994)	0.33 to 0.36

Table 3. Accuracies of genomic predictions for indicator traits of feed efficiency.

Trait	Paper	Average accuracy
Dry matter intake	de Haas et al. (2012)	0.35
	de Haas et al. (2015)	0.37
	Mujibi et al. (2011)	0.20
	Boloorma et al. (2013)	0.32
Residual feed intake	Pryce et al. (2012)	0.40
	Mujibi et al. (2011)	0.43
	Boloorma et al. (2013)	0.43

Interactions Between Energy and Protein (Amino Acids) in Lactating Dairy Cows

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Summary

Energy is required for cows to efficiently convert amino acids (**AA**) into milk protein, and crude protein (**CP**) is needed by cows to efficiently convert gross energy into net energy for lactation (**NEL**). In early lactation when cows are in negative energy balance, milk protein yield is likely to increase when supply of the proper AA is increased independent of energy; however, in later lactation, response to AA (or CP) supplementation is dependent on energy supply. In later lactation, if energy allowable milk is approximately equal to actual milk, increasing supply of AA or CP will not greatly affect milk protein yield; however, if energy allowable milk exceeds actual milk, milk protein yield should respond to improved protein nutrition. Because of both economic and environmental reasons, lower concentrations of dietary CP in lactation diets are often encouraged, and in many situations, they have been implemented successfully. However, reducing dietary CP concentrations can reduce NEL intake via reduced digestibility and DM intake. Reducing CP via reducing rumen degradable protein (**RDP**) appears to have the greatest negative effect on digestibility, even when the resulting RDP concentration still appears adequate (e.g., ~10% of diet DM). Therefore, RDP should be maintained in lower protein diets. The negative effects of reducing dietary protein also likely depend on what nutrient or nutrients replace the CP that is being removed from the diet. If CP is replaced with forage NDF, intake often will decrease. In higher starch diets, replacing CP with starch may reduce digestibility, and in lower starch diets, replacing CP with byproduct NDF likely will reduce digestibility.

Introduction

An "interaction" between two nutrients can be defined as a non-additive response when the supply of the two nutrients is altered. For example, if you add 1 additional unit of protein to a diet, you increase milk protein yield by 1 unit and if you add 1 unit of energy you increase milk protein yield by 1 unit; however, if you add 1 unit of both nutrients, rather than getting the additive response (2 units of milk protein), you get 3 units of protein; that is an interaction. Interactions can be positive (greater response than the sum of the expected responses) or negative (lesser response than the sum of the expected response). Interactions between energy and protein would be expected because all synthetic reactions, such as milk protein production, require energy (e.g., ATP) and because enzymes are involved in essentially all biochemical reactions, AA are needed to extract energy from the diet.

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Effect of Energy on Response to Dietary Protein

Early lactation

Because of the way many (all?) mammals evolved, diet is not the only source of energy to support lactation. Dairy cows are designed to mobilize body energy reserves in early lactation to support high milk production prior to the cow's ability to consume adequate dietary energy. Because of energy mobilization and repletion, the relationships or interactions between dietary energy and protein will be different in early lactation (defined as the first ~ 4 weeks of lactation) than in mid or late lactation. A cow requires about 3.9 Mcal of metabolizable energy (**ME**) to make 1 lb of milk protein. About 2.5 Mcal of that energy is in 1 lb of milk protein and 1.4 Mcal of that energy is needed to run the reactions and is lost as heat. Conversely, if a cow mobilizes 1 lb of body protein, about 3.7 lb of fat are typically mobilized, releasing a total of 18.6 Mcal of metabolizable energy (**ME**). Because of the cow's ability to mobilize substantially more energy than protein, protein, not energy, is usually first limiting in early lactation.

In theory, if the supply of needed AA is increased in early lactation, milk protein yield should increase and loss of body energy reserves should increase, resulting in a decrease in body condition score (**BCS**). On the other hand, increasing dietary energy but keeping AA supply fixed should not increase milk protein yield greatly but reduce BCS loss. This is exactly what a group of researchers from Norway found (Schei et al., 2005). They fed a control diet that contained adequate energy and protein to meet the requirements of cows during the first 4 wk of lactation. They also fed a diet that had both energy and protein reduced by about 25% compared to the control (Low/Low) and a third diet that reduced energy 25% but kept protein supply equal to control. Milk protein yield was reduced by cows fed Low/Low, but it was not reduced when only energy was reduced (Figure 1). In addition, the low energy/adequate protein diet greatly increased plasma NEFA and ketones, indicating that body fat mobilization increased. This relationship was illustrated even more dramatically in an experiment in which casein was infused into the abomasum of early lactation cows (Galindo et al., 2015; Larsen et al., 2015). In those experiments, infusing casein AA increased milk protein yield by about 0.6 and 0.7 lb/day measured at 5 and 29 days in milk. At the same times, cows infused with casein compared to control cows had calculated NEL balance about 8 Mcal more negative at 5 days in milk and about equal negative energy balance at 29 days (Figure 2). This shows that in very early lactation, if the supply of proper AA is increased, cows will mobilize body energy so that those AA can be incorporated into milk. As lactation progresses, DMI increases so that the effect of increased supply of AA on milk protein yield is maintained but mobilization of body energy is reduced. In other words, with reasonable diets and typical cows in very early lactation, yield of milk protein is mostly dependent on dietary AA supply and is almost independent of dietary energy supply.

Later lactation

The relationship between dietary energy and protein is different for cows past early lactation. In theory, at peak and later lactation, one would expect essentially no response in milk protein yield to increasing the supply of the correct AA if energy was limiting and you would expect little response to additional energy if AA were limiting. As supplies of both were increased, milk protein yield should increase with the response following the law of diminishing returns. Experimental data follow the expected pattern almost exactly. Researchers from France

(Brun-Lafleur et al., 2010) fed diets that varied in MP and NEL from deficient to excess based on the French ration formulation system. When protein was deficient, milk protein yield had almost no response to increasing energy from very deficient to excess (Figure 3). When energy was deficient, increasing MP from deficient to adequate yielded a small increase in milk protein (~0.1 lb/day) with no additional response as energy increased above requirement. When both energy and MP increased from deficient to excess, milk protein yield increased by almost 0.4 lb/day. The response to increasing MP was linear within each level of energy. However within each level of MP, response to increasing energy followed a diminishing return function; milk protein yield increased linearly as energy increased until the energy requirement was met, then no additional increasing milk protein yield was observed as energy increased. What this means is at peak and later stages of lactation, maximum response to AA supplementation or increased dietary MP requires that energy must be fed at rates equal or greater than requirement. This also means that if we want to improve our ability to estimate responses to changes in AA supply, we need to be able to accurately estimate feed energy and energy requirements.

Effects of Protein on Feed Energy Supply

Most equations used to estimate diet NEL concentrations include concentrations of standard nutrients and digestibility and efficiency coefficients, and the equations generally follow the classic net energy scheme (gross energy to digestible energy to metabolizable energy to net energy). Equations in use today do not capture all the effects protein has on energy concentrations in diets which means that estimated energy values may be erroneous when dietary protein deviates much from typical values. This may become important if we start formulating extensively for AA and that results in lower protein diets.

Dietary protein concentration may affect diet energy values because:

- Protein has 1.3 times more gross energy (**GE**) per pound than carbohydrates
- Average protein is more digestible than fiber (**NDF**) but less digestible than starch
- Increased dietary protein is associated with increased fiber digestibility
- Increasing dietary protein is associated with increased DM digestibility
- Increasing dietary protein usually increases urinary energy loss
- Increasing dietary protein usually increases heat increment

When dietary protein is increased, carbohydrate (fiber and starch) concentration usually is reduced by the same amount. Because protein has about 1.3 times as much energy per pound as carbohydrate, increasing dietary protein concentration usually increases the concentration of GE. On average (this will vary depending on the source of protein in the diet), true digestibility of protein by dairy cows is about 83%, whereas starch and NDF have average digestibilities of about 92 and 48% in lactating dairy cows. Based on differences in GE and digestibility, increasing the concentration of protein in a diet by 2 percentage units and reducing the concentration of NDF or starch by 2 units would change the digestible energy (DE) concentration of the diet by approximately 0.05 or 0.02 Mcal/lb, respectively. Those changes are equal to a 3.5 and 1.4% increase over the DE concentration of an average dairy cow diet.

Effects on digestibility

Protein concentration can affect digestibility of NDF and DM. Adequate RDP is needed to maximize ruminal bacterial growth which is essential for good fiber digestibility. Digestibility of NDF and DM is often reduced

when RDP is deficient. For example, when RDP balance (based on the NRC, 2001) system was about 200 g/day deficient, NDF digestibility was reduced 20% compared to a diet that had 27 g/day extra RDP (Lee et al., 2011), and in another experiment (Lee et al., 2012), NDF digestibility was reduced 9% when the diet was 42 g/day deficient in RDP (Table 1). The low RDP diets had 14 to 14.9% CP and 9 or 9.6% RDP. In both experiments, diets deficient in RDP also had significantly lower DM digestibility (7 and 2% lower). Digestibility of DM is similar to energy digestibility; therefore, it is safe to assume the low RDP diets in those studies reduced DE by more than 7 and 2% when changes in GE are factored.

In those studies, RDP was deficient (based on NRC 2001), and one could argue that the response in improved digestibility is simply a result of correcting a deficiency. However, other studies have shown linear increases in NDF and DM digestibilities as CP and RDP increased at concentrations well above expected requirements (Broderick et al., 2008). In that study, diet CP increased from 14.8 to 18.6% and RDP (NRC, 2001) increased from 10.0 to 12.3%. Those increases were associated with a linear increase in NDF digestibility from about 52 to 59% and an increase in DM digestibility from about 68 to 71%. This could suggest that either the RDP requirement is underestimated or that RDP (or protein) has some stimulatory effect on bacteria or the cow even after requirements are met. An alternative possibility is that the effect was not caused by increasing protein concentrations but rather by decreasing starch concentrations. In Broderick et al. (2008), CP replaced starch so that starch concentration decreased from about 28 to 23% as protein increased (Broderick et al., 2008). Increasing dietary starch is associated with decreased NDF digestibility (Ferraretto et al., 2013). This illustrates an important concept in nutrition studies; when the concentration of one nutrient increases as least one other nutrient must decrease and we never know if the response was caused by the increase in the 'test nutrient' or the decrease in what it replaced. Regardless of the mechanism, increasing dietary CP and RDP often increases the DE concentration of diets. This needs to be considered when low protein diets are fed.

Efficiency of converting digestible to metabolizable energy

Dietary protein concentration and AA profile affects the efficiency of converting DE to ME. In an adult cow, the vast majority of AA that are not secreted in milk are eventually oxidized to provide energy. In 2 and 3 year old cows, some AA are retained in the body as growth but that amount is small (<100 g/day) relative to intake of protein. When AA are oxidized, most of the released N is excreted in urine which increases urinary energy loss. On average, each gram of urinary N is associated with about 14.3 kcal of energy. Using the OARDC digestibility database (~500 observations), for a cow that averaged 50 lb of DMI and 76 lb of milk, and fed a diet that averaged 16.5% CP, urinary N excretion averaged 182 g/day or 30% of N intake. The estimated urinary energy for that dataset is 2.6 Mcal/day or about 4% of the average DE intake. On average, for every additional gram of N consumed by a cow (equal to 6.25 g of CP), urinary N will increase by about 0.3 to 0.7 g depending on how much milk protein yield increases (greater urinary N increase when milk protein response is less). For example, if two cows had similar DMI (50 lb/day) but were fed either a 15 or 17% CP diet, intake of CP would be 1 lb greater for the cow fed the high CP diet (Table 2). This is equal to 73 g of N. If milk protein yield was not different, then the cow fed higher CP would excrete about 51 g more N in urine (73 g of N intake x 0.7g urinary N/g intake N). The 51 g of increased

urinary N is equal to 0.73 Mcal of increased urinary energy. Overall, the effect on dietary ME from increasing CP is small within the range of dietary CP concentrations that are typically fed to dairy cows.

Efficiency of converting metabolizable to net energy

In addition to the energy contained in the N compounds excreted in urine, energy is required to synthesize those compounds and that energy is measured as heat production. Increased metabolic heat production decreases the efficiency of converting ME to NE. Heat production per unit of GE intake has a positive, but weak, correlation to urinary N excretion, indicating that increasing dietary CP is generally associated with increasing heat production and decreasing efficiency of converting ME to NEL. Using the example above, increasing dietary CP by 2 percentage units would increase urinary N excretion by 51 g and on average that would increase daily heat production by 0.8 Mcal/day (with a very large associated uncertainty). To put this in perspective, on average, a cow eating 50 lb of DMI and producing 75 lb of milk produces about 22 Mcal of non-maintenance heat (i.e., heat increment) per day.

Based on all these assumptions, when CP is increased and starch or NDF is reduced concomitantly, dietary NEL concentration would be reduced by 0.01 to 0.03 Mcal/lb, which is less than the accuracy of our energy estimation equations. When potential associative effects are considered (e.g., Table 1) effects of increasing dietary CP on diet NEL may actually be positive. The bottom line is that with reasonable diets, changing the concentration of CP probably has only a very minor effect on the concentration of NEL in the diet. This should not be interpreted to mean that changing dietary CP is energy neutral. Intake of NEL, not NEL concentration is what matters. On average, increasing dietary CP is associated with increasing DMI (Allen, 2000). The effect on DMI likely is related to what other nutrient changes when CP changes; however, this has not been teased out. If forage NDF is increased as dietary CP decreases, a greater negative effect on DMI would be expected than if byproduct NDF was increased. Increasing starch can have variable effects on DMI depending on stage of lactation and energy needs of the cow. Overall, formulating diets for AA rather than CP should reduce the CP concentrations of diets, but this has the distinct potential of reducing NEL intake which would result in reduced milk component yields and/or reduced body condition.

Conclusions

Interactions between dietary protein and energy and stage of lactation dictate whether cows will respond to dietary changes. In early lactation, increasing AA supply can increase milk protein yield independent of any change in energy intake, but in later lactation, milk protein yield will only respond to increased AA supply when adequate energy is available. On the other hand, energy intake is affected by dietary protein. Increasing dietary CP, especially RDP above requirement, can increase NEL intake via enhanced digestibility and DM intake. Maintaining adequate RDP concentrations and carefully considering what other nutrients (e.g., fiber or starch) will change when dietary CP concentrations change is essential to obtain good results from lower protein diets.

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Table 1. Effect of reducing dietary protein concentration on production and nutrient digestibility.

	Control	Low Protein
Experiment 1 (Lee et al., 2011)		
Diet CP, % of DM	16.7	14.8
Diet RDP, % of DM	10.6	9.8
RDP Balance (g/day)	141	-42
DMI, lb/day	54.3*	52.4
Milk, lb/day	86.5*	79.6
Milk protein, lb/day	2.46	2.49
DM digestibility, %	69.7*	68.4
NDF digestibility, %	54.0*	49.2
MUN, mg/dL	12.5*	8.3
Experiment 2 (Lee et al., 2012)		
Diet CP, % of DM	15.6	14.0
Diet RDP, % of DM	10.0	9.1
RDP Balance (g/day)	27	-203
DMI, lb/day	54.8	54.1
Milk, lb/day	86.2	83.8
Milk protein, lb/day	2.62*	2.46
DM digestibility, %	60.9*	56.6
NDF digestibility, %	42.8*	34.1
MUN, mg/dL	10.0*	8.4

*Treatment means within an experiment differ ($P < 0.05$)

Table 2. Example of how an increase in CP concentration could affect dietary digestible energy (DE) and metabolizable energy (ME) values when DM intake was 50 lb/day (no difference between diets)[1]

	15% CP	17% CP
CP intake, lb/day	7.50	8.50
CP GE intake, Mcal/day	19.3	21.8
CP-DE intake, Mcal/day	12.5	14.2
Change in CP-DE intake, Mcal/day	0	1.7
If starch was replaced as CP increased		
Change in starch intake, lb/day	0	1.0
Change in starch DE intake, Mcal/day	0	-1.8
Net change in DE, Mcal/day	0	-0.1
If NDF was replaced as CP increased		
Change in NDF intake, lb/day	0	1.0
Change in NDF DE intake, Mcal/day	0	-0.9
Net change in DE, Mcal/day	0	0.8
Change in urinary N, g/day	0	51
Change in urinary N energy, Mcal/day	0	0.73
Net change in ME intake		
When starch is replaced, Mcal/day	0	-0.83
When NDF is replaced, Mcal/day	0	+0.07

[1]Assumed apparent digestibility of CP, starch, and NDF as 65, 92, and 48% (OARDC digestibility database) and no negative or positive associative effects were applied. The energy content of CP was assumed to be 2.57 Mcal/lb and 1.91 Mcal/lb for starch and NDF, respectively.

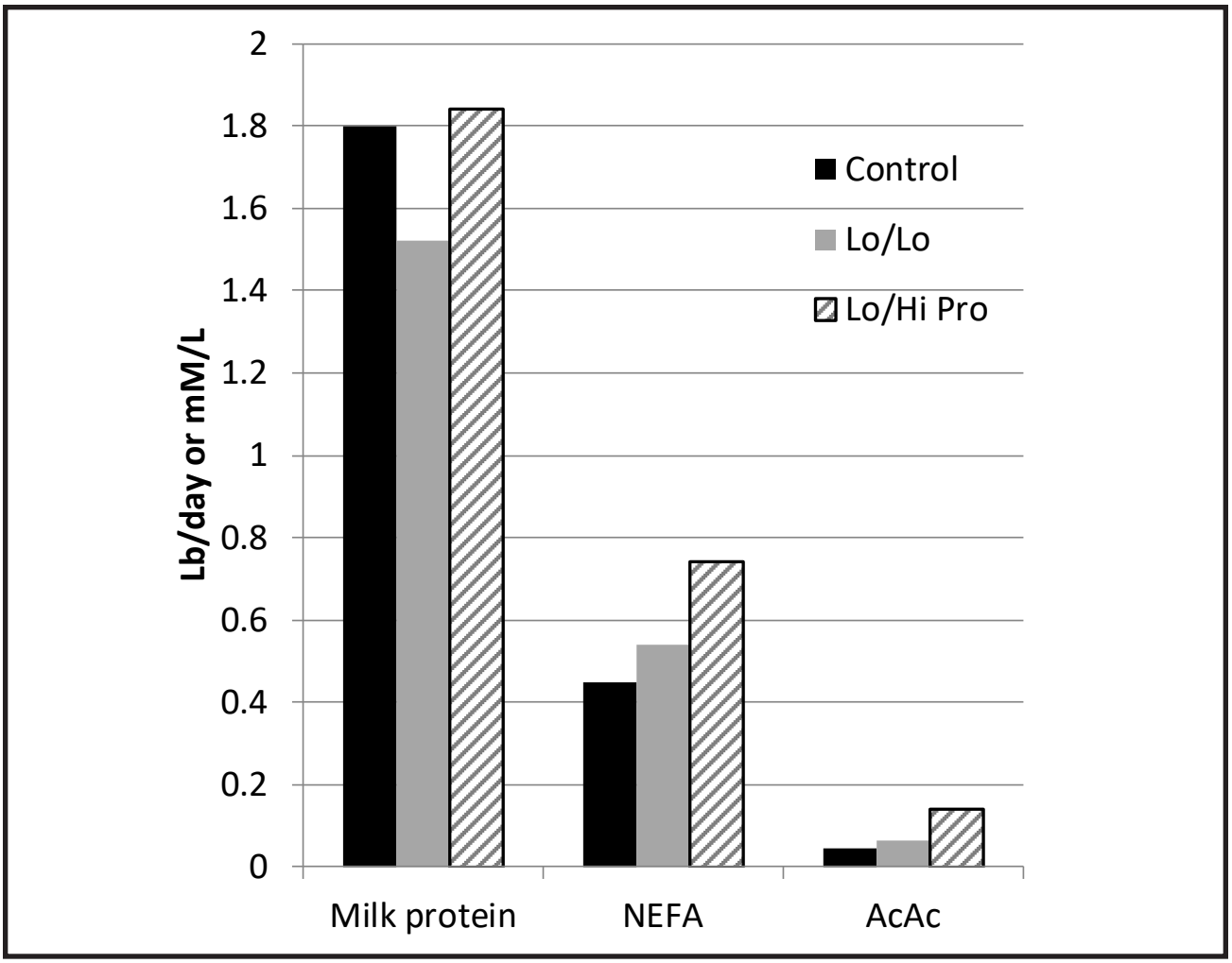

Figure 1. Effect of diets that were adequate in energy and protein (Control), deficient in both energy and protein (Lo/Lo), or deficient in energy but adequate in protein (Lo/Hi Pro) on milk protein yield (lb/day), plasma non-esterified fatty acids (NEFA) and plasma acetoacetate (AcAc). Diets were fed the

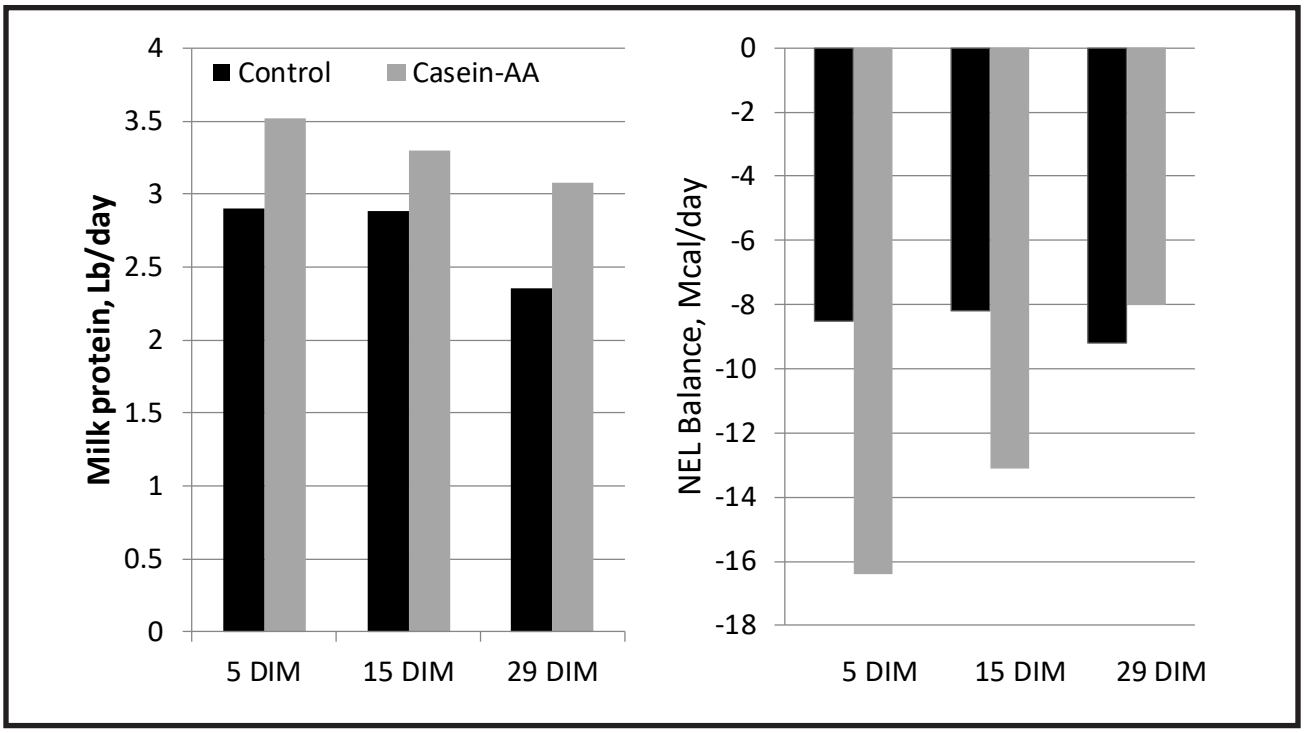

Figure 2. Effect of infusing casein amino acids (Casein-AA) into the abomasum of early lactation cows. The infusion greatly increased milk protein yield but also greatly increased mobilization of body energy (Larsen et al., 2015; Galindo et al., 2015).

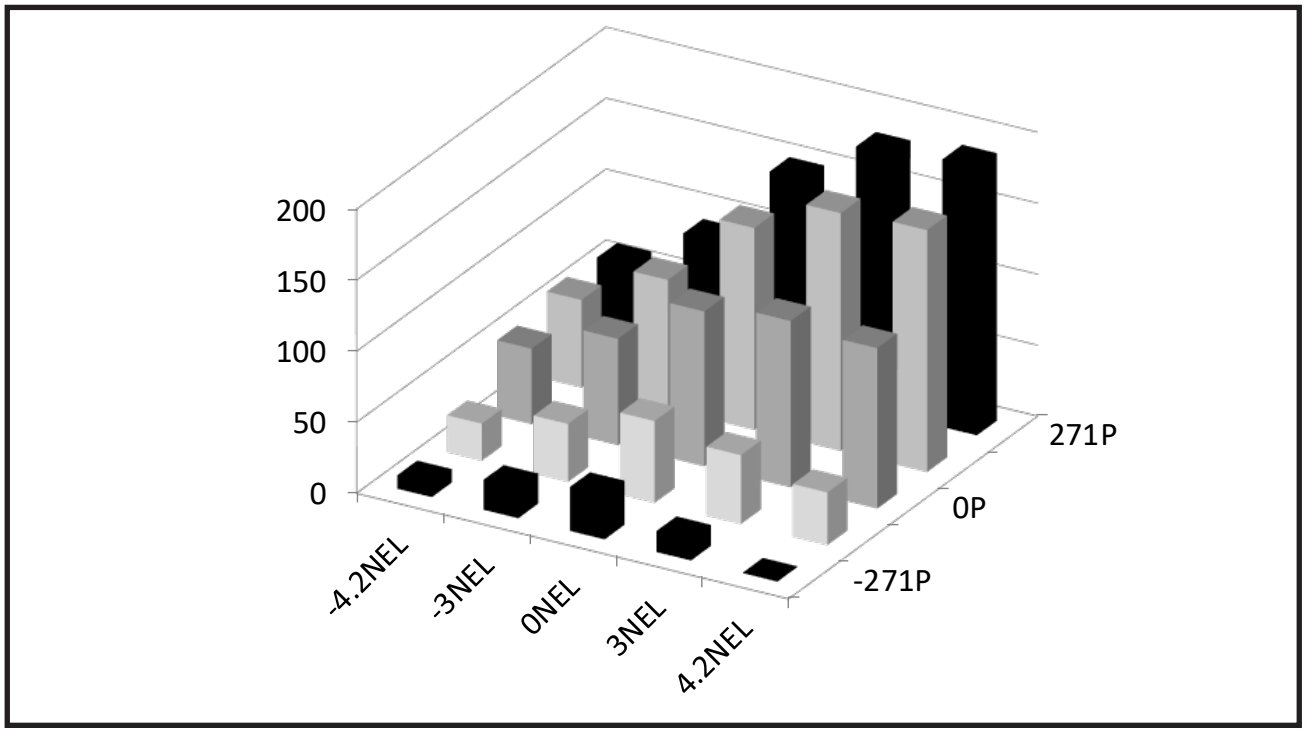

Figure 3. Response to changes in intake of NEL and metabolizable protein (MP) in midlactation dairy cows. Energy and protein were calculated using the French system (Brun-Lafleur et al., 2010). When protein was deficient, essentially no response was observed with increasing NEL, and when NEL was deficient, response to protein was muted.