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PROCEEDINGS

Diamond V Pre-Conference Paper

Diamond V Next Generation Technology: Helping Cows Reach Their Genetic Potential Every Day. T.J. Oelberg, Ph.D. Diamond V.

Allowing dairy cows to reach their genetic potential milk production every day requires management focus on transition cows, cow comfort, heat abatement, milking procedures, forage quality, feeding management, and heifer raising to name a few. This manuscript will discuss reducing total mix ration (TMR) variation, improving feed access, and enhancing rumen function and immune system with Diamond V® next generation technology.

Reducing TMR Variation

The first step in reducing TMR variation is pushing and lifting faced silages with skid-steer or payloaders into well-mixed piles. Figure 1 shows nearly a 2.5-fold reduction in standard deviation levels of crude protein in alfalfa haylage (5) before it included in a total mixed ration. This is a key step in reducing variation in the TMR because alfalfa haylage and corn silage make up 50-60% of the TMR on many dairies across the U.S. Also, many dairies blend bales of alfalfa hay by lifting and pushing bales with strings removed into piles before loading into a TMR. These practices are now common-place on many dairies across the U.S.

Figure 1. Lifting and pushing faced alfalfa haylage into a pile reduces variation in crude protein.

10 Samples from Face
CP Range 7.1CP % pts
CP SD 2.54

10 Samples from Blended Pile
CP Range 2.5 CP % pts
CP SD 1.08

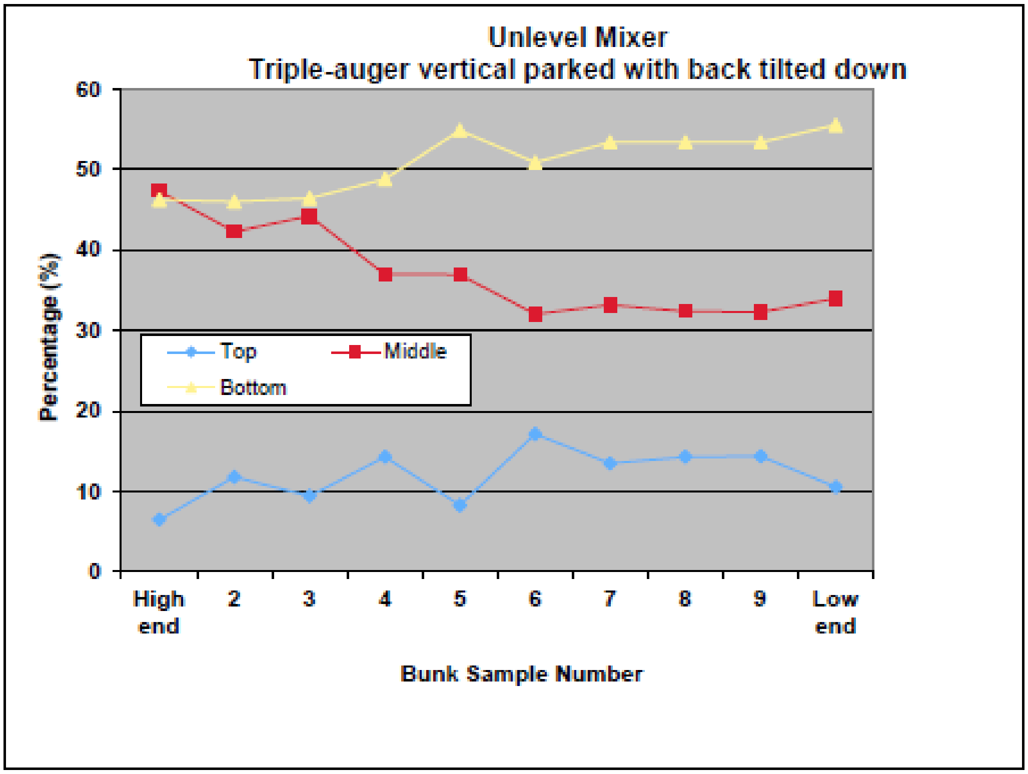
The second step in reducing TMR variation is following the mixing basics described in detail by Oelberg and Stone (4). An on-farm method of taking ten (10) equally-spaced samples of freshly delivered TMR along the feed bunk and subjecting each sample to the Penn State Particle Separation (PSPS) procedure allowed the discovery of the mixing basics shown below:

1. Worn mixer augers, kicker plates and/or knives
2. Unlevel mixer during TMR mixing
3. Mix time after the last added ingredient
4. Loading position on the mixer box
5. Load size
6. Hay quality and processing
7. Loading sequence
8. Liquid distribution
9. Vertical mixer auger speed
10. Vertical mixer auger timing

11. Forage restrictor settings on vertical mixers

The effect of mixing a TMR in an un-level mixer is shown in figure 2. There is 10-point difference in the amount of material in the bottom screen between the high end (start of unloading) and the low end (end of unloading). Because cattle are quite territorial, the cows on the opposite ends of the feed bunk will consume different TMRs and instead of the one formulated by the nutritionist. This will have an impact on rumen health and energy-corrected milk production. Each of the 11 factors listed above can impact TMR consistency and the cow's genetic potential milk production.

Figure 2. Unlevel mixer boxes force heavy dense ingredients to the **bottom** screen of the Penn State Particle Separator (PSPS) which flow to lower section of the mixer (5).

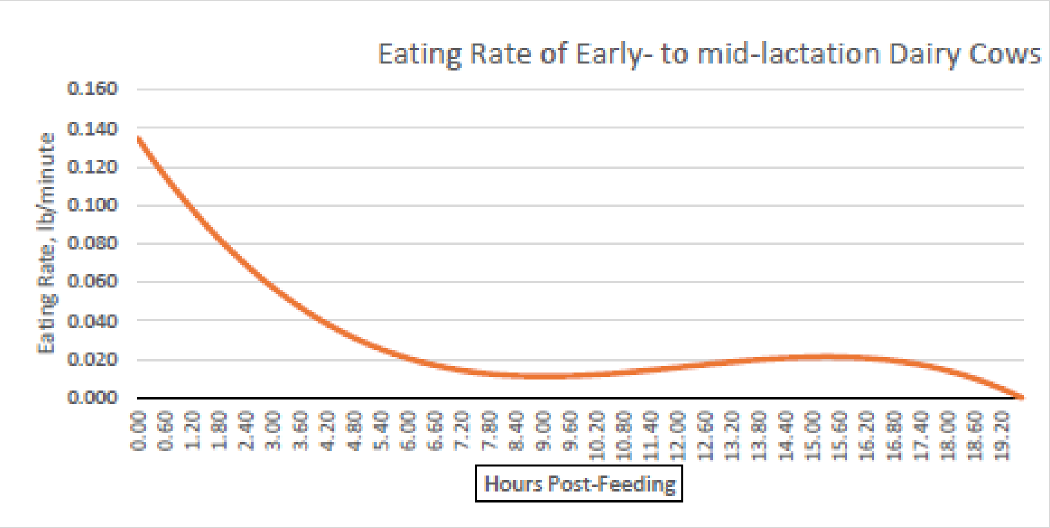


Making TMR More Accessible to Cows

The use of time-lapsed photography to observe feed bunk levels throughout the night and over several days has given great insight on the TMR accessibility to dairy cows (5). Delivering the first-feed drop of TMR at the same time every day and keeping the TMR pushed up so that cows can reach the TMR at all times are critical for maximum dry matter intake (DMI) and genetic potential milk production. First-feed drop TMR should be delivered within 20 minutes of the target time each day so that cows have 24-hour access to fresh feed every day. Refusals need to be pushed out and weighed for each pen so that DMI is accurate. This assumes that feed was continually pushed up throughout the 24-hour period so that all cows within the pen had access to feed and the net intake (feed offered minus feed refused) reflects their true appetite.

The biggest challenge for many dairy producers is to keep the TMR pushed up so that all cows always have access to feed, especially during the nighttime and early morning hours. Figure 3 shows the eating rates of dairy cows in one experiment conducted by Oelberg (3). Multiparous Holstein cows in early- to mid-lactation were fed once daily around 8:00 a.m. Meal patterns and feed intake were recorded every minute for 20 hours. The eating rates varied from 0.05 to 0.13 lb/minute during first few hours post-feeding and varied by diet and cow. However, eating rate reached a steady level of 0.02 lb/minute 6-to-20 hours post feeding, which is the time frame where many dairies fail to push up feed. This would be considered a conservative estimate of eating rate since dairy cows in 1985 were lower production cows than the current modern dairy cows.

Figure 3. Eating rate of early- to mid-lactation dairy cows (3)



Using the 0.02 lb/minute eating rate during the nighttime hours, the potential DMI lost can be calculated for the hours that cows are either out of feed or cannot reach the feed, as shown in Table 1. Knowing the number of cows in the pen and the percentage of the feed bunk where cows could not reach feed for so many hours, one can calculate potential milk production loss for the pen.

Table 1. Hours out of feed 6-to-24 hours post-feeding (once daily) and potential DMI lost.

Hours out of feed	Potential DMI lost, lb
1	1.2
2	2.4
4	4.8
8	9.6
16	19.2

Supporting Gut Health

Making the TMR consistent and available 24 hours per day will allow cows to reach their genetic potential milk production. Many nutritionists supplement the dairy TMR with feed additives that support rumen fermentation and/or support the cow's immune system. Shen et al. (9) showed a marked improvement in rumen pH of beef heifers fed barley-based grain and barley silage diets containing 54% starch and 29.7% neutral detergent fiber (NDF) when fed Diamond V next generation beef product NaturSafe® compared to control diets or to diets containing beef industry standard antibiotics. They also reported highly significant increases in rumen NDF digestibility levels of 52.9%, 39.3%, and 41.3% for NaturSafe, antibiotics, and control respectively. Total tract NDF digestibility levels followed the same patterns of increase (9).

Shi et al. (10) reported reduced acidosis index in transition dairy cows as they were switched from a pre-fresh diet to a 28% starch control lactation diet or to the control containing Diamond V next generation dairy product, NutriTek®. Acidosis index was calculated as the time in minutes that rumen pH was 5.8 or less divided by DMI in kilograms. The DMI of the NutriTek-treated cows was more consistent and stable during the transition period due to a more stable rumen pH.

Oba (2) reported on a study done at University of Alberta studying the effects of level of starch (22.0 vs 28.3%) on post fresh with and without NutriTek. Cows were fed a 13.9% starch diet with or without NutriTek four (4) weeks before calving. Cows were then fed either the low-starch or high-starch diet with or without NutriTek the first three (3) weeks post-calving. Finally, all cows were switched to a 28% starch diet with or without NutriTek six (6) weeks after calving. Milk production was increased for the high-starch diet and for the cows fed NutriTek. The most interesting result was a significant increase in blood glucose levels at day 42 post-calving with the NutriTek cows. The increase averaged 5.6 mg/dl across the low- and high-starch diets.

Reedy et al. (7) measured levels of acetate, propionate, and total volatile fatty acids (VFAs) in an in vitro rumen system evaluating a wide variety of forages from around the world varying in neutral detergent fiber levels from 32.3% to 70.2%, and starch levels ranging from 0.4% to 40.7%. On average, total rumen VFAs were increased 17% with NutriTek. Recently, Reedy et al. (8) reported changes in rumen VFAs for corn silage samples representing the beginning, middle, and end of the ramp of bunker silo after the silage had been stored for nine (9) months. The rumen VFA levels were significantly lower for silage at the beginning of the ramp compared to silage in the middle and end of the ramp. Supplementing the in vitro system with NutriTek significantly increased VFA levels of the corn silage representing all sections of the ramp. NutriTek can enhance rumen fermentation and help cattle transition more smoothly through forage changes.

Supporting the Immune System

Maintaining a strong immune system to handle many of the challenges a dairy cow encounters throughout is essential for her to reach her genetic potential milk production. Shi et al. (10) reported lower lipopolysaccharide (LPS) levels in the rumen fluid of beef heifers fed NaturSafe compared to animals fed either a control diet or a diet containing antibiotics commonly used in feedlot diets. LPS is a component of the outer wall of gram-negative bacteria and can create major health issues for cattle if the LPS enters the blood stream. Oba (2) reported significantly lower serum haptoglobin levels at day 7 post-calving in transition cows fed NutriTek compared to control cows. Haptoglobin is a biological marker for inflammation. Olagaray et al. (6) reported lower somatic cells in transition dairy cows fed NutriTek compared to controls during the first six (6) weeks after calving. Finally, Jiang et al. (1) reported improved milk production of dairy cattle fed aflatoxin contaminated diets containing NutriTek and a clay, compared to cattle fed a positive control diet without aflatoxin or an aflatoxin contaminated diet containing clay.

NutriTek has improved 3.5% fat-corrected milk in several university transition cow studies (2, 6, 10), and in over a dozen on-farm field studies. The on-farm field studies use randomized pen studies comparing NutriTek-treated cows to control cows starting approximately three (3) weeks post-calving to 12 weeks post-calving.

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Update on Trace Minerals and Vitamins for Dairy Cows

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Introduction

Providing adequate trace minerals and vitamins to dairy cows is essential for high production and good health. However feeding excess trace nutrients inflates feed costs and could be detrimental to production and cow health. Unfortunately quantifying the supply of available trace nutrients and their requirements is extremely difficult which leads to a high degree of uncertainty relative to diet supplementation. This paper provides suggested strategies for formulating diets to provide adequate but not excessive amounts of vitamins and trace minerals under a variety of conditions. When this paper was written (December, 2019), the NRC was in the process of updating the Nutrient requirements of Dairy Cows publication. The upcoming NRC may or may not reflect the opinions in this paper.

Mineral Supply

A major change that occurred in NRC (2001) was that requirements were calculated for absorbed mineral rather than total mineral. This was a major advance because we know mineral from some sources are more absorbable than minerals from other sources. However the use of absorbable mineral has limitations:

Measuring absorption of many minerals is extremely difficult

Actual absorption data are limited; therefore most AC are estimates

Absorption is affected by physiological state of the animal and by numero0us dietary factors (many of which have not been quantified).

For many of the trace minerals, the AC is extremely small and because it is in the denominator (i.e., Dietary mineral required = absorbed requirement/AC) a small numerical change in the AC can have a huge effect on dietary requirement.

Concentrations of Minerals in Basal Ingredients

For most minerals of nutritional interest good analytical methods that can be conducted on a commercial scale at reasonable costs are available. Assuming the feed sample is representative, a standard feed analysis (using wet chemistry methods for minerals) should provide accurate concentration data for Ca, P, Mg, K, Na, Cu, Fe, Mn, and Zn. Labs can also routinely measure sulfur and chloride but often these are separate tests. Most labs do not routinely measure Cr, Co and Se because the concentrations commonly found in feeds are lower than what commercial labs can reliably measure or because of contamination caused by routine sample processing such as using a steel feed grinder (a major concern for Cr). Although we can get accurate total mineral concentrations data for basal ingredients, you must be careful

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when evaluating and using the data. Concentrations of minerals in feeds, even most macrominerals, are low. For example 1 ton of average corn silage (35% dry matter) only contains about 2.5 grams of Cu (to put this in perspective a penny weighs about 2.5 g).

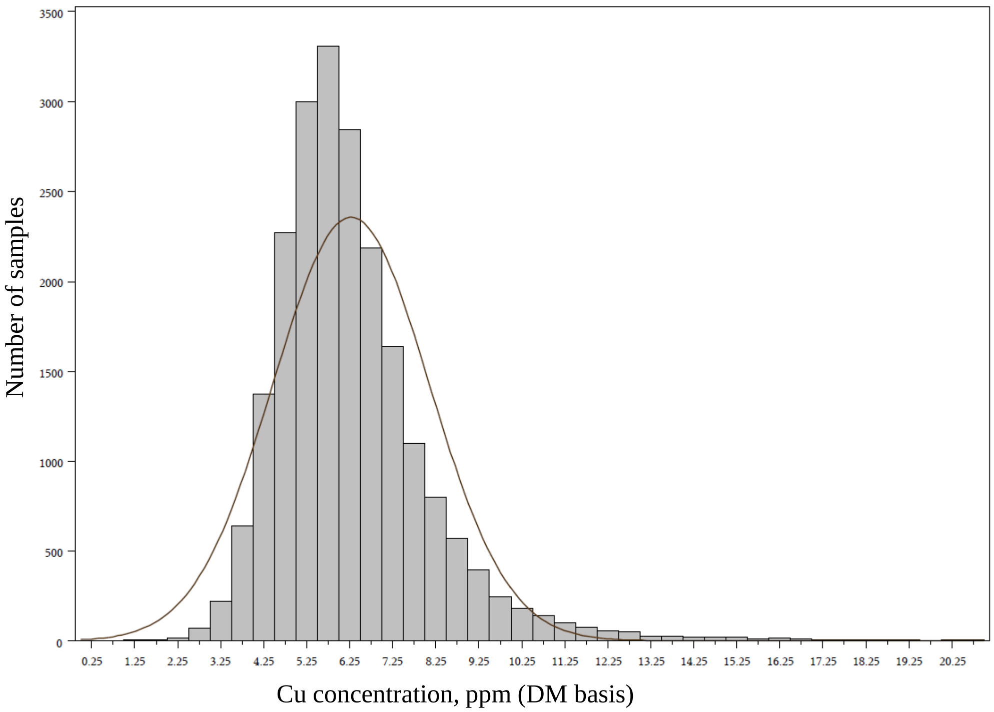

Figure 1. Distribution of Cu concentrations in corn silage grown throughout the U.S. The smooth line indicates a normal distribution would while the bars indicate the actual distribution. (Knapp et al., 2015).

Sampling error is a problem for most nutrients and when concentrations are low, sampling error is usually larger. From a survey we conducted, sampling variation for trace minerals was greater than true variation. This means that mineral concentration data from a single sample should be viewed very suspiciously. Mineral concentration of soils is a major factor affecting the concentrations of most minerals in forages. Therefore averages of samples taken from a farm over time (up to a few years) or from a group of farms within a small geographic area (e.g., a few counties) should be a truer estimate of the actual mineral concentration of a forage than a single sample.

In a normal distribution (the classic bell shaped curve) about half the samples have less than the mean or average concentration, about half the samples have more than the average, and about 95% of the samples are within ± 2 standard deviation (SD) unit of average. This means that if you know the average concentration and the SD you have a good description of the population. This information helps with risk assessment.

If a feed has an average concentration of Mg of 0.4% and an SD of 0.01% and the distribution is normal, about 95% of the samples of that feed should have between 0.38 and 0.42% Mg. With that information you should probably conclude it is not worth analyzing that feed for Mg, because even if your sample is 2 or 3 SD units from the mean it will have no effect on the diet or the animal. However when distributions are skewed, the average and the SD may not be good descriptors of the population. For many minerals, concentrations within feeds are not normally distributed (Figures 1 and 2). Often the distributions have long tails because concentrations cannot be less than 0 but can be extremely high for various reasons. Some samples have high concentrations of certain minerals because of soil contamination. The more skewed the data, the less valuable the average and SD become in describing the feed. The median is the concentration where half of the samples have a lower mineral concentration and half of the samples have more mineral, and in a normal distribution the mean and the median are essentially equal. For concentrations of trace minerals and some macro minerals, the median is usually less than the average because their distributions are skewed. What this means is that for most situations, using average trace mineral concentration (e.g., feed table data), overestimates the trace mineral concentration in the majority of samples. For skewed populations, the median is a better descriptor of the population than the mean; however simply replacing average concentration with median concentration does not fix all the problems associated with a skewed distribution.

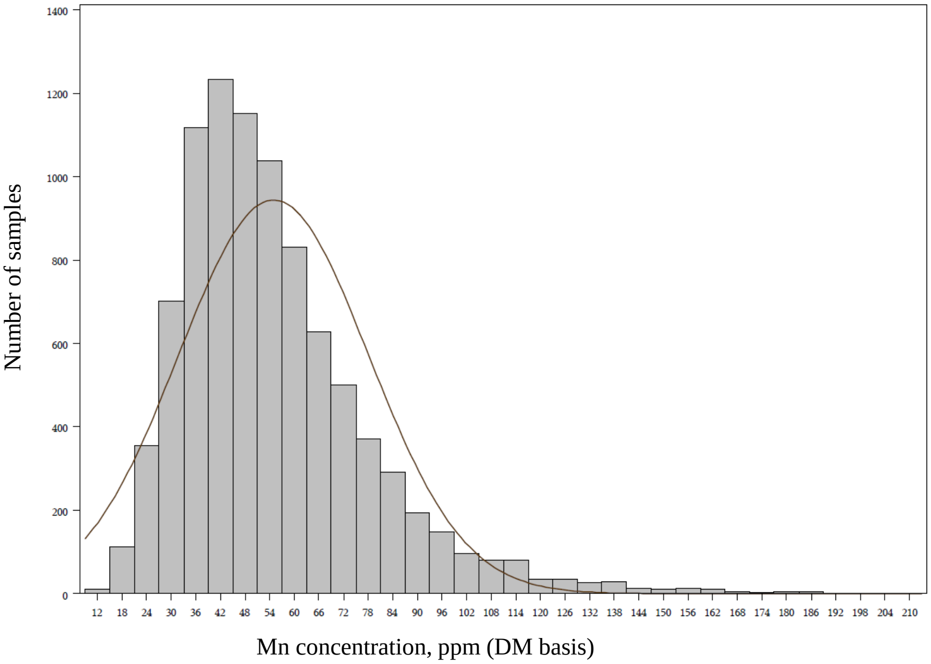

Figure 2. Distribution of Mn concentrations in mixed, mostly legume silage grown throughout the U.S. The smooth line indicates a normal distribution would while the bars indicate the actual distribution (Knapp et al., 2015).

As a distribution becomes more skewed, the risk that a specific feed will contain excess mineral increases. The Mn data shown in Figure 2 is a good example. That data has an average of 55 ppm and an SD of 23. Assuming a normal distribution, one would expect about 2.5% of the samples to have more than about 100 ppm (55 + 2 SD unit) and about 2.5% of the samples to have less than about 9 ppm. However, no samples had less than 9 ppm and 5.2 % had more than 100 ppm. If your particular sample of mixed mostly legume silage was in the 5 out of every 100 samples with a very high Mn concentration, your diet would contain substantially more Mn than expected. Excess dietary Mn is rarely a problem for cows but excess dietary Cu can be (discussed below). Corn silage in Figure 1 had a mean Cu concentration of 6 ppm with a SD of 1.8. With a normal distribution about 2.5% of the samples should have more than about 10 ppm Cu. However, about 5% of samples have more than 10 ppm Cu (i.e., twice the risk). If you formulate a diet assuming corn silage is 6 ppm Cu but it really has 12 ppm, and corn silage comprises a significant portion of the diet, over the long term (months) excess dietary Cu could become a problem. The bottom line is that averages for trace mineral concentrations in forages (and perhaps other feeds) found in tables should be used with caution. Because of substantial sampling variation, data from a single sample should not be used. The best advice is to generate median values for trace minerals for forages grown within a limited geographical area.

Do Trace Minerals in Feeds have Nutritional Value?

Essentially every feedstuff used in dairy diets contains some minerals. The question is, are those minerals biologically available to cows? Although survey data of nutritionists are lacking, based on personal experience it is not uncommon for nutritionists to set trace mineral concentrations in basal ingredients or at least forages, at 0. This approach would be valid if the trace minerals in feedstuffs were not biologically available to cows. Although substantial uncertainty exists regarding the absorption coefficients for most minerals in feeds, a portion of the trace minerals found in most (all?) feedstuffs is clearly available to cows. Tissues from wild ruminants such as deer (Wolfe et al., 2010) contain trace minerals indicating that absorption of basal minerals occur.

The NRC (2001) estimates that Cu, Mn, and Zn from basal ingredients are 4, 0.75 and 15% absorbable. The AC assigned to basal ingredients are usually lower than AC for the sulfate form of minerals even though most of the trace minerals contained within plant cells would be in an organic form. The lower AC for trace minerals in basal ingredients may reflect an adjustment for soil contamination. Some trace minerals in basal feeds, especially forages, are in soil that is attached to the feed and those minerals are often in the oxide form (low availability). Feeds with substantially greater ash and trace mineral concentration than typical likely have AC that are lower than the NRC values for trace minerals. Concentrations of trace minerals substantially greater than median value should be discounted but an exact discount cannot be calculated at this time, but those feeds would still contain some available mineral.

On average (and remember the issues with using averages), unsupplemented diets for lactating cows in the US based mostly on corn silage, alfalfa, corn grain and soybean meal contain 7 to 9 ppm Cu, 25 to 35 ppm Mn, and 30 to 40 ppm Zn (specific farms may differ greatly from these ranges). For an average Holstein cow (75 lbs of milk/day and 53 lbs of dry matter intake) using NRC requirements, basal ingredients supply about 80%, 235% (do not believe this), and 75% of requirements for Cu, Mn, and Zn. Ignoring minerals supplied by basal ingredients can result in substantial over formulation for trace minerals.

Form of supplemental trace mineral (newer information)

In the U.S. the primary form of most supplemental trace minerals is the sulfate salts but some chloride salts are also used. These forms of trace minerals are a good compromise between price and availability. Other commercially available forms of trace minerals include organic and hydroxyl metals (identified as specialty minerals). Several different commercial forms of organic minerals (mostly Cu, Cr, Co, Mn, and Zn) are available and in those products the metal is usually chelated or complexed to a specific amino acid, a blend of amino acids, a sugar or an organic acid. Hydroxy trace minerals are inorganic but their chemical structure differs from sulfates. The metals in sulfates and chloride salts are linked with ionic bonds that readily dissociate in the rumen and hydroxyl and some organic minerals are linked with covalent bonds which are less likely to be broken in the rumen. Potential differences between specialty minerals and sulfate minerals include increased absorption by the cow, different metabolism within cells, and effects on microbes within the rumen and hind gut (i.e., digestive tract microbiome).

Measuring absorption of trace minerals is extremely difficult and we have almost no data on actual absorption of specialty minerals but we do have relative absorption information, especially for Cu (change in liver Cu is an indicator of absorption). In general, organic Cu is more available than copper sulfate and differences often are greater in the presence of antagonists. Because we do not have a good index of relative absorption for Zn or Mn data are lacking on whether specially Zn or Mn is more bioavailable than the sulfates. Some cell culture data suggests that amino acid complexed Zn may be taken up at greater rates than Zn chloride (Sauer et al., 2017).

Clinical and production responses to organic trace minerals compared with sulfates or chlorides generally is positive (Siciliano-Jones et al., 2008; Rabiee et al., 2010). Because multiple trace minerals are usually supplemented we do not know whether the form of one or several minerals is eliciting the responses. Mode of action is not known. It could be increased bioavailability but if that was the primary mechanism, we would expect to see some responses to increased supplementation rates of conventional minerals (i.e., above NRC) but we seldom observe positive effects. Specialty minerals could be metabolized differently within the body but data is lacking showing this. Form of mineral does, however, affect the microbiome and this is a rapidly developing area of research. In vivo digestibility of NDF by dairy cows was less when sulfate trace minerals were fed compared to when hydroxyl minerals were fed (Faulkner and Weiss, 2017). We hypothesized that the sulfate minerals dissociated within the rumen and were toxic to rumen bacterial and reduced fiber digestibility. We also found that feeding Zn glycinate rather than Zn sulfate reduced the fecal abundance of a microbe that is associated with digital dermatitis. We hypothesized that reduced hoof exposure to the pathogen when cows walk in manure could be a mode of action for improved hoof health with organic Zn.

Recommendations

Chromium

Chromium is a required nutrient, however, the NRC (2001) did not provide a quantitative recommendation. Furthermore, feeding diets with more than 0.5 ppm of supplemental Cr or from sources other than Cr propionate is not currently legal in the U.S. Cr is needed to transport glucose into cells that are sensitive to insulin. Because of analytical difficulties (e.g., normal grinding of feeds prior to chemical analysis can contaminate them with Cr) much of the data on Cr concentrations in feed are not valid. However, a limited data base based on proper analytical techniques is available (Spears et al., 2017). Some studies with cattle have shown that supplemental Cr (fed at 0.4 to 0.5 ppm of diet DM) reduced the insulin response to a glucose tolerance test (Sumner et al., 2007; Spears et al., 2012). Elevated insulin reduces glucose production by the liver and enhances glucose uptake by skeletal muscle and adipose tissue. These actions reduce the amount of glucose available to the mammary gland for lactose synthesis and this may be one mode of action for the increased milk yield often observed when Cr is supplemented. Most of the production studies evaluating Cr supplementation (studies used Cr propionate, Cr-methionine, Cr-picolinate and Cr yeast) started supplementation a few weeks before calving and most ended by about 6 wk. Supplementation rates varied but most were 6 to 10 mg/day (approximately 0.3 to 0.5 mg Cr/kg of diet DM). The median milk response from 30 treatments from 14 experiments was +4.1 lbs/day (the SD among responses was 3.5 lbs/day). About 75% of the treatment comparison yielded an increase in milk of more than 2 lbs/day. Although a comprehensive meta-analysis is needed, based on this preliminary analysis of studies, increased milk yield of at least 2 lbs/day is highly probably when approximately 0.5 ppm Cr is supplemented to early lactation cows. Whether this response would be observed throughout lactation is not known. The potential return on investment from milk can be calculated by using the value of milk and cost of feed plus the cost of the supplement and assuming a median response of about 4 lbs of milk and an expected increase in DMI of about 2.8 lbs. At this time, a milk response should only be assumed to occur up to about 42 DIM.

Cobalt

The current NRC requirement for Co is expressed on a concentration basis (i.e., 0.11 ppm in diet DM) rather than mg of absorbable Co/day basis. This was done because Co is mostly (perhaps only) required by ruminal bacteria and the amount they need is a function of how much energy (i.e., feed) is available to them. Although Co concentration data for feeds is very limited, the NRC requirement is for total Co and in many cases, basal ingredients would provide adequate Co. In studies conducted in WA, basal diets contained 0.2 to 0.4 ppm Co (Kincaid et al., 2003; Kincaid and Socha, 2007) but basal diets from WI contained 1 and 2 ppm Co (Akins et al., 2013). Data using growing beef animals (Stangl et al., 2000) found that liver B-12 was maximal when diets contain 0.22 ppm Co (approximately twice as high as current recommendation). With dairy cows, liver B-12 concentrations continued to increase as supplemental Co (from

Co glucoheptonate) increased up to 3.6 ppm (Akins et al., 2013). In that study elevated liver B-12 did not translate into any health or production benefits. Indicating that maximal liver B-12 may not be necessary. Milk production responses to increased Co supplementation have been variable. One study reported a linear increase in milk yield in multiparious cows, but no effect in first lactation animals when supplemental Co increased from 0 to about 1 ppm. Older cows tend to have lower concentrations of B-12 in their livers which could explain the parity effect. Based on current data, the NRC (2001) requirement does not result in maximal liver B-12 concentrations in dairy cows. Across studies, when total dietary Co (basal plus supplemental) was about 1 to 1.3 ppm, maximum milk responses were observed. In some locations, basal ingredients may provide that much Co.

Copper

The NRC (2001) requirement for Cu is expressed on a mg of absorbable Cu/day basis and over a wide range of milk yields (40 to 150 lbs), requirements range from about 7 to 15 mg of absorbed Cu /day under normal conditions. Because Cu is secreted in low concentrations in milk, as milk yield increases, the NRC requirement for Cu increases slightly. However, DMI (and Cu intake) usually increase as milk yield increases to a greater extent than secretion of Cu in milk. Therefore the dietary concentration of Cu needed to meet the requirement may actually decrease as milk yields increase. Dry cows require less milligrams of Cu per day than a lactating cow, but because of dry matter intake differences, the concentration of Cu in dry cows diets may need to be greater than those for lactating cows.

Copper is stored in the liver and liver Cu concentrations are currently considered the gold standard for evaluating Cu status. Adult cattle liver Cu concentrations are deemed “adequate” between 120 – 400 mg/kg on a DM basis or approximately 30 – 110 mg/kg on a wet weight basis (McDowell, 1992). Over supplementation of Cu can result in Cu toxicity. Therefore, the range of adequate Cu status reflects both the minimum (110 or 30mg/kg) and maximum (400 or 120mg/kg) recommended concentrations of liver Cu on a DM or wet wt. basis, respectively. The recommended range for liver Cu is the same for both Jerseys and Holsteins; however, livers from Jersey cows will usually have a greater concentration of Cu than those from Holsteins when fed similar diets. Liver Cu concentrations decrease when cattle are fed diets deficient in Cu and increase in a systematic manner as dietary Cu supply increases (Yost et al., 2002)making it a good marker of mineral status.

All trace minerals have antagonists that reduce absorption but often these do not occur in real situations. All trace minerals are toxic but for most of the minerals the intakes needed to produce toxicity are usually quite high. Copper, however, is unique among nutritionally important minerals in that it is toxic at relatively low intakes which should dictate caution regarding over supplementation. On the other hand, Cu has numerous real world antagonists which mandate the need to over supplement in several situations. The NRC requirement assumes no antagonism (e.g., dietary S at 0.2% of

DM); however several situations commonly exists which result in reduced Cu absorption including:

Excess intake of sulfur (provided by the diet and water)
Excess intake of molybdenum (effect is much worse if excess S is also present)
Excess intake of reduced iron (may reduce absorption and increase Cu requirement)
Pasture consumption (probably related with intake of clay in soil)
Feeding clay-based ‘binders’

Most of these antagonisms have not been quantitatively modeled, and specific recommendations cannot be provided. When dietary sulfur equivalent (this includes S provided by the diet and the drinking water) is >0.25 to 0.3%, additional absorbable Cu should be fed. At higher concentrations of dietary equivalent S (0.4 to 0.5%), cows may need to be fed 2 to 3 X NRC requirement when Cu sulfate is used. As a general guide, for an average lactating Holstein cow, for every 100 mg/L (ppm) of S in water add 0.04 percentage units to the S concentration in the diet to estimate dietary equivalent S. For example, if your diet has 0.26% S and your water has 500 mg/L of S, dietary equivalent S = 0.26 + 5*0.04 = 0.46%. Note that some labs report concentrations of sulfate, not S. If your lab reports sulfate, multiply that value by 0.333 to obtain concentration of S. In most situations dietary S will be <0.25% of the DM. Diets with high inclusion rates of distillers grains and diets that contain forages that have been fertilized heavily with ammonium sulfate can have high concentrations of S. Water S concentration is dependent on source. Water should be sampled and assayed on a regular basis (at least annually) to determine whether water is adding to the S load in the diet.

Although the presence of antagonist justifies feeding additional absorbable Cu or using Cu sources that are more resistant to antagonism, no data are available indicating that the current NRC requirement is not adequate under normal conditions. Because of uncertainties associated with AC and the actual requirement, a **modest** safety factor should be used when formulating diets. Under normal situations, feeding 1.2 to 1.5 X NRC can be justified for risk management and it also should prevent excessive accumulation of Cu in tissues over the life of the cow. For an average lactating cow, NRC requirement for absorbed Cu is about 10 mg/day. Applying the 1.2 to 1.5 X safety factor, the diet should be formulated to provide between 12 and 15 mg of absorbed Cu/day. For an average Holstein cow fed a diet without any antagonists and using Cu sulfate as the source of supplemental Cu, the diet should be formulated to contain 12 to 15 ppm of **total** Cu (i.e., basal + supplemental). If using a Cu source that has higher availability than Cu sulfate, the safety factor would be the same but because of a greater AC, the concentration of total Cu in the diet would be less because less supplemental Cu would be needed.

If antagonists are present, the NRC (2001) overestimates absorbed Cu supply and Cu supply will need to exceed NRC requirements. For an average Holstein cow fed a diet with substantial antagonists, total dietary Cu may need to be 20 ppm, or perhaps more, to provide 12 to 15 mg/d of absorbed Cu. Some specialty Cu supplements are less affected by antagonism (Spears, 2003) and under antagonistic conditions, those sources of Cu should be used.

Adequate absorbable Cu must be fed to maintain good health in dairy cows, however excess Cu is detrimental to cows. Acute Cu toxicity can occur but of a greater concern are the effects of long term overfeeding of Cu. When cows are overfed Cu, liver Cu concentrations increase. If Cu is overfed for a short period of time (i.e., a few weeks) the change in liver Cu may be insignificant but when Cu is overfed for many months, liver Cu concentrations can become dangerously elevated. Jerseys are at higher risk of Cu toxicity because they accumulate greater amounts of Cu in the liver than Holsteins (Du et al., 1996), however toxicity can occur in Holsteins.

In non-lactating cows that were in good (or excess) Cu status and fed diets with approximately 20 ppm total Cu, liver Cu accumulated at an average rate of 0.8 mg/kg DM per day (Balemi et al., 2010). Although milk contains Cu, because of differences in DMI (and subsequent Cu intake), this accumulation of liver Cu is likely similar to a lactating cow fed a diet with 20 ppm Cu. Over a 305 day lactation, a cow fed a diet with ~20 ppm Cu (without antagonists) could accumulate ~250 mg/kg DM in the liver. Over 2 or 3 lactations, liver Cu concentrations would become extremely high. Classic toxicity is thought to occur when liver Cu concentrations are >2000 mg/kg DM. Beef cattle are tolerant to extremely high liver Cu concentrations, and many of the studies used to establish the upper limit for liver Cu used beef cattle. However, beef cattle usually have short lifespans and may not be good models for dairy cows. Chronic copper poisoning is subclinical and can cause liver degeneration, which is evident based on elevated liver enzyme (AST and GGT) activities in plasma (Bidewell et al., 2012). Accumulating evidence suggests problems may start occurring at much lower concentrations of liver Cu (500 or 600 mg/kg DM). Activity of AST, and GGT were significantly greater in heifers and bulls that had average liver Cu concentrations of 640 mg/kg DM compared with animals with average liver Cu of 175 mg/kg DM (Gummow, 1996). What was considered acceptable overfeeding of Cu (e.g., ~20 ppm supplemental Cu) may result in problems because of the duration of the overfeeding.

Manganese

The 2001 NRC greatly reduced the requirement for Mn compared with the earlier NRC. Based on NRC (2001) most lactating cows need between 2 and 3 mg/d of absorbable Mn and based on typical DMI translates to 14 to 16 ppm of total Mn in the diet. However, the 2001 NRC probably greatly overestimated the AC for Mn. Seventy percent of the calves borne from beef heifers fed a diet with about 16 ppm Mn for the last 6 month of gestation displayed signs of classic Mn defiency (Hansen et al., 2006). Using Mn balance studies in lactating cows (Weiss and Socha, 2005; Faulkner, 2016), we estimated that lactating cows (average milk yield in the experiment = 84 lbs/day) needed to consume about 580 mg of Mn to be in Mn balance. Based on the DMI in those experiments, that translated into a dietary concentration of ~30 ppm for total dietary Mn. As discussed above uncertainty exists and reasonable safety factors (i.e., 1.2 to 1.5 X) should be applied. For Mn, the starting point is 30 ppm and after the safety factor is applied, diets for lactating cows should have 36 to 45 ppm **total** Mn.

VITAMINS

Because of very limited data, the term requirement should not be used for vitamins. Rather we should use the term 'Adequate Intake' or AI. This is the quantity of vitamin that has been shown to prevent health problems or result in statistically reduced prevalence or severity of disease. Some vitamins increase milk yields, but because effects on milk yields must be put into economic context (i.e., price of milk, price of feed and cost of the vitamin) milk yield response should not be a major factor when setting AI. However this does not mean that supplementation rates that increase milk yield but do not affect health should not be used in situations where they are profitable. Data on concentrations of vitamins in basal ingredients is extremely limited or lacking entirely which adds to uncertainty. Concentrations of certain vitamins in feeds can be extremely variable (e.g., concentrations of tocopherol in hay crop forages can range from almost 0 to more than 150 ppm). Because supply of vitamins from basal ingredients will almost never be known, AI are usually based on supplemental vitamins. Adequate data are available to determine AI for biotin, niacin, and vitamins A, D, and E.

Vitamin A

NRC (2001) recommendations for vitamin A appear adequate for average cows (i.e., 110 IU of supplemental vitamin A/kg of BW). For a typical Holstein cow that equals about 70,000 IU per day. Milk contains about 0.3 mg of retinol/kg (about 1000 IU/kg or 450 IU/lb); therefore, high producing cows can secrete substantial amounts of A into milk. However this is not obligatory (i.e., feeding less retinol reduces the concentration of retinol in milk). The average cow in the NRC (2001) database produced about 35 kg of milk/d (77 lbs). For cows producing >77lbs of milk, feeding an additional 450 IU of vitamin A/d per lb. of milk >77 lbs will replace what is normally secreted in milk. For example for a Holstein cow producing 70 lbs of milk/d, the adequate intake of vitamin A is 70,000 IU but for a cow producing 100 lbs of milk, the adequate intake would be 70,000 + [(100-77)*450] = 80,350 IU/day. No data are available showing NRC (2001) vitamin A recommendations for dry and prefresh cows are not adequate. However because of reduced dry matter intake by prefresh cows, concentrations of vitamin A should be increased to maintain the AI of vitamin A (approximately 83,000 IU/d for a dry Holstein cow).

Vitamin D

Calcium homeostasis was long considered the primary function of vitamin D, but its effects on cells and animals go far beyond Ca including effects on immune function and health. The 2001 NRC requirement (14 IU of supplemental vitamin D/lb. BW or about 20,000 IU/d for a Holstein cow) is adequate with respect to Ca; however it may not be adequate for optimal immune function. Using a plasma concentration of 30 ng of 25-hydroxyvitamin D/ml to indicate sufficiency, 45 or 50 IU/kg BW (about 30,000

IU/d) may be needed for lactating cows (Nelson et al., 2016). Cows that spend a few hours outside during summer months probably synthesize adequate vitamin D but sun exposure in spring, fall, and winter (in the US) probably lacks intensity for adequate synthesis rates.

Vitamin E

The 2001 NRC recommendations of 500 and 1000 IU/d of supplemental vitamin E for lactating and dry cows are adequate; however, sufficient data exists to justify increasing supplementation during the last 14 to 21 d of gestation. Studies that evaluated the effect of additional vitamin E during the prefresh period used supplementation rates of 2000 to 5000 IU/d and in most studies some improvement in health was noted. Because of cost, the lowest supplementation rate that showed benefits (2000 IU/d) is the recommended AI for prefresh cows. This rate of supplementation has reduced early lactation mastitis and metritis.

Other vitamins

Adequate consistent data exist to set the AI for supplemental biotin at about 20 mg/day. This inclusion rate often improves hoof health and milk production (Lean and Rabiee, 2011). Niacin has been extensively researched but data are equivocal; about half the studies report a benefit and half report no effect. Supplementation at 12 g/d is more likely to elicit a production response (increased milk yield and milk component yields) in early lactation cows than the commonly used rate of 6 g/d. The majority of data do not support the use of niacin to reduce ketosis. Therefore, in most situations, the AI of supplemental niacin is likely 0. Supplemental rumen-protected choline (approximately 13 g/d of actual choline) increases milk yield in early lactation by 4 to 5 lbs/day (Sales et al., 2010; Arshad et al., 2019) and may help reduce fatty liver. In most studies, DM intake also increased with choline. A meta-analysis indicated that the milk yield response to supplemental choline was less as supply of metabolizable methionine increased (Arshad et al., 2019). Most production studies evaluated effects of choline the first 30 to 60 days of lactation and it is likely that response to supplementation would decrease as lactation progressed and DM intake increased. However, supplementing choline only the first 3 weeks of lactation resulted in increased energy-corrected milk yields (about 4 lbs.) for at least the next 9 wk after stopping supplementation (Zenobi et al., 2018). The common supplementation rate is 12-15 g of actual choline/d but the choline must be rumen protected. Because the data on health is equivocal at this time, choline does not have an AI, but it often is profitable because of its effect on milk yield.

Conclusions

Adequate supply of trace minerals and vitamins improves the health and productivity of dairy cows; excess or inadequate trace nutrients can have the opposite effect. The 2001 NRC requirements for Cu, Zn, Se and vitamin A are adequate in most situations and only a modest safety factor should be applied for risk management.

Because of regulations, no safety factor can be applied to Se. For Cu, numerous antagonists exist and in those cases, diets need to provide substantially more Cu than recommended by NRC or a high quality organic Cu should be fed. Although many situations dictate higher concentrations of dietary Cu, be aware of excessive Cu supplementation. Modest overfeeding Cu for months or years can result in high liver Cu concentrations that may be negatively affecting cow health. Manganese requirement is likely much higher than 2001 NRC and Co requirement also likely needs to be increased. Cows benefit from greater amounts of supplemental vitamin E during the prefresh period and lactating cows without great sun exposure may benefit from additional vitamin D supplementation.

Summary

The NRC (2001) requirements for most trace minerals and vitamins appear adequate but modest safety factors (~1.2 to 1.5 X NRC) should be used to reduce risk
The trace minerals contained in basal ingredients, including forages, have some degree of availability and concentrations should not be set to 0
NRC (2001) requirements for Co and Mn are too low and concentrations need to be increased substantially
Be wary of long term overfeeding of Cu. Health issues may be develop at dietary concentrations as low as 20 ppm when fed over long periods
Supplying vitamin E in excess of NRC (2001) requirement to peripartum cows provides health benefits
Supplying vitamin D in excess of NRC (2001) to cows with limited sun exposure may be needed to maintain adequate D status relative to general health

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Oxidative stress and health disorders in periparturient dairy cows

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INTRODUCTION

Dairy cattle are susceptible to increased incidence and severity of several infectious and metabolic disease during the periparturient period (Kelton et al., 1998). Health disorders occurring during this time may greatly impact the productive efficiency of dairy cattle in the ensuing lactation. A major contributing factor to increased health disorders is thought to be oxidative stress that contributes to dysfunctional immune and inflammatory responses. Oxidative stress occurs when cellular macromolecules (including lipids, proteins, and DNA) are damaged as a consequence of an imbalance between oxidants and antioxidants (Valko et al., 2007). Oxidants consist of reactive oxygen metabolites that are capable of oxidizing macromolecular substrates (Villamena, 2013). Antioxidant defenses scavenge pre-formed reactive oxygen metabolites or delay the formation of oxidants (Valko et al., 2007). At physiological concentrations, oxidants are essential for intracellular signaling and other beneficial cellular processes. When oxidants form at concentrations that overwhelm antioxidant defenses, however, oxidative stress can occur, resulting in damage to immune cell populations needed to protect against infectious pathogens. Indeed, the progressive development of oxidative stress in transition dairy cattle is thought to be a significant underlying factor leading to dysfunctional inflammatory responses associated with several economically important diseases such as mastitis and metritis. Understanding more about the underlying causes of oxidative stress during the periparturient period may facilitate the design of nutritional regimes that will reduce the severity and duration of disease as a function of dysfunctional inflammatory responses.

WHAT IS OXIDATIVE STRESS?

Oxidative stress is generally defined as excessive oxidant challenge that causes damage to cellular macromolecules including nucleic acids, proteins, and lipids (Sies et al., 2017).

Oxidative damage to these cellular constituents is now recognized as a significant pathophysiological event leading to many diseases processes in both human and veterinary medicine. For example, potent pro-oxidants can damage to essential all components of the DNA molecule and lead to gene mutations and abnormal protein synthesis (Valko et al., 2006, Halliwell and Gutteridege, 2007). Indeed, oxidant-induced mutations of nucleic acids is a major underlying cause of many different forms of cancer in humans. Other targets of oxidative damage include the amino acid side chains of proteins. Cysteine and methionine residues of proteins are particularly susceptible to oxidation and can lead to the reversible formation of mixed disulfides between a variety of thiol groups (Eaton, 2006). Oxidative modification of protein thiol groups can regulate the function of proteins and influence multiple metabolic, enzymatic, and receptor-mediated processes of cells. Lipids, and polyunsaturated fatty acids in particular, are most susceptible to the impact of excessive pro-oxidant challenge due to the multiple double bonds structure. Oxidative attack of membrane phospholipids causes a lipid peroxidation chain reaction. Since lipid peroxidation is a self-propagating event, the initial oxidation of only a few lipid molecules can eventually result in significant tissue damage. Peroxidation of lipids within cellular membranes can lead to changes in fluidity and cause damage to intracellular organelles. As such, the destruction of membrane lipids and the end-production of lipid peroxidation chain reactions have been implicated in immune dysfunction that leads to several health disorders of dairy cattle including mastitis and metritis (Sordillo and Raphael, 2013).

Although excessive accumulation of pro-oxidants can cause significant damage to cells and tissues, it is important to distinguish between the concepts of oxidative stress and normal redox biology. Redox reactions are characterized as the transfer of electrons where one chemical species is undergoing oxidation (loss of electrons) while the other is being reduced (gain of electrons). Indeed, redox reactions play important roles in many aspects of biology and medicine. The expression of moderate amounts of pro-oxidants, for example, are critical for many normal physiological responses including those regulated by redox sensitive signaling pathways such as NFkB, NrF2, and the MAPKs. The ability to maintain moderate amounts of pro-oxidants is now recognized as an essential way for cells to respond to the microenvironment and regulate essential physiological functions such as the immune system (Halliwell and Gutteridege, 2007, Sordillo and Aitken, 2009).

WHAT ARE PRO-OXIDANTS?

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen that contribute to this pro-oxidant challenge. In general, the term ROS is used as a collective term to include a wide variety of radicals and non-radical molecules including superoxide, hydrogen peroxide, hydrogen radical, singlet oxygen, peroxyl radical, alkoxyl radical, lipid hydroperoxides, peroxynitrite, hypochlorous acid and many others (Li et al., 2016). Formation of ROS occurs as byproducts of normal cellular metabolism of oxygen. A major source of ROS are the free radicals formed as a normal end product of cellular metabolism arising from either the mitochondrial electron transport chain or from stimulation of NADPH (Valko et al., 2007). The majority of ROS found in most healthy tissues likely results from increased cellular metabolism and energy generation by the mitochondria. The generation of ATP in the mitochondria through the Krebs' cycle and the electron transport chain generates O_2^- and H_2O_2 as byproducts will increase during times of enhanced metabolism. Approximately 1-3 % of electrons during the mitochondrial oxidative phosphorylation reactions are transferred are to oxygen to form superoxide (O_2^-) (Holmstrom and Finkel, 2014). Reactive metabolites also increase during regulated inflammatory processes to levels necessary for effective innate and adaptive immune functions (Sordillo and Aitken, 2009). For example, the NADPH oxidase system found in phagocytic immune cells generates significant amounts of ROS during the respiratory burst to kill microbial pathogens (Babior, 1999). There are various cellular enzymes that also can contribute to the overall ROS pool during inflammation. Some of these include xanthine oxidoreductase, nitric oxide synthase, cytochrome P450 monooxygenase (CYP450), lipoxygenase (LOX), and cyclooxygenase (COX)(Sordillo and Aitken, 2009, Mavangira and Sordillo, 2018). For example, membrane phospholipids can undergo enzymatic oxygenation through the COX, LOX, or CYP450 pathways resulting in not only a highly reactive lipid hydroperoxide, but also superoxide anion as a byproduct of the reaction (Raphael and Sordillo, 2013). The production of some ROS from these various sources is essential for the regulation of normal cellular processes including those that control immune and inflammatory responses (Sordillo, 2018).

WHAT ARE ANTIOXIDANTS?

The production of ROS during oxygen metabolism has necessitated the development of antioxidant defenses that can effectively trap reactive intermediates before causing oxidation to macromolecules or to reduce biomolecules that already have been oxidized. As such, antioxidants can be broadly defined as any substance that delays, prevents or removes oxidative damage to a target molecules (Halliwell and Gutteridege, 2007). Antioxidant defenses are diverse, can be either synthesized *in vivo* or derived from the diet, and are localized transiently throughout tissues and different cell types. The various antioxidant defense mechanisms also can be classified on the basis of several criteria, such as on their solubility in lipids and water or on their chemical and physical characteristics (i.e., enzymatic or nonenzymatic) (Sordillo and Aitken, 2009). Among the most efficient antioxidants are those enzymes that can directly catalyze the reduction of ROS. For example, the dismutation of superoxide to H_2O_2 and $^3O_2$ or H_2O_2 to H_2O and $^3O_2$ are catalyzed by superoxide dismutase and catalase, respectively. The selenium-dependent antioxidant enzymes, however, are the most widely studied systems with respect to dairy cattle health and well-being. Many of the beneficial health effects of Se are mediated by antioxidant selenoenzymes, such as glutathione peroxidase and thioredoxin reductase, which have selenocysteine residues incorporated into their active sites (Sordillo, 2013). The non-enzymatic antioxidants are represented by tocopherols, ascorbic acid, carotenoids, lipoic acid, and GSH to name just a few (Papas, 1999, Halliwell, 2007). Vitamin E, and □-tocopherol specifically, is a predominant antioxidant found in biological membranes. The tocopherols are able to disrupt radical chain reactions that lead to auto-oxidation of adjacent membrane-associated fatty acids. For example, vitamin E can act as a scavenger of both lipid radicals and lipid peroxy radical by donating a hydrogen ion with the formation of a tocopheroxyl radical. The tocopheroxyl radical is then regenerated back to its reduced form by vitamin C. Ascorbic acid (vitamin C) is a water-soluble antioxidant that plays a key role in maintaining the redox state of cells. In addition to recycling vitamin E, ascorbic acid can reduce several other oxidized biomolecules and act as a direct scavenger of free radicals. Thus, ascorbic acid plays a key role in maintaining the redox state of cells in addition to functioning as a free radical scavenger for other oxidized biomolecules (Sordillo and Aitken, 2009). The vitamin A precursor, □-carotene, is another important free radical scavenger. The carotenoids are especially effective at quenching singlet oxygen and can prevent the subsequent formation of secondary ROS. Lipoic acid is a component of the pyruvate dehydrogenase complex and has a central role in energy metabolism. However, lipoic acid also can function as a metal chelator and ROS scavenger. The reduced form of lipoic acid, dihydrolipoic acid, can further prevent ROS accumulation by recycling vitamins C and E (Sordillo, 2016).

Although micronutrients are essential components of the antioxidant defense network, it is significant to note that plasma concentrations of vitamins and mineral tend decrease in dairy cows around the time of parturition. The decrease in available serum-derived micronutrients is likely a combination of reduced dietary intakes as well as increased rates of utilization associated with metabolic stress in transition cows (Sordillo, 2016). Concentrations of vitamins and minerals that should be supplemented to dairy cattle diets should consider not only what is adequate to maximize production efficiency, but also what is required by the immune system to prevent oxidative stress and optimize immune cell functions.

OXIDATIVE STRESS IN TRANSITION COWS

Several studies have documented important changes in the antioxidant potential and prooxidant status in the transition dairy cattle (Bernabucci et al., 2002, Castillo et al., 2005b, Sordillo et al., 2007). Antioxidants can be found as water-soluble or lipid-soluble molecules that are localized transiently throughout tissues and various cell types (Drackley, 1999). Given the multiplicity of antioxidant pathways, their centrality in the prevention of oxidative stress, and the influences of diet on overall antioxidant capacity, it is important to be able to quantitatively measure the total antioxidant capacity or antioxidant power within biological specimens. Impairment of blood and milk leukocyte function has long been linked with increased susceptibility to mastitis around the time of calving when oxidative stress is increased. However, remarkably few studies have examined in any detail the redox status of important immune cell populations during this time. Results from our laboratory indicate that the antioxidant potential of isolated peripheral blood mononuclear cells (PBMC) remained relatively constant from 3 wk prior to calving and through calving, but dropped significantly ($P < 0.05$) by 21 days in milk

(Figure 1). These finding are consistent with reports in both humans and dairy cows that showed a relationship between the physiological changes during the periparturient period with a loss in overall antioxidant potential in several different tissue compartments (Gitto et al., 2002, Bernabucci et al., 2005, Castillo et al., 2005a).

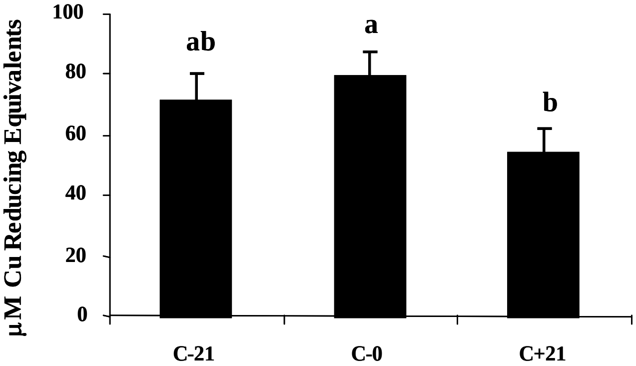

Figure 1. An assay used to determine total antioxidant potential of PBMC obtained from transition cows was based upon the reduction of Cu^{++} to Cu^{+} by the combined action of all antioxidants present in the sample. Changes in total antioxidant potential of white blood cells obtained approximately 21 d prior to calving (C-21), at calving (C-0), and 21 d after calving (C+21). Data are expressed as least square means $\pm$ SE. ^{a,b}Bars with different superscripts differ (P< 0.05) (Sordillo et al., 2007).

Lower antioxidant potential as a consequence of lactation stage can result from an excess accumulation of ROS, a depletion of antioxidant defenses, or a combination of both. One way to determine if ROS-mediated damage is occurring within host tissues is to measure end products of free radical oxidative processes. For example, when ROS react with polyunsaturated fatty acids, lipid peroxidation occurs. Peroxidation of lipids within cellular membranes can lead to changes in fluidity and cause damage to intracellular organelles. The determination of lipid hydroperoxide levels in plasma would be an indication of early stages of this lipid peroxidation damage. We showed that measurement of lipid hydroperoxides increased significant ($P < 0.05$) from calving through the first 3 wk of lactation when compared to the pre-partum measurements (Figure 2). These findings are consistent with other reports in periparturient animals where lipid hydroperoxides and biomarkers of lipid peroxidation, such as thiobarbituric acid-reactive substances (TBARS), were found to increase from calving and through 25 DIM (Bernabucci et al., 2005, Castillo et al., 2005b).

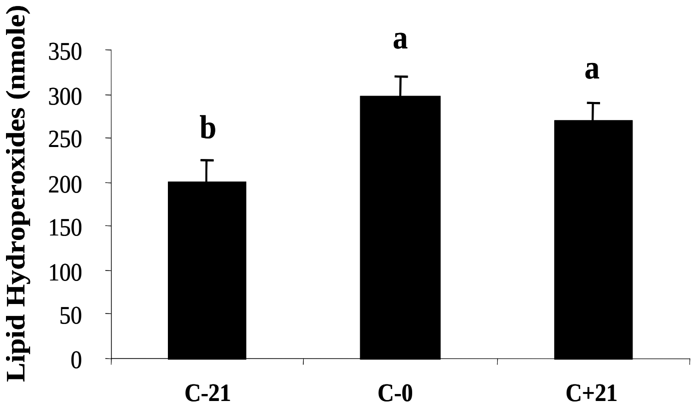

Figure 2. Changes in lipid hydroperoxide levels in plasma samples obtained approximately 21 d prior to calving (C-21), at calving (C-0), and 21 d after calving (C+21). Data are expressed as least square means ± SE. ^{a,b}Bars with different superscripts differ ($P < 0.05$) (Sordillo et al., 2007).

ASSESSING OXIDATIVE STRESS

A major obstacle in mitigating the detrimental impact of oxidative stress on transition cow health is the lack of an accurate way to assess the amount of oxidative injury that is associated with subsequent disease outcomes. Although all cows will experience some degree of oxidative stress around the time of calving, only those cows with severe oxidative injury will succumb to subsequent health disorders (Sordillo and Aitken, 2009). To date, however, there is no standardized way of measuring oxidative stress in cows or assessing critical thresholds of oxidative injury that will lead to health disorders. Primary targets of free radical-induced oxidative damage are cellular lipids that are converted to lipid peroxides. Isoprostanes are a family of prostaglandin-like compounds formed through the free radical-induced peroxidation of arachidonic acid in cellular membranes (Mavangira et al., 2016, Sordillo, 2018). In human medicine, measurement of plasma 15-F_{2t}-isoprostane has proven to be ideally suited for assessing lipid peroxidation and concentrations increased proportionate to oxidative injury and disease incidence (Milne et al., 2015). Indeed, chromatography-based measurement of plasma 15-F_{2t}-isoprostane is considered the gold standard method of assessing oxidative stress in humans (Klawitter et al., 2011). Our group was the first to use chromatography-based analytics (liquid chromatography tandem mass spectrophotometry; LC/MS/MS) to measure 15-F_{2t}-isoprostane concentrations in bovine plasma during the transition period and during mastitis (Mavangira et al., 2016, Kuhn et al., 2018). Unfortunately, threshold concentrations of plasma 15-F_{2t}-isoprostane that could be used as a biomarker for subsequent disease susceptibility in transition dairy cows are currently unknown. The availability of tools to detect health risk early enough in the transition period will allow time to intervene effectively with antioxidant micronutrients before diseases occur.

CONCLUSION

Oxidation and the production of free radicals are an integral part of aerobic metabolism. Considerable evidence supports the contention, however, that oxidative stress during the periparturient and early lactation period may contribute to a number of health disorders in dairy cattle. Dairy cattle management practices and emphasis on genetic selection to maximize milk production has increased the metabolic stresses associated with parturition and the onset of copious milk synthesis and secretion. The antioxidant requirements of cows will likely increase as production demands continue to escalate within the dairy industry. The performance of high producing dairy cattle can be optimized to a certain extent by supplementing diets with optimal levels of micronutrients with antioxidant capabilities. However, oxidative stress continues to be a problem in transitions cows. Innovative approached are needed to enhance the antioxidant defense mechanisms of dairy cattle during times of increased metabolic demands. A better understanding of how antioxidants may preventative immune dysfunction and prevent oxidative damage to host tissues may lead to more effective strategies to control health disorders in the transition dairy cow. However, strategies to mitigate the detrimental impact of oxidative stress on dairy cattle health are limited by available methodologies for accurately evaluating oxidative damage.

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Nutrition and management of the dairy calf to realize the growth and lactation potential

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For many years the rearing of calves has been viewed as a liability. Frequently the goal has been to wean them as early and as inexpensively as possible. However, market conditions, research and the experience of progressive dairy producers indicate that this is frequently not the best management decision for several reasons.

- Growth and lactation yield. Producers have found that feeding calves to achieve their growth potential has numerous benefits including reduced morbidity and mortality and positive impact on milk yield in their first and later lactations.
- Animal welfare. The calf program is an acutely visible indicator of standards for welfare of the dairy herd as evidenced by recent undesirable publicity. Properly designed and managed systems where calves are housed in pairs or groups has shown benefits to animal behavior and comfort, growth and later performance. Additional concerns exist where very young calves are transported long distances to rearing facilities.
- Economics. A survey by Karzes (2013) demonstrated that, although daily costs for the preweaned calf were highest ($3.13), they comprised less than 15% of total cost and 8% of the total growth cost. Reductions in rearing costs are improved more significantly by reducing the size of the replacement herd and managing the heifer to calve at an earlier age.

The net result of these conditions is that many dairies are raising fewer heifer calves, breeding lower ranking cows to beef, feeding and managing their calves in a more biologically normal manner and developing calf rearing systems which emphasize improved conditions for calves, enabling more effective and efficient use of labor resources.

The challenge in rearing the preweaned calf is that there a multitude of factors influencing success. Success can be defined from differently when evaluated from the perspective of calf health, labor efficiency, herd manager responsibilities, owner assessment and perception of dairy product consumers. Ideally the goal should be to rear calves to achieve maximum genetic potential for growth and lactation performance in an economical manner. Integral to this endeavor are favorable welfare of the animal, labor efficiency and effectiveness in a manner which is perceived desirably by the consumer. Unfortunately, our research conducted on the preweaned calf has been very segmented with little consideration given to the goals of the replacement program. This presentation will attempt to review the breadth of conditions affecting the successful rearing of calves.

Maternal influence on the calf. We will make the assumption that effective feeding management of the lactating and dry cow is appropriate for development of the unborn calf.

- Production of high-quality colostrum can be compromised especially during the fall and early winter (Gavin et al, 2018). Affected cows (especially Jerseys) produce little to no colostrum prior to or after calving. Utilization of replacers should be considered essential adjuncts to passive transfer of immunity.
- Heat stress during the prepartum period results in premature calving and less efficient absorption of maternal colostrum by the calf. These calves are also less adapted to heat stress once they mature (Dahl et al, 2016).
- A diverse microbiome in the meconium of the newborn calf suggests that microbial colonization of the calf may begin in utero. It has been established that biome of the calf is influenced by the maternal vaginal, oral and fecal microbiomes. This active area of research indicates a strong relationship of the microbiome to calf health and productivity.

Maternity management. The first minutes or hours of the calf's life have immediate and long-term impacts on health and performance of the calf.

- In addition to the maternal influence on the developing microbiome of the calf, the birthing environment is a significant contributor. Ingestion of large quantities of bacteria prior to or during colostrum consumption has a negative impact on IgG absorption by the calf (Godden et al, 2012; James et al, 1978).

Colostrum management.

- The first requisite is sanitary harvesting of adequate quantities of high IgG colostrum in a timely fashion. Delays in feeding or in rapid cooling of colostrum result in excessive microbial growth which can impair IgG absorption.
- Colostrum is a source of both desirable and undesirable microbes and viruses. Where risk for disease is evident, timely pasteurization has been shown to improve efficiency of IgG absorption (Godden et al, 2012) and risk or disease transfer to the calf.
- Consumption of fresh colostrum from the dam which enabled absorption of immune cells from the dam had a positive impact on immune function of the calf. (Langel et al, 2016)
- There are positive influences of non-Ig and non-cellular components of colostrum on development of the digestive system of the calf. Consumption of bovine colostrum initially and for several feedings has been documented to improve growth of intestinal villi, absorption of glucose, support of insulin action and promotion of post-natal growth (Hammon, 2019)
- Consumption of adequate quantities of colostrum early in life has been a long-standing recommendation. Achieving 10 mg of IgG / ml of blood was once indicative of successful passive transfer of immunity to the calf. New standards as shown below have been proposed.

Category	Serum IgG g/L	Total protein g/dl	Brix %	Consensus % calves
Excellent	>25	>6.2	>9.4	40
Good	18.0-24.9	5.8 – 6.1	8.9-9.3	30
Fair	10.0 – 17.9	5.1 – 5.7	8.1 – 8.8	20
Poor	<10.0	<5.1	<8.1	<10

- Ideally successful colostrum management involves:
    - Timely harvest of colostrum post calving to optimize yield.
    - Birth environment to minimize potential contamination by undesirable microorganisms.
    - Prompt feeding of at least 4L high IgG colostrum (>50g/dl) with low bacteria count (<20,000 cfu/ml).
    - Ideally, 4L of fresh colostrum from the dam fed as soon as possible after birth.
    - When there is a risk of disease transfer, pasteurize colostrum to reduce undesirable bacteria.
    - Harvest and feed “transition” milk from fresh cows to the youngest calves as long as the supply lasts.
    - Implement use of effective colostrum replacers when sufficient colostrum resources are not available.

Nutrition and management. Historically, we have managed the preweaned calf with the goal of stimulating rumen development and early weaning. These practices were achieved by limiting milk or feeding milk replacers with lower fat content thereby reducing daily and total preweaning feed expenses. However, dairy is one of very few animal agriculture industries that follows this practice. Instead calves should be fed to meet their nutrient requirements for maintenance and growth while encouraging low morbidity and mortality.

The table below illustrates the impact of environment on maintenance requirements.

	Temperature ^{o}F			
Body weight	14	32	50	68
77	8.8	7.7	6.2	4.8
100	11	8.8	7.3	5.7

Research conducted at Virginia Tech (Bascom et al, 2007) indicated that maintenance requirements for Jersey calves are as much as 20% higher per unit of body weight than considered in this table. Additionally, maintenance requirements increase as temperature rise above the thermoneutral zone. It is not a common practice to increase nutrient intake when calves are exposed to conditions outside of the thermoneutral zone.

Traditionally milk replacers containing 20% protein and 20% fat were fed to preweaned calves. These replacers were developed to reduce cost and encourage starter intake and early weaning and not necessarily to meet the requirements of the growing calf. The table below demonstrates the energy allowable gain and cost per lb of gain at two different feeding rates for milk or milk replacer at different temperatures.

Calf	Whole milk		20:20 milk replacer	
	68F	32F	68F	32F
88 lb. calf – week 1 1 lb of DMI Cost = $1.49/day	.9 LB. ADG $1.65/lb gain	.3 lb. ADG $4.96/lb gain	.7 lb. ADG	Lose weight
88 lb. calf week 1 2.2 lb DMI Cost $2.98	2.6 lb. ADG??	2.1lb ADG $1.42/ lb gain	2.2 lb/day	1.7 lb/day

In viewing these tables, it is apparent that there is insufficient energy to meet the calf's requirements for maintenance and growth under cold environmental conditions. This is especially risky during the first weeks of life when the calf is incapable of consuming sufficient energy or protein from calf starter and likely impairs immune function during this time. These data also illustrate the impact of feeding rate on economic efficiency of gain. Although not as great a problem, excessive environmental temperatures also increase maintenance requirements. This can be problematic as calves typically have reduced appetites in this environment.

Traditionally, calves have been offered limited amount of milk (~2L/feeding) early in life and only gradually increased milk intake with the logic that their digestive systems were incapable of consuming more milk or that it would lead to more diarrhea. Use of ad lib milk feeding from autofeeders early in life has dispelled this theory but it was thought that higher milk feeding was not feasible for 2x bottle or bucket-fed herds. In a field study with over 1,000 calves on 5 dairies, Knauer et al (2018) found that calves offered a fixed amount of milk post colostrum feeding consumed 14L more milk than calves in which milk was gradually increased over 7 to 14 days.

Estimated energy and protein available for growth for the average FIX (6.8L/day) and INC (5L) milk intake per enrolled calf. (Knauer et al, 2018)

Variable	Summer FIX	Summer INC	Winter FIX	Winter INC
Total DMI (kg)	0.85	0.63	0.85	0.63
DMI req for maintenance kg/day	0.29	0.29	0.44	0.44
DMI available for growth kg/day	0.56	0.34	0.41	0.18
Energy allowable ADG	0.99	0.65	0.76	0.39
Protein allowable ADG	0.74	0.52	0.74	.52

Over the first three weeks, calves receiving a FIX amount of milk or milk replacer weighed 1.35 kg more and were .3cm taller with no difference in morbidity or mortality.

Calf behavior research has yielded new information of significance to the dairy industry. Feeding behavior patterns are learned and develop early in life with long-term implications. More liberal milk feeding: reduces stress, improves immune function and feed conversion and appears to improve lactation performance. More liberally fed calves consume their solid feed at slower rate, in smaller meals with longer pauses between bouts of eating and a lesser response to feed delivery. Provision of chopped forages early in life appears to promote solid feed consumption and encourages development of a more stable developing rumen environment. Slug feeding eating patterns appear to be learned and persist later in life. Additionally, grouping calves promotes solid feed intake and growth. Research conducted at Guelph University (Miller-Cushon et al, 2016) has demonstrated that paired housed calves consumed more starter concentrate before and after weaning. They are less likely to experience the post weaning “slump” commonly observed in calves fed more liberally prior to weaning. These findings indicate that our system of housing calves individually and feeding twice day at irregular intervals enhance development of undesirable behaviors.

Calf starters: Probably the one of the most important characteristics of a starter would be its palatability. In most research conducted on calf starters, calves were housed individually, limit-fed milk replacer and/or were weaned abruptly at an early age (less than 6 weeks). We know that these conditions are a strong stimulus for dry feed intake and that these changes are probably very disruptive to the intestinal microbiome. More recent research with more liberally fed calves using step down weaning has shown potential benefits of adding small amounts of chopped forage to aid in the development of the rumen and transition away from milk consumption (Terre et al, 2013, 2016).

Water: Although water quality is a critical component of calf rearing, it is often overlooked. Desired characteristics of water quality are shown in the table below. Water for calves should be tested twice a year.

Item	Expected	Borderline	Concern
pH	6.8 – 7.5	Under 6 and over 8.4	Under 5.5 and over 8.5
Total Dissolved Solids	<500 ppm	Over 500 ppm	Over 1000 ppm
Calcium	0-43 ppm	>100ppm	> 200ppm
Chloride	0-100ppm	>100ppm	>300ppm
Copper	0-0.2ppm	>0.2ppm	>0.5pm
Iron	0-0.2ppm	>0.2ppm	>0.3ppm
Manganese	0.05ppm	>0.05ppm	>0.05ppm
Magnesium	0-29ppm	>50 ppm	>125 ppm
Nitrate	0-44ppm	>50ppm	>100ppm
Nitrate-nitrogen	0 – 10 ppm	>11.4ppm	>22.7ppm
Sodium	0-3ppm	>55ppm	>200ppm
Zinc	0-5ppm	>5ppm	>25ppm
Total bacteria/ml	<2	>10	>10,000
Fecal coliform/100ml	Less than 0.1	Over 0.1	Over 1

Developed by Dr. Don Sockett, Dr. David Beede, Dr. Tim Johnson, and Mr. Bob Riesberg.

Successful calf rearing requires the development of systems which assure that all the desired components of calf management are addressed consistently. They are:

Prepartum dairy cow

- Nutrition to support desired calf growth and produce adequate amounts of colostrum with high IgG content.
- Heat stress remediation to promote efficient IgG absorption by the calf

Calving management

- A clean, well-bedded calving area to promote desired development of the biome in the calf.
- Convenient location for observation, timely harvest of colostrum with low bacteria count and further processing prior to feeding the calf.
- Timely feeding of either fresh or processed colostrum to supply at least 200g of IgG as soon as possible but within 6 hours of birth.

Nutrition of the pre-weaned calf

- Feed sufficient milk or milk replacer to meet nutrient requirements for maintenance and desired growth (double birth weight by 56 days). This may require as much as 2.0 lb. of DM from milk solids/day.
- Provision of a palatable calf starter grain within the first weeks of life with at least 20% protein. Provision of chopped palatable forage by the 4th week of life.
- Fresh water with desirable characteristics.
- Step-down weaning over at least 7 days.

Welfare friendly facilities

- Although individual housing systems will continue to be popular, the adoption of paired housing and or group housing systems should be considered as enabling social development and enhancing calf performance.
- Although there are no regulations concerning transportation of calves, it is likely that regulations will come in the future. In Canada after February of 2020, it will be forbidden to transport calves less than 8 days of age. In addition, calves over 8 days may not be transported more than 12 hours from their last feeding at the source farm. These policies may likely be considered in the US in the future.
- Labor will continue to be a challenge on dairies. Implementation of facilities which address comfort and efficacy of labor should be planned

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Balancing for amino acids in support of lactation.

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Protein is an Essential Nutrient

Protein is one of six essential nutrients needed to survive. It is composed of carbon, hydrogen, oxygen, nitrogen (**N**), and sulfur. The latter two elements are what differentiate protein from carbohydrates, which contain only carbon, hydrogen, and oxygen. Generalizing protein as one comprehensive category allows simple categorization of a substrate that humans and animals must consume in their diets. However, this mindset does not accurately depict what they need to synthesize body tissue (skin, muscle, organs, etc.), gestational tissue, milk protein for young, and to carry out countless other processes required for basic life functions.

Protein is actually composed of smaller molecules called amino acids (**AA**), which are strung together to form a long chain. The AA can be envisioned as links in the chain. However, this chain is unconventional, as its links come in 20 different shapes (the 20 different AA). Because the length of the chain and the order of AA in each chain vary, and both dictate the characteristics of the protein, an essentially infinite number of proteins can be created. For example, if a protein is composed of 200 AA (a smaller sized protein), there a 20^{200} ($1.6 * 10^{260}$) possible combinations of the AA and thus 20^{200} different proteins.

Animal cells make more than 20,000 different proteins. These proteins provide the structural framework of the cell and conduct much of the work that allows the cell to metabolize nutrients, grow, divide, and maintain itself. Examples of unique proteins include:

- hair and wool fibers, which are composed of a number of long protein chains wound together by the hair follicle cell and secreted as a hair or wool fiber;
- a mesh of translucent proteins forming the retina of the eye;
- a series of fibrin proteins bound together to form a muscle fiber;
- actin protein, which acts as the motor to cause muscle fiber contraction;
- keratin sheets created by skin cells before dying to create the outer skin layer;
- and the caseins, beta-lactoglobulin, and alpha-lactalbumin proteins secreted with milk.

Metabolizable Protein and Amino Acids

Metabolizable protein (**MP**) represents the true protein available to the cow absorbed from the intestine. Metabolizable protein includes digested microbial protein (**MCP**) and protein escaping degradation in the rumen (**RUP**). The Dairy NRC (2001) includes endogenous protein in this term, but this is not a correct representation as it is derived from previously absorbed microbial and ruminally undegraded feed protein, and thus does not represent “new” protein.

Although animal N requirements are commonly stated in terms of MP, the true requirements are for the specific AA resident in that protein. The animal actually does not have a protein requirement, per se. Because there is a diversity of AA composition in the absorbed protein, stating animal requirements in terms of MP inherently causes requirement over-prediction to compensate for variation in AA composition. This is perhaps most apparent when feeding diets constructed largely from maize products which are inherently low in lysine (Polan et al., 1991). Such a diet could be created to meet MP requirements, but animals typically still respond to the addition of a protected lysine source or more protein that also provides lysine to the ration (Vyas and Erdman, 2009).

When one or more AA are deficient, production will generally decline relative to a sufficient diet. When such diets and their corresponding milk production are used to generate MP requirement prediction equations, the AA deficient diets will categorize as MP deficient resulting in a solution that calls for more MP than if all of the diets had a good mix of AA. This results in higher MP requirements which serve to ensure the most limiting AA in the diet always exceed requirements. Thus, we are often overfeeding many AA when basing requirements on MP alone.

Animals can be successfully fed a lower MP diet if the AA composition of that diet is better matched to AA requirements. This was demonstrated by Haque et al. (2012) using diets with less than 13% crude protein (**CP**). The idea of having a perfect mix of AA is generally referred to as the “ideal protein” concept. Basing requirements on the individual AA, rather than on MP alone, clearly identifies which AA are being wasted and those that are insufficient. This allows construction of diets which achieve the “ideal” profile. Feeding an ideal protein profile at 100% of the animal’s AA requirements will yield maximum N efficiency (percentage of dietary N that is converted to a marketable product). This is beneficial from an environmental standpoint, as dairy cattle excrete any unused dietary N in their urine and feces.

Though some of the N in stored manure may be captured by growing plants and microbes in the soil after land application, much of it is lost as ammonia. This is evident by the smell of ammonia associated with barn floors and manure storage facilities. The ammonia is primarily derived from catabolism of urea in urine. It is rapidly converted to ammonia by action of the enzyme urease which is present in feces. As soon as the urine and feces mix on the barn floor, the conversion of urea into ammonia begins, and ammonia then vaporizes from the manure. The loss of ammonia continues during manure storage, resulting in half or more of the N in manure being lost to the air.

Ammonia emissions are a form of environmental pollution (Uuml et al., 2001, Agle et al., 2008). Ammonia in the air combines with sulfur and nitrogen oxides from car and power plant exhaust to form small particles less than 2.5 micrometers. These particles cause the haze visible in the air and evidence is growing that they contribute to lung problems including asthma.

Economic and Environmental Benefits of Precision N Feeding

In addition to the environmental impact of ammonia emissions, there is also a large financial cost. Protein is an expensive dietary nutrient representing approximately 42% of the cost of a lactating cow ration in the United States (St-Pierre, 2012). In a survey carried out on 103 large-scale dairies across the US (613 ± 46 cows; 34.5 ± 0.3 kg of milk per cow per day), nutritionists reported feeding diets with 17.8 ± 0.1 % crude protein (Caraviello et al., 2006). A meta-analysis of 846 experimental diets found a similar mean diet CP content and identified that conversion efficiencies for dietary and metabolizable N (based on NRC, 2001) to milk protein averaged 24.6 % and 42.6 %, respectively (Hristov et al., 2004). Assuming the same intake and diet composition (22.1 kg/d DMI and 17.8 % CP), over a 10-month lactation, the 9 million dairy cattle in the US excrete an estimated 1.3 million metric tons (mmt) of N per year (Livestock, Dairy, and Poultry Outlook: August 2012, LDPM-218, Dairy Economic Research Service, USDA). It is likely a survey today would observe slightly lower protein diets than what the aforementioned researchers observed given the significant increase in protein cost since 2006. However, the efficiency of conversion of dietary protein to milk protein does not generally exceed 30% until dietary CP levels fall below 15.5%. It is unlikely that the current average dietary CP% on US dairy farms is this low.

The dairy extension group at Ohio State determines the cost of a pound of MP in dairy rations on a bi-monthly basis (Buckeye Dairy News (www.dairy.osu.edu/newsletter/buckeye-dairy-news). Over the past 5 years, this value has remained relatively constant at $0.43/lb, although the most recent analyses shows a much lower value, presumably due to the impact of Chinese tarrifs on imported soybean products. Presumably this value will return to the historic trend line when the trade war is resolved. Using the historic trend, a ration for a cow producing 80 lbs of milk should contain approximately 5.44 lbs of MP which can be readily met with a diet containing 16.1% CP resulting in a N efficiency of 28.7%.

Successful removal of 1 lb of MP from the ration would result in a reduction in ration cost of $0.43/cow/d, a dietary CP level of 13.1%, and a N efficiency of 35.1%. Thus, there are both economic and environmental incentives to feed less dietary protein; the challenge is doing so without sacrificing production. To accomplish this, accurate and precise nutrition models that are based on AA rather than on MP must be implemented in ration balancing software. This would allow nutritionists to reliably match the supply of AA to the animal's needs, along with the desired level of production.

Ruminal Outflow of Microbial and Undegraded Feed Protein

Regardless of whether the requirement system is based on MP or on AA, predictions of the supply of MP must be unbiased. Estimates of the supplied AA are derived from predictions of the true feed protein escaping ruminal degradation, and the true microbial protein synthesized in the rumen. If either are biased, then the estimates of the supply of AA associated with each will be biased. Although the 2001 Dairy NRC represented a significant improvement in accuracy and precision over earlier systems, predictions of microbial and undegraded feed protein (RUP) were apparently biased (Roman-Garcia et al., 2016, White et al., 2016, White et al., 2017a, White et al., 2017b). Undegraded feed protein flow was found to be overpredicted on average by 40 g N/d for a typical animal, and the error increased as RUP flow increased. This signals fundamental problems in model structure, which consequently contributes to similar bias in predicting milk production. Bateman et al. (2005) and Broderick et al. (2010) observed similar problems.

Correlation analyses indicated the RUP bias problem was associated with passage rate (K_p) estimates. The K_p equations used by the Dairy NRC (2001) were biased compared to K_p measurements from studies that used indigestible NDF as a marker (Krizsan et al., 2010). A recent study of K_p on forage-based diets also supports bias in prediction of particulate K_p (Gregorini et al., 2015). Recent work by our group has demonstrated that the derivation of new estimates for K_p from observed RUP flows resulted in very modest gains in precision but substantial reductions in bias.

The revised estimates for RUP led to similar adjustments in RDP (calculated by difference from total N flows). The latter were used by Moraes et al. (unpublished) along with new estimates of ruminal starch and fiber degradation to derive an updated prediction of microbial protein outflow. The approach taken provides an integrated representation of ruminally degraded N and carbohydrate sources that substantially improved precision and accuracy relative to the previous equation. However, the potential contribution of recycled blood urea in microbial protein (Reynolds and Kristensen, 2008) was not represented. This likely is only important when attempting to feed very low protein diets (less than 13% CP), and consequently may be ignored for now.

Ruminal Outflow of AA and Absorption from the Intestine

A number of updates and revisions to the NRC 2001 system for predicting AA flows were undertaken (Fleming et al., 2019). These included corrections for hydration and incomplete recovery of AA from acid hydrolysis of proteins (Lapierre et al., 2016), as well as revised estimates of the composition of AA in microbial protein (Sok et al., 2017). Past efforts have largely ignored the difference in mass of AA when bound in protein versus when in free form. In the former, a molecule of water is removed across each peptide bound holding the AA together to form the protein resulting in a mean mass loss of 15%. This was reflected in research models such as Molly (Baldwin et al., 1987), but had been overlooked by the prior NRC committee (2001). The AA composition of proteins is almost always reported in a hydrated form given that the measurements are made on free AA after acid hydrolysis. Correction for the mass of hydration, incomplete recovery of AA from acid hydrolysis, and updated microbial AA composition resulted in removal of much of the mean bias observed by NRC (2001). These corrections mostly aligned predicted AA flows with observed total AA outflows from the rumen. The model tended to overpredict several AA, including methionine, which was felt to possibly reflect technical challenges including incomplete recovery from hydrolysis due to inadequate temperature control during digestion or introduction of oxygen in the hydrolysis tubes leading to some oxidative losses.

Although this work improved estimates of RUP AA flows from the rumen, predictions of absorbed AA are likely still biased and less precise than desired due to the assumption that all AA are digested to the same extent as the protein contained in the microbes and

RUP. We have recently adapted an approach used by Maxin et al. (2013) to assess the absorbed supply of each AA from individual dietary ingredients (Estes et al., 2018, Huang et al., 2019). The method makes use of an infusion of a ^{13}C labeled AA mixture derived from enriched algae to assess the entry rate of each AA. The labeled AA are introduced into the jugular vein, consequently, measurements reflect appearance in blood thereby accounting for all losses before blood appearance, i.e. ruminal and intestinal.

We have assessed AA entry as a fraction of the AA in the source ingredient for 9 ingredients: corn silage, grass hay, alfalfa hay, corn grain, soyhulls, distiller's grains, brewer's grains (Huang et al., 2019), blood meal, and feather meal (Estes et al., 2018). In all cases except feather meal, the proportions of AA in the source ingredient that entered the blood pool varied considerably across the essential AA. Thus, either the composition of the RUP deviates significantly from the ingredient or the digestibility of individual AA in the RUP vary considerably, or both. In the case of the feather and blood meals, the modified 3-step and Ross assays failed to reflect the observed digestibility of those ingredients indicating those methods are not reliable for evaluation of AA availability (Estes et al., in preparation). Additional work is required to establish methods of AA availability assessments that can be conducted in real or near-real time for field application.

Fixed AA Use Efficiencies and the Ideal Protein Concept

A portion of the problem in predicting milk or milk protein responses to MP supply noted above is the model assumption that the conversion of MP to milk protein, after subtracting maintenance use, is a constant 65%. In a summary of literature data, Lapierre et al. (2007) found that the highest MP conversion efficiency was 43%. Efficiency decreased as milk protein output (and MP supply) increased. In a summary of publications that reported responses to post-ruminally infused casein, Hanigan et al. (1998) found a similar maximum efficiency of conversion of about 45% with an average conversion efficiency of 22%. Whitelaw et al. (1986) abomasally infused casein at 3 different levels and observed responses at each level with efficiencies of conversion ranging from 40 for the first increment to 15% for the last increment. Each of these demonstrates that the maximum efficiency is less than the assumed 65% and varies depending on the overall supply and other factors. These problems are not resolved if MP is replaced with individual AA. In fact, adherence to the same scheme for the multiple essential AA (EAA) maintains the slope bias associated with too high of an assumed efficiency and introduces significant mean bias.

Historically, we have used the concept of a first-limiting AA as the basis for determination of requirements. The concept is based on a hypothesis which has become known as the Law of the Minimum. Sprengel (1828) formulated this concept based on plant growth responses to soil minerals. The original thesis stated that a nutrient can limit plant growth, and when limiting, additional growth will be proportional to additional supply. This is a logical approach rooted in the concept of conservation of mass. Clearly, nutrient output cannot exceed nutrient input if it cannot be synthesized, and output will be less than input if the transfer is less than 100% efficient, which is often the case.

This concept is strongly supported by volumes of data over the past 175 years. Von Liebig (see Paris, 1992 for a translation) subsequently restated and expanded the hypothesis indicating that if a nutrient was limiting growth, responses to other nutrients could not occur (von Liebig, 1862). Mitchell and Block (1946) used von Liebig's extension of Sprengel's thesis to develop the concept of the order of limiting AA, which was described using an analogy of a water barrel with broken staves. Based on this formulation, if any nutrient is limiting milk production, then only the addition of that nutrient to the diet will result in a positive milk yield response, e.g. the single-limiting nutrient paradigm. The ideal protein concept loosely aligns with this framework in that it is assumed there is an ideal AA profile that should be provided to an animal and that profile will remain largely fixed as production levels change.

Whereas the observations of Sprengel are well supported, that of von Liebig and the subsequent derivations have not fared as well. The single-limiting AA theory and the ideal protein concept hold true provided the efficiencies of use of absorbed AA remain constant regardless of supply. For example, if the supply of methionine is doubled, the allowable protein production from methionine must also double (and the reciprocal for halving supply). Additionally, doubling the supply and use of methionine should exactly double the need for histidine. If this is true, then one can easily determine which nutrient is most limiting by calculation of the allowable protein yield from each AA based on the composition of the protein and the known efficiencies, and this can be extended to energy and other required inputs. If the result of that calculation indicates that if inadequate histidine is being provided, then one would predict a response to the addition of histidine, and the same for any other nutrient that is apparently deficient.

However, the transfer efficiency of absorbed AA to milk protein is not fixed. Because AA removal from blood is regulated in concert with needs for milk protein synthesis (Bequette et al., 2000), the efficiency of AA transfer from the gut to milk protein is variable. This complicates application of the ideal protein calculations and undermines the concept of a single-limiting nutrient. If there are interactions among nutrients or among nutrients and the environment that affect efficiency, the predictions of which nutrient is first-limiting will be faulty as will predictions of allowable protein production.

Work at the cellular level over the past 30 years has clearly demonstrated that protein synthesis and AA transport in support of such synthesis are highly regulated by the mix of AA available in the cell, the energy status of the cell, and hormonal signals denoting overall energy status in the animal (Bequette et al., 2000, Appuhamy et al., 2009, Appuhamy et al., 2011, Appuhamy et al., 2012). These regulatory mechanisms control protein synthesis, and therefore tie rates of protein synthesis to substrate supply and energy state in the animal. One can think of them as the accelerator pedal in a car. Pressing on the pedal does not, in and of itself, make the car go faster. It controls fuel entry to the engine, and more fuel does make the car go faster. Thus the pedal controls car speed. A shortage of one or more AA causes a reduction in the regulatory system which results in a slowing of the rate of protein synthesis and secretion, i.e. less milk protein. The cells sense the deficiency and respond by increasing the activity of transporters for those AA to minimize the deficiency and limit the response to a very modest decline in protein synthesis.

High insulin concentrations outside of the cell denote adequate energy supply in the animal, which stimulates protein synthesis and consequently stimulates AA transport into the cell. Inadequate supplies of ATP due to a shortage of acetate and other energetic precursors deriving from ruminal fermentation and absorbed nutrients causes a reduction in protein synthesis and in AA transport, although the effects of this latter signal are modest perhaps becoming more potent under severe shortages (Arriola Apelo et al., 2014a, Castro et al., 2016a) (Bequette et al., 2000). The signals arising from each of these factors are integrated in an additive manner via a set of regulatory mechanisms within the cells resulting in the ability to respond to several factors at a time. As a result, the cells have some latitude in adjusting their activity in their attempts to maintain production. Returning to our analogy it is equivalent to multiple gas pedals and the average of them being used to set the rate of fuel delivery to the engine.

Such variation in AA transport and use causes variable efficiency of transfer from absorbed AA to protein production. This undermines the ideal protein concept by creating a range of inputs that can achieve similar efficiency. This also contributes to muted responses to AA as the efficiency declines as the supply increases. The additivity of the responses across factors also is inconsistent with the single-limiting nutrient concept. If provision of more than one nutrient or hormone can partially or completely offset the loss or deficiency of another, there is almost an infinite number of combinations of AA, energy substrates, and hormonal concentrations that will result in the very same amount of milk. This concept is demonstrated *in vitro* by Clark et al. (1978) and (Arriola Apelo et al., 2014b), and *in vivo* by Rius et al. (2010a), Hanigan et al. (2000), Liu et al. (2017), and Yoder et al. (in press, JDS) clearly demonstrating that the response surface is complex and not well represented by the “Law of the Minimum” when applied to lactational responses to AA. Therefore, current protein and AA requirement models for lactation inappropriately represent the underlying biology, which leads to inflated prediction errors and large bias in predicted responses.

Milk Protein Output from Energy and AA Supplies

Work to define the mechanisms controlling mammary AA uptake and subsequent use for milk protein production has progressed considerably over the past 15 years. Fairly robust mechanistic models of mammary metabolism capture the independent and additive effects of key essential AA, energy supply, and insulin (Hanigan et al., 2000, Hanigan et al., 2001, Hanigan et al., 2002, Castro et al., 2016b).

These concepts are at least partially captured if one represents milk protein production as an additive function of several key EAA and energy supply. In such an equation, the partial effect of each EAA and energy can be captured in the model. Evaluation of over 950 treatment means from the literature that included protein infusions, individual AA infusions, and rumen-protected amino acids **(RPAA)** feeding studies resulted in a very robust set of equations that contained 7 EAA plus digestible energy, animal body weight, and digestible NDF. The AA were well defined by the data and the estimates were very stable during cross-evaluation. They included arginine, histidine, isoleucine, leucine, lysine, methionine, and threonine. Phenylalanine estimates were not stable on cross-evaluation likely indicating inadequate, independent observations of responses to that AA. Tryptophan (**Trp**) generally solved for negative responses, but it was unclear if that reflected a true inhibition or if it was an artifact of little independent manipulation of Trp. Valine responses were poorly supported by the data.

A key component to the prediction equation is the inclusion of digestible energy. It is a very strong driver of milk protein output and reflects the linkage between energy and protein supplies to the animal. Milk and body proteins cannot be synthesized at maximal rates if the energy supply to the animal is inadequate. Inclusion of energy in the milk protein equation reflects this and mirrors the inclusion of ruminally digestible carbohydrate in the microbial growth equation.

Evaluation of the new equations shows a near halving of the prediction errors and complete removal of the slope and mean bias present in the prior system. Although milk protein is not directly predicted by the NRC (2001), one can generate a prediction using the observed milk protein concentration from each treatment and the predicted milk volume from the model. Using this approach yielded an error of prediction of 24.9% for MP plus NEL with 18% of the error due to mean bias and 21% due to slope bias. When each of the EAA were also considered using the first-limiting nutrient approach, the overall error became 29.0%, and the mean and slope bias proportions were 46 and 5%, respectively. This contrasts with an error of 13.9% for the new equation based on supplies of the EAA and energy with no mean or slope bias.

Initial testing of this system in a ration balancer environment has shown that given a range of RPAA at prices similar to those available today, diets can be balanced for cows producing 100 lbs of milk per day with less than 14% CP and at costs less than the NRC (2001) MP based costs. We are in the process of setting up an animal study to test these predictions.

Conclusion

Rations can be balanced at levels well below 15% CP, probably even below 13%, if we are able to reliably match AA supply with true animal needs. Current models of AA requirements used in field application programs have significant accuracy and precision problems due to less robust parameter estimates and a post-absorptive system framework that is inconsistent with the known biology. We have addressed both issues resulting in a new system that better represents the biology and exhibits much greater accuracy and precision, allowing us to achieve N efficiencies of 35% or greater in lactating cattle.

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Managing Invasive Range Plants in Beef-Cattle Grazing Systems: The Tale of Sericea Lespedeza (*Lespedeza cuneata*) in Native Tallgrass Prairie[2]

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ABSTRACT: North-American tallgrass prairie provides an array of ecosystem services including carbon sequestration, biodiversity preservation, and forage for grazing livestock. Once covering 68 million ha, only 4% remains today. The largest remnant (~1.5 million ha) lies in the Kansas Flint Hills, home to ~1.3 million yearling cattle and ~90,000 beef cows annually. Unfortunately, the functionality of this ecosystem is threatened by an exotic invader - sericea lespedeza (*Lespedeza cuneata*). Known colloquially as sericea, it is a perennial forb with prodigious capacity to proliferate. Sericea selection by grazing cattle is poor; condensed-tannin concentrations in wild-type sericea can approach 20% of plant DM. Total-tract N digestibility by beef cows consuming sericea-contaminated tallgrass-prairie hay was documented at < 0%; moreover, adaptability of beef cattle to high-tannin diets appears to be limited. Sericea control has been attempted using herbicides. This has not limited proliferation and has resulted in collateral damage to non-target lifeforms. Attempts to naturalize sericea to the ecosystem via enhanced herbivory were evaluated. Supplementation of beef cow diets with tannin-binding feedstuffs resulted in ≥ 29% increases in sericea selection compared with non-supplemented cows. Co-grazing beef cows and goats was associated with > 20% more defoliation of sericea than beef-cow grazing alone. Sequential grazing of yearling steers followed by mature ewes resulted in > 92% defoliation of sericea compared with < 2% in pastures grazed by steers alone. Unfortunately, widespread adoption of these techniques by the ranching community hasn’t occurred because of increased costs or logistical constraints. More recently, prescribed fire as a low-cost means of control was evaluated. Prescribed fires in late summer greatly diminished sericea proliferation compared with prescribed fires in spring (i.e., the traditional prescribed-fire season). No changes in peak forage biomass or C4 grass-species abundance were observed; moreover, native legumes and nectar-producing forbs increased ≥ 2-fold in response to summer fire. Cultural acceptability of prescribed fire in the region is high; significant adoption by the ranching community has been observed.

Key words: condensed tannins, invasive plants, *Lespedeza cuneata*, native tallgrass prairie

INTRODUCTION

Sericea lespedeza (*Lespedeza cuneata* [Dumont] G. Don; hereafter **sericea** or **sericea lespedeza**), a perennial, warm-season, leguminous forb, was introduced into the United States in the late 19th century from the Sino-Indian region of Asia for its soil conservation properties on farmland and mine spoils and perceived value for wildlife habitat (Mosjidis, 1997). The plant was introduced into the American Midwest in the 1930’s to reclaim strip-mined land (Ohlenbusch et al., 2007). In the ensuing decades, sericea propagated, adapted environmentally, and moved westward into the tallgrass

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prairie region of Kansas and Oklahoma (Hobbs and Humphries, 1995). In the lag between introduction of sericea and public awareness of its invasive tendencies, sericea seed was harvested inadvertently from infested rangelands and planted on land enrolled in the U. S. Department of Agriculture Conservation Reserve Program (Eddy et al., 2003). The subsequent and continued spread of sericea in pastoral lands of the tallgrass prairie region is of ongoing concern because it threatens to alter fundamentally one of the most endangered ecosystems on earth (Sampson and Knopf, 2018).

Sericea tends to be a highly-competitive invader in the tallgrass prairie region for a variety of reasons: lack of sufficient grazing pressure, mild allelopathy, canopy dominance, environmental adaptability, and high fecundity. Dietary selection of sericea by beef cattle grazing native rangelands is limited due to its elevated condensed tannin content (Preedy et al., 2013b; Sowers et al., 2019); therefore, control of propagation via grazing is unlikely because pastoral production systems in the tallgrass prairie region are overwhelmingly dominated by beef cattle (USDA, 2017). When condensed tannin levels in cultivated varieties of sericea reached 5 to 12% of plant dry matter, depressed intake, diet digestibility, and animal productivity were documented (Mosjidis, 1997; Aerts et al., 1999). Preedy et al. (2013b) reported that condensed-tannin concentration in *wild-type* sericea ranged from 10 to 19% of whole-plant plant DM during the growing season.

The root system and seed coat of sericea lespedeza have mild allelopathic properties that reduced growth and productivity of both cultivated grasses and native grasses (Logan et al., 1969; Kalburtji and Mosjidis, 1992; Dudley and Fick, 2003; Coykendall and Houseman, 2014). The influence of this allelopathy on forb and shrub growth has not been widely investigated. In addition, sericea tends to produce a robust canopy when mature that prevents sunlight from reaching understory plants (Ohlenbusch et al., 2007; Vermeire et al., 2007; Allred et al., 2010); it can also thrive in shallow and acidic soils that will not support vigorous populations of native plants (Mosjidis, 1997).

Natural characteristics of sericea have led researchers to make generalizations about its environmental adaptability. Ohlenbusch et al. (2007) indicated that herbivore avoidance of individual plants with naturally greater condensed-tannin concentrations may have led to selection for elevated condensed-tannin levels in wild-type sericea. It produces both self-fertilizing (i.e., cleistogamous) flowers and flowers that require cross-pollination (i.e., chasmogamous). The latter allow sexual recombination of genetic material between diverse parent plants and are a possible reason why sericea has adapted so quickly to the tallgrass prairie region (Donnelly, 1979; Mosjidis, 1997).

Finally, sericea is a prolific seed producer (Ohlenbusch et al., 2007). Lemmon et al. (2017) indicated that single stems produced an average of 864 seeds annually over a 4-yr period. Notably, non-scarified sericea seed may remain viable in the soil for many yr, leading to prolonged germination (Cummings et al., 2006).

To date, sericea has degraded more than 2,500 km^2 of native tallgrass prairie (KDA, 2016). In pastoral beef production systems of the region, this degradation has manifested itself as diminished carrying capacity (Preedy et al., 2013b; Sowers et al., 2019); however, detrimental effects of invasion on grassland fauna have also been documented. Eddy and Moore (1998) observed decreased invertebrate diversity in sericea-invaded tallgrass prairie. Similarly, Brooke et al. (2016) reported disproportionate placement of nests by Northern bobwhite (*Colinus virginianus*) in areas relatively free of sericea, whereas Ogden (2016) documented habitat degradation for certain upland-nesting songbirds and grassland-obligate pollinators in areas moderately infested with sericea.

The role of fire in sericea lespedeza ecology has received only limited attention to date. Fire likely plays a critical role in scarification and subsequent germination of its seed. Herranz et al. (1998) indicated that dry or moist heat effectively scarified seeds of 7 leguminosae species. Subsequently, Vermeire et al. (2007) and Wong et al. (2012) indicated that prescribed fires common to the tallgrass-prairie region appeared to stimulate sericea germination; however, the season in which prescribed fire was applied may have influenced seedling survival. Cummings et al. (2007) reported that application of growing-season prescribed fire at 3-yr intervals decreased the rate of invasion in Oklahoma tallgrass prairie compared with application of dormant-season (i.e., spring) prescribed fire at 3-yr intervals.

The objectives of this manuscript are three-fold: 1) to provide a short overview of condensed-tannin effects on ruminal fermentation; 2) to provide a review of proposed control methods for sericea lespedeza in the tallgrass prairie region of North America; and 3) to make some concluding generalizations about approaches to controlling invasive range plants.

DISCUSSION

Condensed tannins and ruminal fermentation. Condensed tannins are flavonoid polymers with a high binding affinity for proteins (Waghorn, 2008). They are found in a wide variety of plants and are a biodefense mechanism against plant diseases, environmental stress, and herbivory (Min et al., 2003). Condensed tannins limit voluntary intake, diet digestibility, and ruminal protein degradation by ruminants due to formation of tannin-protein complexes *in vivo* (Makkar, 2003). Tannin-protein complexes form during mastication as condensed tannins are released from macerated plant cells (Min et al., 2003). These complexes are stable under ruminal conditions, rendering proteins non-degradable by microbial enzymes (Al-Dobaib, 2009; Hassanat and Benchaar, 2012). As a result, ruminal VFA production is sharply curtailed (Hoehn et al., 2018) and microbial growth rates therein are suppressed (Min et al., 2005).

Condensed tannins in forage crops are a strong deterrent to consumption by beef cattle (Eckerle et al. 2011a; Preedy et al., 2013b). This is likely related to a dearth of ruminally-available N and a corresponding decrease in microbial cell protein production. Non-supplemented beef cows fed a high-tannin forage had total-tract N digestibilities that were < 0% (Eckerle et al., 2011b). Prior exposure to dietary tannins did not meaningfully improve ruminal fermentation parameters (Hoehn et al., 2018). Small ruminants appear to have greater tolerance for condensed tannins than cattle (Pacheco et al., 2012; Lemmon et al., 2017); however, reasons for interspecies differences in tannin tolerance have not been fully elucidated (Robbins et al., 1991; McKiernan, 2015).

Wild-type sericea lespedeza is among the most tannin-dense herbaceous plants globally. Its condensed-tannin concentrations are reported to range from 10 to 25% of whole-plant plant DM during the growing season (Eckerle et al., 2010; Preedy et al., 2013b). Avoidance of wild-type sericea lespedeza by confined beef cattle consuming tallgrass prairie hay (Eckerle et al., 2011a and 2011b), by grazing beef cows (Preedy et al., 2013a and 2013b), and by grazing yearling beef steers (Sowers et al., 2019) has been reported. Eckerle et al. (2011b) reported that beef cows likely developed a flavor-related aversion to sericea lespedeza before a general ruminal malaise occurred. Condensed tannins are astringent and may be perceived by some herbivores as having a bitter flavor (Provenza et al., 1990; Hagerman et al., 1992). Grazing herbivores may learn to avoid sericea lespedeza because of the astringent flavor associated with condensed tannins rather than because of any detrimental effects of condensed tannins on ruminal N metabolism.

Herbicides. Early efforts to control sericea lespedeza were focused on specialty herbicides. Altom and Stritzke (1992) evaluated the efficacy of several post-emergent herbicides applied from mid-May through early June including: triclopyr, picloram, 2,4-D, metsulfuron methyl, dicamba, clopyralid, and fluroxypyr. One yr following treatment, sericea stem density in triclopyr- and fluroxypyr-treated plots ranged from 0 to 21% of non-treated control plots, whereas metsulfuron methyl-treated plots had sericea stem densities that ranged from 0 to 67% of non-treated controls. Other herbicides did not provide satisfactory control.

Koger et al. (2002) later determined the timing of herbicide application influenced sericea lespedeza survival. These researchers applied triclopyr or metsulfuron methyl at 3 different sericea growth stages including simple-stem (mid-June), branched-stem (mid to late July), or flowering (mid to late September). When triclopyr was applied at the simple-stem or branched-stem developmental stages, it routinely reduced sericea to less than 20% of pre-treatment stem density. Conversely, triclopyr was much less reliable when applied at the flowering stage of sericea development. Metsulfuron methyl was ineffective for sericea control when applied at the simple-stem stage of development; however, it proved to be a viable option for control when applied during the flowering stage. Following publication of this work, researchers and management agencies began to recommend what has since become the accepted paradigm for sericea control: apply triclopyr during the vegetative phase of sericea development and apply metsulfuron methyl during the reproductive phase (Ohlenbusch et al., 2007; Vermeire et al., 2007; Farris and Murray, 2009).

Subsequent reports touted success in controlling sericea lespedeza with specialty herbicides (Jordan et al., 2002; Emry, 2008); however, eradication was never realized. Eddy et al. (2003) reported that acreage in Kansas effected by sericea invasion increased 60-fold between 1988 and 2000, in spite of widespread regional herbicide usage during that period. Given the profound fecundity of sericea (Lemmon et al., 2017) and its sustained ability to sprout from the seed bank (Woods et al., 2009) and from existing plant crowns, herbicide treatments do not appear to be an effective means of long-term control.

Perhaps a more important issue ecologically is that attempts to control invasive forbs with herbicides have historically resulted in collateral damage to non-target native forbs. Use of herbicides in native grasslands for the control of broom snakeweed (McDaniel et al., 2000), leafy spurge (Rinella et al., 2009), smooth sumac (Tunnell et al., 2006), and sericea lespedeza (Gatson, 2018) reduced richness and evenness of native forbs. The severity of depressive effects was dependent on the invasive species, the herbicide, and the ecosystem in question. Therefore, the dearth of research on the effects of herbicides on non-target organisms should serve as impetus for further investigation.

Predation. Contemporary with the development of herbicide strategies, an attempt was also made to achieve biological control of sericea lespedeza using the lespedeza webworm (*Tetralopha scortealis* Lederer). The lespedeza webworm is the larval form of a moth native to the southeastern U.S. It forms a dense, silk-like web around sericea which limits photosynthetic activity. The larvae then defoliate the plant (Poos and Hetrick, 1945). Eddy et al. (2003) released lespedeza webworm at various locations in southeast Kansas and measured subsequent effects on sericea stands. Initially, webworm infestations decreased the average number of seeds produced per sericea stem from 644 seeds/plant to less than 6 seeds/plant. Unfortunately, webworms were unable to survive in dry conditions or through the winter. These authors concluded that lespedeza webworms were unlikely to play an important role in sericea control in the tallgrass prairie due to a lack of environmental fitness.

Herbivory. Schutzenhofer and Knight (2007) explored the theoretical possibility of controlling sericea invasions via herbivory. Sericea lespedeza plants of varying sizes were manually defoliated early in the growing season to simulate natural herbivory. Unfortunately, the population growth of sericea remained quite high, even when subjected to as much as 80% defoliation early in the growing season. This finding somewhat limited hopes that the rate of sericea invasion might be diminished if wild or domestic herbivores readily consumed the plant. Nevertheless, it remained plausible that more extensive, repeated, or temporally-targeted herbivory might contribute to a reduction in the abundance and vigor of the plant.

Condensed tannins in sericea lespedeza reduce its acceptability by beef cattle (Cope and Burns, 1971) and inhibit voluntary intake (Wilkins et al., 1953). In spite of these obstacles, there have been attempts to increase sericea consumption by grazing beef cattle. Mantz et al. (2009) demonstrated that feeding confined beef steers polyethylene glycol, a tannin-binding compound (Jones and Mangan, 1977), increased voluntary intake of freshly-cut sericea. Citing cost and regulatory restriction on the use of polyethylene glycol as a feedstuff, Eckerle et al. (2011b and 2011c) evaluated the value of corn steep liquor for the same purpose. They reported that it was likewise effective at increasing voluntary intake of sericea-contaminated prairie hay by beef cattle fed in confinement.

Recognizing the potential for controlling sericea lespedeza in addition to improving animal performance, Preedy et al. (2013a; 2013b) extended this line of research by measuring the effect of corn steep liquor supplementation on dietary selection of sericea by cattle grazing native tallgrass-prairie pastures. They reported increased voluntary intake of sericea during the months of August and September by beef cows supplemented with corn steep liquor compared with non-supplemented cows. Importantly, that time period corresponded with flowering and seed production by the plant. These authors speculated that the seed production would be reduced by increasing herbivory on the plant during that specific phenological stage.

The important work of Preedy et al. (2013a; 2013b) notwithstanding, evidence for slowing invasion and reducing existing infestations of sericea lespedeza through beef-cattle grazing alone remains limited. Although grazing of sericea by non-supplemented cattle is inhibited by high condensed tannin content (Wilkins et al., 1953; Cope and Burns, 1971), this may not be an impediment for domesticated small ruminants (Robbins et al., 1991; Hart, 2001). Accordingly, focused grazing with sheep or goats for inhibiting sericea proliferation has been investigated.

Early research by Hart (2000; 2001) reported that grazing juvenile goats at a stocking rate of 10 goats/ha for 120 d for several consecutive years significantly suppressed sericea growth and seed production. The author indicated that goat grazing was an effective, sustainable, and income-generating method of rangeland improvement but opined that widespread adoption by beef producers of the region was unlikely. Cultural biases were cited as the main reasons for producer reluctance to adopt a small-ruminant grazing enterprise independent of a beef-cattle grazing enterprise (Hart, 2001). Subsequently, Pacheco et al. (2012) studied the effects of co-grazing goats and beef cows on herbivory of sericea. Livestock were grazed on sericea-infested native tallgrass pastures from June through October. Equivalent stocking rate complements of both species (i.e., 8 ha/AUM for cattle and 8 ha/AUE for goats) were evaluated. At the conclusion of the study period, a greater proportion of sericea stems in co-species pastures showed evidence of herbivory than in cattle-only pastures. Final biomass of sericea entering the dormant season, however, was not different between treatments. This may have been an indication that, although goats increased grazing pressure on sericea, the stocking rate of goats must be very high before the total biomass of sericea in a heavily-infested pastures could be meaningfully reduced.

The most popular grazing management practice in the Flint Hills of Kansas involves annual spring burning followed by intensive grazing with yearling beef cattle from April to August (Owensby et al., 2008). Rangelands are then rested for the balance of the growing season. Accordingly, Lemmon et al. (2017) evaluated a leader-follower grazing system in which sheep were grazed subsequent to yearling cattle. In a 4-yr experiment, yearling cattle were grazed on eight 31-ha pastures from mid-April to mid-July at a rate of 235 kg of live weight/ha. Mature ewes were then grazed on half of the pastures for 60 d, while remaining pastures were rested until the following spring. Ewes were stocked at a rate of 325 kg of live weight/ha.

When compared to pastures grazed by yearling cattle only, pastures with added late-season grazing by sheep had substantially increased percentages of sericea plants showing evidence of herbivory (92.1 vs. 1.4%) at the end of the sheep-grazing period (Lemmon et al., 2017). Whole-plant mass of sericea stems entering the dormant season (1,443 vs. 4,424 mg/plant; DM basis) and sericea seed production (114 vs. 864 seeds/plant) were greatly reduced. The authors interpreted these results to indicate that late-season sheep grazing decreased the vigor of existing sericea plants at a phenologically-critical time for the plant and limited the proliferation of new plants via seed. At the conclusion of the study, basal frequency of sericea in pastures grazed by both cattle and sheep was less than that in pastures grazed by yearling cattle alone. Year-end residual biomass was 904 kg DM/ha less on pastures grazed using the leader-follower system compared with those grazed only by beef cattle; however, authors indicated that sufficient biomass was present on all pastures to prevent soil-moisture loss and to allow prescribed burning the subsequent spring.

In spite of substantial evidence to support the soundness of the approach, adoption of small-ruminant grazing enterprises in the tallgrass prairie region for the purpose of sericea lespedeza control has been modest. Cultural biases cited by Hart (2001) may explain part of the regional reluctance to use small ruminants to control sericea; however, other legitimate challenges exist. Kansas has fewer than 124,000 small ruminants (USDA, 2017) and approximately 250,000 ha of native tallgrass prairie that has been degraded by sericea lespedeza (KDA, 2016). Considering the small-ruminant stocking rates suggested by Hart (2001), Pacheco et al. (2012), and Lemmon et al. (2017), 1.25 to 2.5 million small ruminants would be required for adequate sericea lespedeza control through targeted grazing. Adding to this logistical difficulty are issues related to limited market access for small-ruminant products, increased fencing costs compared with beef cattle, and losses due to predation.

Prescribed Fire. The role of fire in sericea lespedeza ecology has received only limited attention to date. Fire likely plays a critical role in scarification and subsequent germination of its seed (Herranz et al., 1998; Vermeire et al., 2007). Traditional spring-season burning of the tallgrass prairie has not resulted in control of sericea and may, in fact, have exacerbated the problem (Cummings et al., 2007; Ohlenbusch et al., 2007). Although burning during the spring may serve to promote invasion, burning during more sensitive times in the life cycle of sericea may have an inhibitory effect. Two recent studies conducted in the tallgrass prairie region have explored this possibility.

Wong et al. (2012) conducted 2 field experiments designed to evaluate the effects of fire timing on sericea lespedeza seed germination and seedling survival. The results of their first field experiment indicated that burning in early November after seed dispersal had occurred decreased the rate of sericea establishment. The authors attributed this result to a direct reduction in the viability of seed exposed to a November fire. In the second experiment, 90 individual 1-m^2 plots were established and sowed with sericea seed in late March. Fifteen individual plots were then burned on 1 of 6 different dates: 21 April, 25 May, 21 June, 21 July, and 4 September of year 1 and 21 April of year 2. Independent of prescribed-fire timing, burning was a strong stimulator of sericea seed germination. This effect may be responsible in part for the rapid increase of sericea in tallgrass prairie pastures that are burned annually in early spring. Seedling survival was much greater in plots burned early in the growing season than those burned in the summer. Although the late-summer burns also stimulated germination of sericea seeds, few late-germinating seedlings survived to the second growing season.

Alexander et al. (2017) took the next steps in applying these observations to the control of sericea lespedeza on a larger scale. Their trials were conducted on a native tallgrass prairie in the northern Flint Hills with a moderate to heavy infestation of sericea. This research was conducted on 9 paddocks (~ 5 ha) that were burned annually for 4 consecutive yr in either early April (i.e., the traditional burning season in the region), early August, or early September. Paddocks burned in early August or early September had precipitous declines in sericea seed production and whole-plant mass at dormancy when compared with paddocks burned in April. This suggested the possibility that late-summer burning could substantially curb the reproductive capabilities of the plant. More importantly, the basal frequency and total biomass of sericea at the conclusion of the experiment were an order of magnitude less in areas treated with August or September fire compared to those treated with April fire (Alexander, 2018). This may be an indication that late-summer fires increased the mortality of mature sericea lespedeza crowns in addition to inhibiting seed-based and vegetative reproduction.

Cultural acceptability of prescribed fire in the tallgrass prairie ecoregion is high; moreover, costs of prescribed fire application are relatively small compared with herbicide-based approaches to sericea lespedeza control (Olson, 2019). As a result of the work of Alexander et al. (2017) and Alexander (2018), there have been noticeable changes in prescribed fire use among tallgrass-prairie land managers, as well as meaningful changes to regional and national conservation policies to promote use of summer-season prescribed fire for sericea lespedeza control. Users of the technique report that summer-season prescribed burns are less complicated to control than comparable early-spring prescribed fires. They also report that summer-season burns are more compatible with ranch labor availability and coincide with more favorable atmospheric conditions for prescribed fire compared with spring-season burns. Significant adoption of the technique by the ranching community has been observed.

Conclusions. The search for a means to naturalize wild-type sericea lespedeza to the tallgrass prairie ecoregion is 4 decades old. Herbicides, insect predation, herbivory by domestic ruminants, and prescribed fire have all shown promise as methods of control and, thus, naturalization. Interestingly, each of these potential control mechanisms was linked to one or more specific life-cycle characteristics of sericea, making temporal aspects of treatment application critical to treatment efficacy. In effect, control strategies were developed based on a gradual growth in the understanding of the basic biology of wild-type sericea lespedeza.

Although several sericea-lespedeza control techniques have been successful from a scientific and statistical viewpoint, not all produce a result that is satisfying from a sensory perspective. Whether or not the *applied-science* community is willing to admit it, we are in the business of attempting to change human behavior. Statistical tests can be a critical component of behavior change; however, long-term adoption of new management practices may rest with users' ability to perceive, visually or otherwise, improvements in ecosystem condition within a short time of making a change in practice. As with other invasive organisms, no single approach is likely to provide user-defined satisfactory control of sericea lespedeza. Multi-faceted control strategies are desirable not only to develop comprehensive and satisfying control but, perhaps more importantly, also to encompass an inclusive range of land-management goals.

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Creating rangeland resiliency for stable ecosystems and healthy ranches

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"Do unto those downstream as you would have those upstream do unto you."
– Wendell Berry

Sustainable agriculture has nearly lost its meaning. And perhaps it never quite meant what the word's etymology suggests. "Sustainable" implies continuing, maintaining that which is. Nathan Sayre has shown that the term sustainability may be a tautology, circular reasoning: that which continues is sustainable (Sayre 2005). If what is is healthy, all is well. If the status quo is not good, sustaining it is unhelpful. What most people mean by sustainable agriculture is agriculture that does not rely on non-renewable natural resources, that incorporates regenerative ecological processes. In either term, we must be concerned with identifying and defining that which we are sustaining or generating again.

Value-laden terms like "resiliency", "stable", and "healthy" have natural appeal. But stable doesn't necessarily mean steady-state. And healthy doesn't necessarily mean maximum profit. Current definitions of resiliency include social elements as well as ecological and economic (Sayre et al. 2013; Bestelmeyer and Briske 2012). Restoration science has often treated humans as only the problem. Agriculturalists have rightly reacted to restoration-minded folk who advocate re-wilding, who insist upon returning to an imagined pre-Columbian ecological nirvana that perhaps never existed, or only appeared utopian because the post-Columbus observations were of ecosystems already out of equilibrium because European diseases had recently decimated the indigenous humans; wildlife populations had spiked temporarily as a result of dramatically decreased harvest pressure (Mann 2005). Mounting evidence indicates North American landscapes were anthropogenic well before Europeans arrived. Restoration efforts based on the belief that removing humans from the landscape will automatically cause degraded ecosystems to spring back to a primitive pristine state have no scientific foundation and cause significant human suffering. True resiliency in animal agriculture, especially ranching on natural plant communities, must, by definition, include people in the regeneration equation.

Over the last century, our scientific understanding of semi-arid ecosystems has changed much; changes in understanding have led to changes in management paradigms which support regenerative livestock grazing. As ecological models changed from the steady state or equilibrium model to ecosystem thinking to the non-equilibrium or state-and-transition model, management goals shifted from commodity production (on rangeland) to ecosystem health to socio-ecological resilience. The steady-state model held that ecosystems "want" to return to a climax state, and that left alone they will do just that, like a spring. Under that model, one could define the health of a given ecosystem by its degree of similarity to the climax plant community for a given site, and it was typically assumed that late successional ecological states/plant communities were ideal for the production of the most desired commodity--forage. Rangeland management focused on creating homogeneity through herbicides, planting, fencing, water provisioning, brush control, and prescribed fire to eliminate brush. Disturbances such as fire, flood, drought, and grazing were seen as negative influences and it was generally believed that reducing their frequency or severity or spatial extent would either maintain the proposed climax state or speed return to climax following retrogression (caused by disturbance). Growing recognition that this model didn't reflect reality for nearly all of the semi-arid Western United States, where variability was a more significant ecological driver than aridity, led to thinking in terms of ecosystem services and more recently toward the ecological patterns and processes that support multiple ecosystem services (Fuhlendorf et al. 2012). We will discuss resiliency and rangeland health concepts before relating this to animal nutrition and grazing management.

Resilience has been defined as "the capacity of an system to absorb disturbance and reorganize while undergoing change so as to retain essentially the same function, structure, identity, and feedbacks" (Walker et al. 2004). For those managing land for maximum resiliency, this involves creating or maintaining desirable ecological states and avoiding tipping over a threshold to a less desirable but potentially stable degraded state (Elmqvist et al. 2003). For example, once a sagebrush-bunchgrass plant community has shifted to sagebrush, invasive annual grass, and invasive forbs, removing the persistent excessive disturbances that drove it there will not permit the site to spring back to the desired stable state. It has tipped over a threshold into a new stable state. A resilient system is able to regain a stable state after significant disturbance and is able to resist the loss of a stable state in response to disturbance. The concept "emphasizes the properties of entire socio-ecological systems, rather than the persistence of particular ecological states linked to historical conditions. The term 'resilience' in resilience-based management pertains to societal well-being . . ." (Bestelmeyer and Briske 2012).

Attributes of these ecological models have been summarized by Bestelmeyer and Briske in this table from their 2012 article "Grand Challenges for Resilience-Based Management of Rangelands":

Table 2. Seven distinguishing attributes of steady state, ecosystem, and resilience-based management models (modified from Chapin et al. 2009).

	Steady state management	Ecosystem management	Resilience-based management
Ecological models	Succession–retrogression	State-and-transition, rangeland health	Multiple social-ecological systems/ novel ecosystems
Reference condition	Historic climax plant community	Historic climax plant community, including historical range of variation	Landscapes with maximum options for ecosystem services
Role of humans	Use ecosystems	Part of ecosystems	Direct trajectories of ecosystem change
Ecosystem services	Meat and fiber products	Several ecosystem services	Options for diverse ecosystem services
Management goals	Sustain maximum yield of commodities	Sustain multiple uses	Sustain capacity of social-ecological systems to support human well-being
Science-management linkages	Top-down from management agencies	Top-down from management agencies	Multiscaled social learning institutions
Knowledge systems	Management experience and agricultural experiments	Multidisciplinary science and ecological experiments	Collaborative groups, spatially referenced, updatable database systems

What makes management on rangelands so complex is that rangelands are unusually productive in terms of provisioning services and all are highly valuable. Rangelands are a unique setting that links agriculture and naturally-occurring plant and animal communities. Managers of rangelands recognize the need to accommodate provisioning services such as meat and fiber, regulating services such as soil carbon (which regulates climate) and pollination, cultural services such as recreation and cultural heritage, and ecological support services like plant production, water cycling, nutrient cycling, etc. (Havstad et al. 2007). Recent scholarship has shown that managing for heterogeneity accomplishes all of these and supports profitable ranching; successful ranches are a key component of a resilient socio-ecological system.

There is wide recognition of nearly universal indicators of rangeland health based on the non-equilibrium ecological model (Herrick et al. 2019; Pellant 2000). The 17 indicators described in the interagency technical reference “Interpreting Indicators of Rangeland Health” describe the relative health of three categories of indicators: soil and site stability, hydrologic function (how well a site receives and stores water), and biotic integrity (the health of the plant community). These indicators rank the resiliency of a rangeland site relative to its own potential to maintain the ecological processes of water cycling, energy flow, and nutrient cycling rather than the current botanical similarity to a believed historic plant community or other reference condition.

We now discuss specific links between heterogeneity and ranch profitability. Sam Fuhlendorf argues in a seminal 2012 paper titled “Conservation of pattern and process: developing an alternative paradigm of rangeland management” that livestock production is one service that results from health rangelands, but that it cannot be the driver of all management decisions. Broader ecosystem characteristics such as biodiversity support ranch profitability, however, in several key ways (Fuhlendorf et al. 2012).

Net primary production (NPP) is an important indicator of rangeland health. NPP includes all plants in an ecological site, not just primary forage plants. Botanically diverse rangelands tend to be more productive than depauperate rangelands dominated by invasive annual grasses and shrubs. Diversity is maintained, in part, by not attempting grazing uniformity across all ecological sites, uniformity which promotes a particular suite of plant species tolerant of that particular defoliation timing, frequency, and severity. A mosaic of plant community types and successional stages is promoted through diversity in grazing use patterns. Different plant functional groups and species within functional groups utilize the soil profile differently through a variety of rooting structures, depths, and symbiotic relationships with soil organisms like bacteria and fungi.

Mature research also has shown that plants contain an array of secondary compounds as well as familiar primary compounds such as protein and carbohydrates. These secondary compounds can be anti-quality factors that reduce consumption but many are also beneficial at low concentrations and are sought out by wild and domesticated animals (Provenza 2008; Provenza et al. 2007; Provenza and Villalba 2010). Animals consuming a wide variety of plant species are healthier and require less medical treatment (Provenza et al. 2007). The grazing patterns that promote diverse plant communities permit animals to be selective about what they consume. Research consistently shows that animals consume plants and plant parts that meet their nutritional needs, optimizing animal gain, body condition, and per head profitability. High functional group diversity and species diversity maintains ecological resilience—when disturbance such as drought or variation in precipitation timing occurs different species are expressed in that year. For example, with warmer, drier spring conditions needle-and-thread (Stipa comata) may be abundant rather than Columbia needlegrass.

Plant communities boasting a wide variety of species but dominated by perennials exhibit a broader growth curve because perennials have deeper root systems, facilitating access to soil moisture later into the hot, dry season in the Pacific Northwest. Perennial grasses are important because they are abundant, they stabilize soils, they are often more competitive against invasive exotic plants than shrubs, and they serve vital ecosystem functions (Chambers et al. 2014; Germino, Fisk, and Applestein 2019; Chambers et al. 2016). This has multiple benefits: reduced fire risk, higher forage production, higher forage quality in summer and into fall, resistance to invasive annuals.

Conserving naturally diverse patterns and processes on rangelands requires rethinking grazing management. Fuhlendorf recommends several principles that promote landscape-scale rangeland health:

1. Maintain large continuous tracts of rangelands so that disturbance processes can interact with complex plant communities on a variety of spatial and temporal scales.
2. No single grazing intensity is the right one. A variety of grazing intensities are actually important to conservation of biodiversity.

3. Uniformity of grazing distribution is not the goal of range management. It's too expensive, decreases biodiversity, and inhibits the creation of a mosaic of ecological conditions.
4. "Shifting mosaics are necessary for maintaining ecosystem structure and function and achieving multiple objectives."
5. Rangeland conservation should consider all species of animals and plants.
6. Disturbance regimes (fire, grazing) are vital to ecosystem structure and function. These processes interact with soils and climate to produce biodiversity.

Recent scholarship reinforces the not-so-new idea that adaptive management, including flexible stocking decisions, is the key to grazing management that supports rangeland health and the production of ecological goods and services. A 2018 paper by an all-star researcher team which set out to determine what management strategies on commercial ranches were associated with high rangeland health long-term, i.e., in places where those strategies had been in place for a long time and the ranch's environment would reflect accurately the efficacy of the approach. They found that flexibility, adaptive learning, and aiming for long-term goals were more highly correlated with diverse plant species composition and healthy, diverse wildlife populations than specific grazing management techniques (Wilmer et al. 2018). This flexibility is echoed by others who maintain that stocking rate is still the most influential grazing decision but that there is not a single stocking rate that is "proper".

The future of public lands grazing is with ranchers who are able to manage for non-livestock production goals such as wildlife habitat, clean water, open space, biodiversity. Not coincidentally, these factors also promote ranch profitability and social acceptance. Ranchers stand in better stead with the American public and global citizens than they think (Paul F. Starrs 1998; Brunson and Huntsinger 2008). And the old ways may be the new ways, if large landscapes and cost barriers to extensive infrastructure like miles upon miles of fence lead us back to herding and more direct supervision of grazing herds and flocks of domestic livestock (P. F. Starrs 2018). There is renewed cultural and scientific interest in the merits of herding and transhumance, merits which include animal health, land health, attachment to place, local agricultural economies, and more. Either way, increased understanding of ecological patterns will benefit stock-raising. Producing food and fiber on naturally occurring plant communities where we can also have wildlife habitat, open space, clean air and water is a very good human endeavor and we should pursue doing it well.

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Challenges and opportunities in nutritional management of robotic milking herds

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Introduction

With the rapid adoption of automated (robotic) milking systems (AMS), we have experienced a fundamental shift in nutritional management, with the division of the ration into a partial mixed ration (PMR) and the AMS pellet. In addition, the nature of the PMR, allocation of the PMR, type of pellet, and feeding strategy of the pellet delivered in the AMS differ. The large diversity coupled limited controlled research regarding feeding management have led to recommendations being largely based on survey studies or based on anecdotal data from single-farm case studies. However, research on feeding management strategies for cows managed in AMS has increased, and this paper will describe the current state of knowledge along with areas where research is needed.

Does Cow-traffic Design Influence the Feeding Management Approach?

There are two main goals when considering the nutritional program for cows milked with AMS. The first, as with all planned nutritional programs, is to provide a diet that meets nutrient requirements for maintenance and production. However, with AMS, there is a perception that this goal can be shifted from the pen level to the cow level. Thus, producers could be providing a different diet for each cow within the same pen by adjusting the amount of pellet provided in the AMS. The second goal, which is unique to AMS, is to stimulate cows to voluntarily enter the AMS by dispensing pellet in the AMS. A disproportionately large focus has been placed on the AMS pellet, considering that the PMR provides the majority of the dry matter and nutrients consumed. For example, assuming a static dry matter intake (DMI) of 28 kg, the PMR could be estimated to contribute between 89 and 71% of the total dietary dry matter for cows offered three and eight kg of pellet in the AMS (dry matter basis), respectively.

Current survey data suggest that producers with free-flow traffic barns program greater AMS pellet allocations than those with guided-flow traffic barns (Salfer and Endres, 2018). Feeding greater quantities of pellet in the AMS, by default, also indicates the PMR will be less nutrient dense. While this may not be considered to be a problem, recent research has demonstrated that feeding a PMR with a greater proportion of forage increases the ability of cattle to sort that PMR (Menajovsky et al., 2018; Paddick et al., 2019). Providing more pellet in the AMS with free-flow barns is typically done because cows can choose when, and if, they voluntarily enter the AMS, whereas with guided flow barns, cows are ultimately directed to the commitment pen and the AMS using automated sorting gates. While the survey data indicate that producers with free-flow barns provide more pellet in the AMS, it is not known whether those cows consume more AMS pellet because the amount actually delivered and the amount consumed are not reported. The difference between the computer programmed value, amount delivered, and amount consumed for the AMS pellet is of major importance. Moreover, survey-based studies have neglected to evaluate PMR composition and do not have the ability to evaluate PMR intake at a cow level (Bently et al., 2013; Tremblay et al., 2016;

Salfer and Endres, 2018). Thus, caution should be applied when considering survey-based data as a means to evaluate potential recommended feeding strategies.

Salfer and Endres (2018) reported that the upper limit for pellet allocation in AMS (computer programmed value) in their survey was 11.3 kg /cow/day. Assuming cows could consume 11.3 kg/day, each cow would need to consume over 2.8 kg/milking (assuming 4 milkings/day) equal to 350 to 400 g/minute if milking duration was between seven and eight minutes. This high rate of pellet feeding may outpace the ability of cows to consume pellet while milking, and likely would result in a significant quantity of pellet that is either not delivered to the cow (Penner et al., 2017) or delivered in the AMS but not consumed by the cow (Bach and Cabrera, 2017).

Unfortunately, there is a lack of data evaluating whether traffic flow truly affects the amount of pellet required to be offered in the AMS. A study conducted in a feed-first, guided-flow barn reported no effect on voluntary attendance or milk yield when the amount of pellet delivered varied from 0.5 to 5.0 kg of DM/day (Paddick et al., 2019), whereas similar treatments in a free-flow barn resulted in more frequent voluntary milkings (Schwanke et al., 2019). It would be nice to conclude that these data provide support for allocating greater quantities of AMS pellet under free-flow systems; however, the AMS pellet composition, PMR composition, total DMI, and days in milk also differed between the two studies thereby preventing a direct comparison. Moreover, Bach et al. (2007) reported that the amount of pellet provided in a free-flow system did not affect voluntary attendance or milk yield. As a result, studies should not be interpreted to indicate the absolute amount of pellet provided because the amount likely differs on a farm-to-farm basis.

Does Increasing the AMS Pellet Allocation Increase Voluntary Attendance and Milk Yield?

One of the most common claims with AMS feeding strategies is that increasing the amount of pellet delivered in the AMS will stimulate voluntary attendance and milk yield. The approaches used to increase the AMS pellet allocation should be considered because there are two very different nutritional strategies. First, producers need to decide how much pellet is required from a basal level and this basal amount must consider the formulation of the PMR. Previous studies have been conducted to evaluate how the amount of pellet offered in AMS affects production responses when the total dietary nutrient supply is equivalent. In other words, with this strategy, increasing the amount of pellet provided in the AMS requires an equal reduction in the amount of pellet in the PMR such that the total diet (PMR + AMS) does not differ. The first study published using this nutritional strategy compared treatments with computer programmed values of three or eight kg of pellet in the AMS in a free-flow barn design (Bach et al., 2007). In that study, despite having programmed values of 3 and 8 kg/day, pellet delivery was 2.6 and 6.8 kg/day (dry matter basis) and the amount of pellet delivered did not affect milk production or milk component production. In two recent studies conducted in a feed first guided-flow barn at the University of Saskatchewan, AMS pellet delivery ranged between 0.5 and 5.0 kg of dry matter/cow/day (Hare et al., 2018; Paddick et al., 2019). Altering the amount of AMS pellet while maintaining equal dietary nutrient composition did not affect voluntary visits, milk yield or milk component yield. In contrast, a recent study conducted at the University of Guelph in a free-flow barn reported that with total diets (PMR + AMS pellet) that were the same in nutrient composition, increasing the AMS pellet from 3 to 6 kg/day (and correspondingly reducing the same pellet in the PMR), stimulated greater DMI, increased voluntary visits by 0.5 milkings/day, and numerically increased milk yield by 1.5 kg/day (Schwanke et al., 2019).

It might seem counter-intuitive that increasing the AMS pellet allocation does not necessarily stimulate voluntary visits or milk yield. However, simply providing more pellet in the AMS does not necessarily translate to greater DMI. For example, Hare et al. (2018) reported that for every 1 kg increase in AMS pellet delivered, there was a corresponding decrease in PMR DMI of 1.58 kg. Bach et al. (2007) reported a 1.14 kg reduction in PMR DMI and Paddick et al. (2019) reported that PMR DMI decreased by 0.97 kg for every one kg increase in AMS pellet delivered. The large or at least equal reduction in PMR DMI with increasing AMS pellet intake demonstrates that nutrient intake may not be positively affected. These effects of greater concentrate consumption in the AMS and subsequent PMR substitution rate may also vary due to the energy density of the PMR; Menajovsky et al. (2018) reported a 0.78 and 0.89 kg/d reduction of PMR for every 1 kg of concentrate, depending on PMR energy density (low or high). In contrast, Schwanke et al. (2019) reported that for every 1 kg increase in AMS pellet intake there was only a 0.63 kg reduction in PMR DMI (Table 1). In that that case, providing more pellet in the AMS resulted in greater total DMI and likely explains their numerical improvement in milk yield. The variable and currently unpredictable substitution rate may challenge the ability to formulate diets for individual cows in the same pen given that only the amount or types of pellet in the AMS can differ.

Table 1. Effect of increasing pellet in the automated milking system (AMS) on the reduction in PMR intake (DM basis).

Study	DIM (mean ± SD)	Cows, parity, and study design	Traffic and diet, dietary scenario	Substitution ratio, kg PMR/kg AMS concentrate
Bach et al., 2007	191 ± 2.13	69 primiparous Holstein, 46 multiparous Holstein Completely randomized design	Free Isocaloric	1.14
Hare et al., 2018	227 ± 25 123 ± 71	5 multiparous Holstein 3 primiparous Holstein	Guided Isocaloric	1.58
Henriksen et al., 2018	32-320 14-330	22 primiparous Holstein, 19 multiparous Holstein 11-week study	Free Static PMR with 2 concentrate	0.58 – 0.92
Henriksen et al., 2018	29-218 17-267	14 primiparous Jersey 28 multiparous Jersey 11-week study	Free Static PMR with 2 concentrate allocations	0.69-0.50
Menajovsky et al., 2018	141 ± 13.6	8 multiparous Holstein Replicated 4x4 Latin square	Guided Low energy PMR High energy PMR	0.89 0.78
Henriksen et al., 2019	Early (5 to 14) Mid (15 to 240) Late (241 to 305)	128 cows (68 Holstein + 60 Jersey) Continuous lactation study	Free Static PMR with 2 differing concentrate allocations	5 1.1 2.9
Paddick et al., 2019	90.6 ± 9.8	8 primiparous Holstein Replicated 4x4 Latin square	Guided Isocaloric	0.97

Schwanke et al., 2019	47.1 ± 15.0	15 primiparous Holstein cows, crossover design	Free, Isocaloric	0.63

As a second strategy, the energy density of the diet for an individual cow can be changed by increasing or decreasing the AMS pellet allocation without changing the composition of the PMR. This approach is one strategy to apply precision feeding management. There has been limited research with this strategy; however, in a recent study where cows received two or six kg of AMS pellet (dry matter basis), there were only subtle differences in milking frequency and only numerical improvements for milk and milk protein yield (Menajovsky et al., 2018). At a farm level, Tremblay et al. (2016) reported a negative relationship between the amount of pellet offered in the AMS and milk yield. Their rationale was that poor forage quality requires more pellet; however, there was no information provided on PMR characteristics. To our knowledge, there is still a lack of research focusing on the use of precision feeding strategies, particularly with high-yielding and early lactation cows.

A challenge with adopting precision feeding strategies is that predictions are needed for the amount of PMR and AMS pellet that the cow will consume on a daily basis. The data are clear that increasing the quantity of AMS pellet offered in the AMS increases the day-to-day variability in the consumption of the AMS pellet and hence creates more dietary variability (Hare et al., 2018; Menajovsky et al., 2018; Paddick et al., 2019; Schwanke et al., 2019). Based on the available data, the coefficient of variation (CV) in AMS pellet delivered averages 13.6%.

In most studies, a fundamental assumption is that as AMS pellet delivered, and presumably consumed, increased, PMR intake would decrease with an equal magnitude. We know this assumption is not true as substitution rates (amount of decrease in PMR intake for every 1 kg increase in AMS pellet intake) range from 0.63 to 1.58 kg (Table 1). Obviously, the reduction in PMR intake with increasing AMS pellet allocation will change the nature of the total diet and depending on the direction and magnitude of the PMR substitution, the proportions of forage neutral detergent fibre (NDF) or physically effective NDF may become marginal coupled with increases in ruminally degradable starch.

In AMS systems, there are three values that are relevant when considering AMS pellet delivery. The first value is the computer programmed target value. This value is the maximum amount that can be offered to cows in the AMS, assuming that carry-over of pellet is not included in the equation. The second value is the amount that is delivered to the cows in the AMS. The third value is the amount consumed in the AMS. The amount of pellet programmed in the computer does not correspond with the amount delivered (Figure 1). For example, Bach et al. (2007) allocated either 3 or 8 kg/day in the AMS but only 2.6 and 6.8 kg/day were delivered, respectively. Halachmi et al. (2005) offered either 7 kg/day or 1.2 kg/visit to cows and reported that cows offered 7 kg/day were only delivered 5.2 kg/day while those offered 1.2 kg/visit received 3.85 kg/day. Pellet delivery and pellet consumption below that of the formulated diet are major concerns. Evaluating the deviation between the amount programmed and the amount offered is an important management tool because it demonstrates the ability to deliver the formulated diet to the cows. The deviation between the amount programmed and the amount delivered increases as the amount programmed increases (Figure 1). While it cannot be evaluated on farm easily, residual pellet left in the AMS feeder also increases with increasing pellet allocation in the AMS (Bach and Cabrera, 2017). Differences among the amount of pellet programmed, amount delivered in the AMS, and amount consumed by cows in the AMS can pose a challenge to dairy producers and their nutritionists, and diminish the ability to formulate diets that reasonably predict production outcomes.

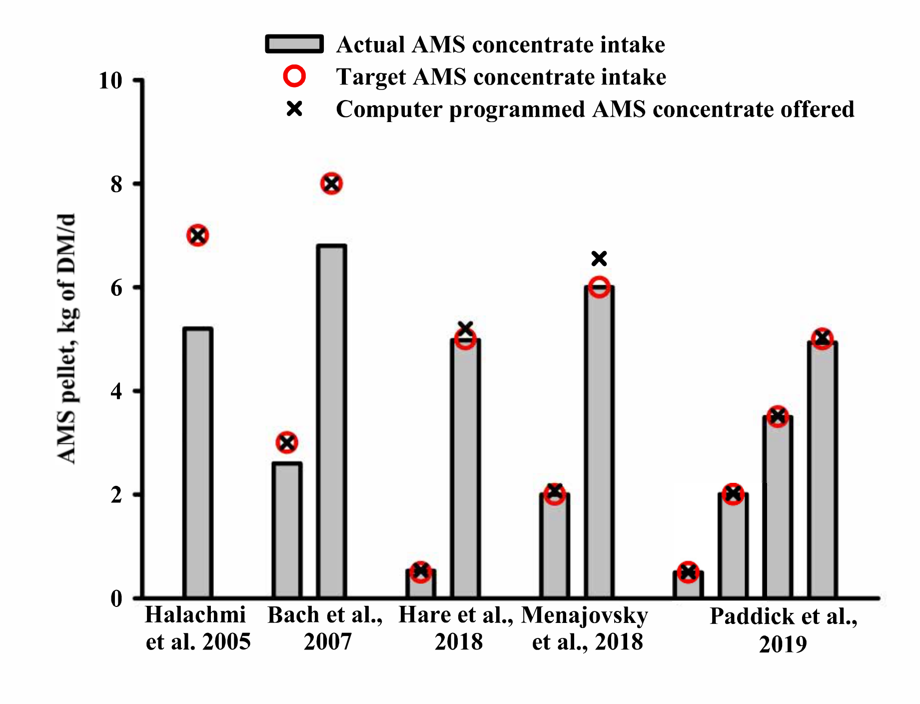

Figure 1. Comparison of computer programmed target AMS pellet allocation and AMS pellet consumption. The circles indicate the target quantity of AMS pellet desired, the ‘x’ indicate the computer programmed quantity, and the grey vertical bars indicate the average quantity that cows are delivered (adapted from Paddick and Penner, 2018).

How Important is the Type of Supplement Provided in the AMS?

In addition to general feeding management, palatability of the pellet provided in the AMS is also important. Madsen et al. (2010) evaluated pellets containing barley, wheat, a barley-oat mix, maize, artificially dried grass, or pellets with added lipid with all cows fed a common PMR. They observed that AMS pellet intake and voluntary visits were greatest when the pellets contained the wheat or the barley-oat mix. However, pelleted barley and wheat are expected to have a rapid rate of fermentation in the rumen and feeding substantial quantities would be expected to increase the risk for low ruminal pH. To reduce fermentability, pellets could be prepared with low-starch alternatives (Miron et al., 2004; Halamachi et al., 2006; 2009). Substituting starch sources with soyhulls did not negatively affect voluntary attendance at the AMS or milk yield (Halamachi et al., 2006, 2009), and may slightly improve milk fat and reduce milk protein concentrations (Miron et al., 2004).

Producers may also choose to use home-grown feeds in the AMS. In a recent study at the University of Saskatchewan, it was tested whether feeding a pellet was required or if they could deliver steam-flaked barley as an alternative (Greg Penner, personal communication) in a feed-first guided-traffic flow barn. In that study, the pellet comprised only barley grain and the same source of barley grain was used for the steam-flaked treatment. In all cases, cows were programmed to have 2.0 kg of the concentrate in the AMS delivered. While PMR (27.0 kg/d DM basis) and AMS concentrate intake (1.99 kg/d DM basis) did not differ among treatments, cows fed the steam-flaked barley tended to have fewer visits (2.99 vs. 2.83; P = 0.07) to the AMS, tended to have a longer interval between milking events (488 vs. 542 minutes; P = 0.10), and spent 28 minutes more in the commitment pen prior to entering the AMS (P = 0.01) than those fed pelleted barley. While this did not translate into differences in milk yield (average of 44.9 L/d), it may be expected that with a longer-term study, production impacts would be observed. In contrast, Henriksen et al. (2018) reported greater voluntary visits when a texturized feed (combination of pellet and steam-rolled barley) was provided in comparison to a pellet alone. Regardless, utilization of a pellet as the sole ingredient or part of the mix may limit the ability of producers to use home-grown feeds in the AMS.

Partial Mixed Ration: the major, but forgotten component of the diet

As mentioned previously, all surveys that have been published to date focus on AMS feeding with little or no information collected to describe PMR composition or intake. The lack of focus on the PMR is likely because only group intakes can be determined and many of the studies have been conducted using retrospective analysis. However, drawing conclusions or making recommendations for feeding management without considering the PMR could lead to erroneous decisions. We recently completed a study where we varied the formulation of the PMR such that we increased the energy density of the PMR by a similar magnitude to that commonly used when increasing the amount of pellet in the AMS (Menajovsky et al., 2018). Feeding the PMR with a greater energy density tended to increase milk yield (39.2 vs. 37.9 kg/d; P = 0.10) likely because of greater energy supply.

Management of the PMR may be a key factor in success of AMS, largely due to the fact that milking activity in AMS is largely tied to PMR feeding activity (DeVries et al., 2011; Deming et al., 2013). Stimulation of PMR eating behavior, through frequent feed delivery and push up across the day may, thus, be important for optimizing AMS usage. Interestingly, in recent observational study of AMS herds, Siewert et al. (2018) reported that farms with automatic feed push-up produced 352 kg more milk/robotic unit and 4.9 kg more milk/cow per day than farms that manually pushed up feed. This effect may not be directly attributable to the use of an automated feed pusher, but rather that those farms using such automated equipment had more consistent feed push-up, and thus continuous feed access, than those pushing up feed manually.

Early lactation challenges?

Automated milking systems provide the ability to milk and feed cows individually based on production potential and stage of lactation. However, individualized milking may not only lead to more frequent milking and greater milk yield in early lactation, but may lead to issues with negative energy balance and metabolic disorders. Tatone et al.

(2017) reported that AMS herds in Ontario, Canada had higher within-herd prevalence of SCK (26%; as measured through milk ketone levels) than did conventional herds (21%). Those researchers also reported that multiparous cows in AMS herds were more likely to have SCK than in conventional herds (Tatone et al., 2017). Higher SCK prevalence may be the result of increased frequency of milking during early lactation or inadequate supplemental feeding of concentrates in the robot. In a field study King et al. (2018) reported that development of SCK in AMS cows was associated with greater production of milk relative to the amount of feed consumed in the AMS, suggesting that inadequate supplementation was occurring at that time. This provides evidence that robot feed supplementation must be based on stage of lactation and production level.

Conclusions

The adoption of AMS systems continues to rise and sound feeding management practices are needed to support efficient and cost-effective milk production. Feeding strategy in AMS herds must take into account the stage of lactation and production level. It is well established that the feeding strategy at the AMS will impact PMR consumption levels, thus this needs to be accounted for when formulating dietary plans. Finally, encouraging PMR feeding will help drive total intake and milking activity.

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The SAGA continues – searching for the source of gut-derived inflammation

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Introduction

Every ruminant nutritionist worth his/her salt over the past 40 years has been taught about ruminal acidosis – its causes, consequences, and strategies to avoid what can be an extremely detrimental condition. First, the focus was on the acute, clinical form of acidosis, where lactic acid-producing bacteria proliferate and a rapid increase in lactic acid concentrations in the rumen can drive pH below 5 and kill most ruminal microbes. The feedlot industry certainly benefitted from understanding this condition, and strategies derived from this research continue to influence nutrition of finishing cattle.

In the dairy industry, the need to support adequate milk fat yield means that greater amounts of fiber are fed, and it is rare to find evidence of lactic acidosis. However, dairy nutritionists began to refer to sub-acute ruminal acidosis (**SARA**) to describe a condition where pH does not spiral out of control, but where the ruminal microbiota experiences extended hours each day at pH < 5.8. This scenario was proposed to be associated with impaired ruminal digestion (especially of fiber), increased risk of some clinical diseases, and a general impairment of health and productivity. Substantial research has been carried out under the SARA framework, much of which has improved our ability to formulate and deliver optimal dairy cattle diets.

However, a third paradigm has emerged in the last 10 years, focused more on the potential for hind-gut acidosis and disrupted barrier function to induce systemic inflammatory responses. In recognition of the potential for both foregut and hindgut acidosis and dysbiosis to create health challenges, some have proposed that we shift the focus away from a narrow emphasis on SARA to considering sub-acute gastrointestinal acidosis (**SAGA**). In this paper I'll review why this concept has emerged, what recent research has taught us about SAGA, and consider the implications for feeding management of dairy cattle.

Mechanisms for whole-body responses to SARA

It is easy enough to understand why a dramatic (or even modest) decline in ruminal pH could have detrimental effects on gut microbes. Many in vitro microbiology experiments have demonstrated that controlled acidification of culture media can harm or kill bacteria and protozoa, particularly the fiber-degrading species. In turn, the impact on nutrient digestion and absorption of products from microbial metabolism is logical enough. For example, a very large meta-analysis suggests that for every 1% increase in dietary starch concentration, total-tract NDF digestibility declines by about 0.5 units (Ferraretto et al., 2013), presumably due at least partially to decreased ruminal pH.

It is less obvious, though, why systemic effects of SARA should be observed. The abomasum can clearly handle an extremely low pH without ill effect, although not all regions of the gastrointestinal tract have the same epithelial structure. It is really the case that mildly acidic rumen contents can harm the cow?

A critical insight into this question was provided more than 40 years ago, with the demonstration that rumen fluid had high endotoxin activity, particularly from cattle fed predominantly grain (Nagaraja et al., 1978). Although endotoxin is always found in gut microbial ecosystems, situations that favor the growth of Gram-negative bacteria and those that trigger rapid death of these microbes (and release of their cell wall constituent lipopolysaccharides) could plausibly harm intestinal epithelium. Damage to the ruminal epithelium could theoretically generate a direct inflammatory response to impact whole-animal physiology, or epithelial barrier function could be disrupted, resulting in systemic delivery of not only microbial toxins but even intact pathogens such as *Fusobacterium necrophorum* (Garcia et al., 2017).

There continues to a variety of opinions regarding whether loss of ruminal epithelium barrier integrity is a common occurrence or not. For starters, the ruminal epithelium is vastly better fortified than the intestinal epithelium, with 4 layers of protection vs. 1 in the intestines (**Fig. 1**).

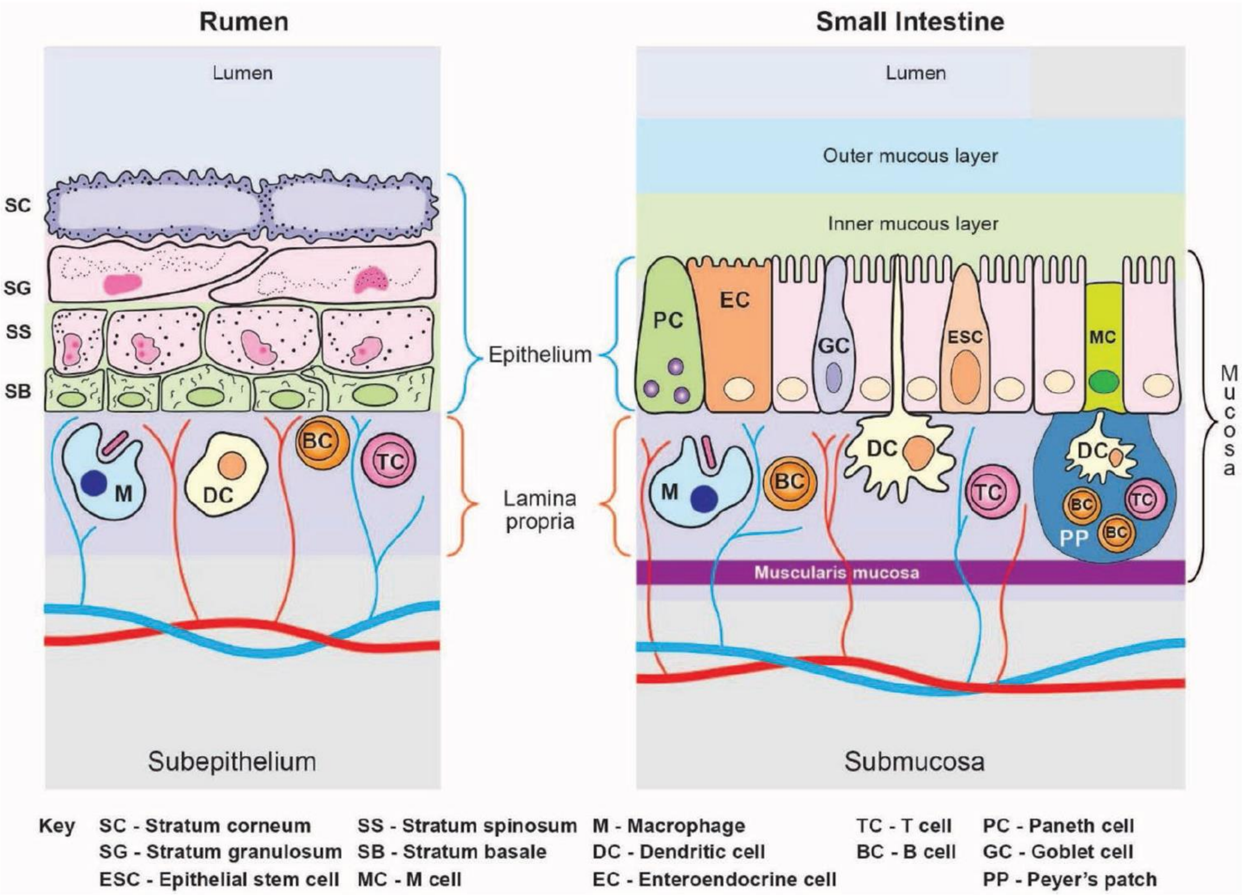


Figure 1. Morphology of the epithelium in the rumen vs. the distal intestine. From Garcia et al. (2017).

Despite the anatomic resilience of the rumen epithelium, commercial abattoir surveys have demonstrated that approximately 10% of dairy cows have active or healed ulcerations of the rumen at slaughter (Rezac et al., 2014). These gross lesions are certainly of sufficient size and severity to enable translocation of microbes and their products. Additionally, a recent report may explain how more microscopic disruption of the epithelium can also contribute to movement of microbes or microbial toxins. Meissner and colleagues (2017) collected ruminal tissue for ex vivo experiments to evaluate barrier integrity against a variety of molecules. Interestingly, maintaining the ruminal tissue at pH 5.1 rather than 6.1 had only minimal effects on electrical conductivity and transit rates of a high-molecular-weight fluorophore. However, when the ruminal tissue was maintained at pH 5.1 with a physiological concentration of volatile fatty acids (VFA; 100 mM), both tissue conductance and fluorophore transit increased dramatically, indicating enhanced epithelial permeability. This finding was further supported by substantially decreased abundance of multiple tight junction proteins (Meissner et al., 2017). Despite the solid construction of the ruminal epithelium, it appears that chemical and/or physiological impacts of high VFA concentrations can lead to a decline in the “mortar” that holds cells tightly together. This mechanism may establish microlesions that can eventually develop into full-blown ulcerations. Either way, the loss of barrier integrity provides a path for intra-ruminal LPS or other toxins to impact the cow.

Confounded results

Despite a growing mechanistic underpinning connecting ruminal dysbiosis to systemic inflammation and illness behavior, there are also reasons to question some long-held assumptions regarding links between ruminal fermentation and whole-animal responses. First of all, it is clear today that nearly any significant disruption of normal ruminal fermentation also has substantial effects on the distal small intestine. For example, let’s consider a case where some problem with diet formulation or presentation results in a dairy cow consuming much more starch than intended. We would expect ruminal pH to decline rapidly, which in turn would disrupt fermentation of potentially-digestible NDF (pdNDF). What is less commonly considered is that this increased outflow of pdNDF to the abomasum and eventually the hind gut will provide much more substrate to gut microbes in that ecosystem. As a result, we would expect at least some increase in acid production and a decrease in pH in the hind gut. In a more extreme scenario where even ruminal starch fermentation is impacted (or bypassed, for example by feeding intact corn), the impact on hind-gut pH may be even more dramatic than in the rumen. The point is that we have hundreds of published studies with various dietary challenges documenting declines in rumen pH and associated systemic responses including host release of inflammatory molecules, decreased feed intake, and other illness behaviors. Unfortunately, very few of those studies simultaneously measured fecal pH (much less ileal or colonic) at the same time. It’s important to acknowledge that a correlation between ruminal acidosis and other responses does not imply a causal relationship, especially when disruptions to other regions of the gastrointestinal tract occur essentially simultaneously.

As demonstrated above, the intestinal epithelium is the chain-link fence to the rumen's Great Wall. Although more or less ignored for decades in the study of ruminants, the distal intestine is a focus of intense research in species used as models for human health. As a result, we now know about many factors that can contribute to disruptions of the intestinal epithelium, including heat stress, dysbiosis, and even short-term feed restriction. Following up on this monogastric work, lactating Holstein cows undergoing feed intake restriction (50-60% of *ad libitum* intake) showed clear signs of LPS translocation out of the gut by day 5 and altered intestinal tissue morphology on day 7 of the challenge (Kvidera et al., 2017).

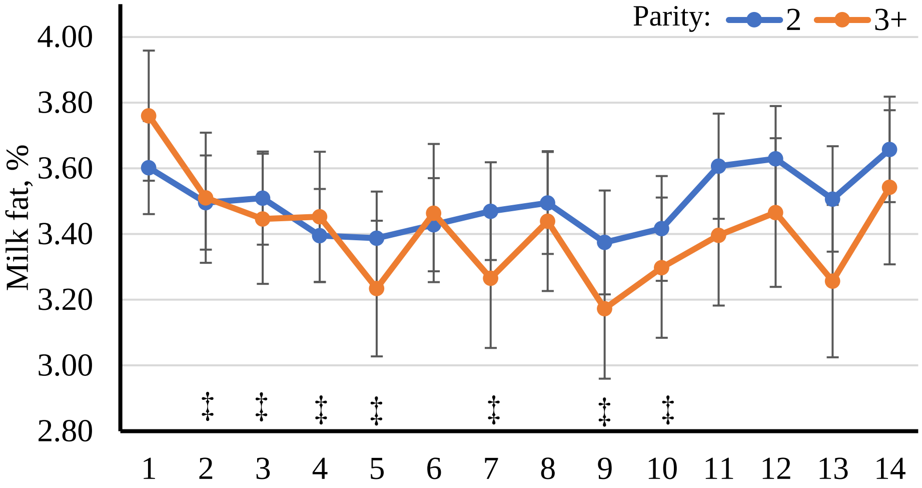
With the increasing interest in the hind-gut as the possible site connecting SAGA with systemic effects, several intensive studies have been conducted to challenge the hind gut microbiome with excess starch supply and see if classic acidosis responses (or even hemorrhagic bowel syndrome) could be induced (Gressley et al., 2011, 2016). In general, although fermentation and pH can certainly be affected by postruminal carbohydrate supply, these studies failed to consistently observe health effects of these rather extreme treatments. It seems unlikely that excessive postruminal carbohydrate supply *alone* is sufficient to induce illness behavior in most cattle.

SAGA: a case study

Our research group recently dealt with an outbreak and resolution of digestive disorders among 15 control cows enrolled in a larger production study. Over 14 weeks, cows were individually fed, with milk yield and composition, blood variables, and health observations recorded. The diet included drought-stressed corn silage that introduced difficulties including low energy density, high dry matter content (making it unstable at feedout), and mycotoxin contamination. The diet included 31% NDF, but only 16% forage NDF, and particle size was marginal. Retrospective mycotoxin analysis showed TMR concentrations of ~1000 ppb trichothecenes and ~70 ppb zearalenone.

By weeks 4–5 on the study, sporadic diarrhea began to appear and milk fat content had dropped from 3.7% to 3.4%, on average (**Fig. 2**). Coincident with the onset of summer heat stress (mean daily THI > 65), three cows developed severe digestive disorders, resulting in a displaced abomasum in one cow. Fecal samples were collected to enumerate viable clostridia bacteria (Arm & Hammer, Waukesha, WI), revealing a mean of 10^3 CFU/g *Clostridium perfringens*, with individuals as high as 10^5 CFU/g. Furthermore, blood analyses showed significant increases in the inflammatory biomarker haptoglobin and the dysbiosis marker D-lactate (**Fig. 2**).

At that point, the diet was changed to replace some corn silage with wheat straw (3.5% of DM), a direct-fed microbial was added to the diet (Biofix Plus Pro; Biomin America, Overland Park, KS), and organic acid treatment (Ultra-Curb, Kemin, Des Moines, IA) of the silage face was initiated. Within a month after these changes were implemented, essentially all signs of digestive problems resolved, including milk fat content, fecal consistency, and blood plasma concentrations of haptoglobin and D-lactate. This case study points to multiple factors that likely combined to lead to microbial and gastrointestinal disruptions resulting in clinical disease in a subset of cows.



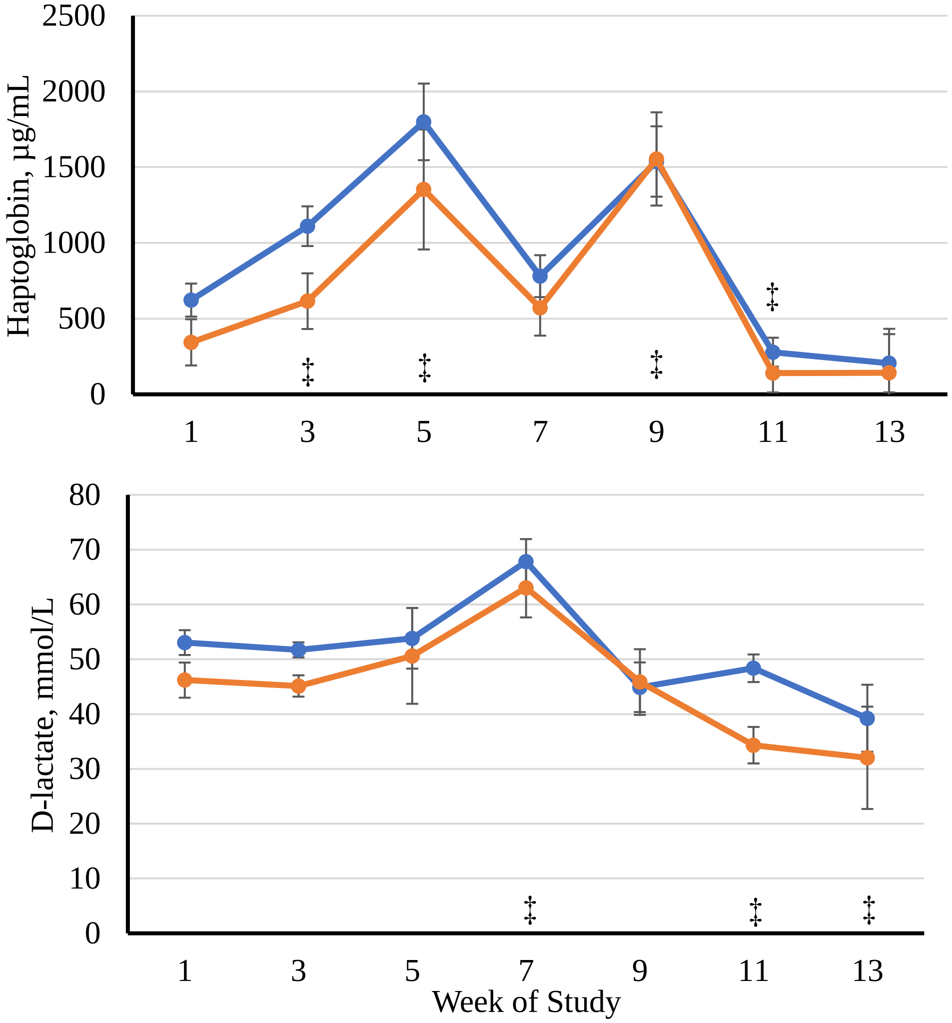


Figure 2. Milk fat, plasma haptoglobin, and plasma D-lactate concentrations of cows fed a ration low in peNDF and naturally contaminated with mycotoxins during the onset of summer heat stress. In week 8, dietary peNDF was increased with 3.5% wheat straw and a direct-fed microbial was added to combat mycotoxicosis. Values are means ± standard errors, $n = 15$. ‡ $P < 0.05$ vs. week 1.

Where do we stand?

Ruminal acidosis certainly can and does occur, and we have good evidence now that many dairy cattle exhibit physical signs of damage to the ruminal wall during their lifetime. As a result, at least some systemic negative effects of SAGA are likely due to direct translocation of microbes or microbial products through a disrupted ruminal epithelium. However, it’s also the case that the intestinal epithelium is susceptible to disruption, and dysbiosis in the hind gut is likely just as common as it is in the rumen. Furthermore, the intestine is likely more susceptible to compounded stressors, given the reported impacts of factors like mycotoxins and heat stress on intestinal health across species. Tracking down the root cause of a specific digestive health issue may need to expand beyond a careful review of diet formulation and presentation, to include other factors that can adversely affect microbial or intestinal stability.

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Management considerations for beef cows with emphasis on offspring performance and cow nutrient requirements

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Introduction

The beef cattle industry in the western United States is dependent on forage production; however, variable environmental conditions pose significant production challenges for the region's cow/calf producers (DelCurto et al., 2000; Reeves et al., 2013). These include concerns around forage quality and availability and how subsequent animal performance is affected. In addition, these challenges often result in significant fluctuations in cow weight and body condition score (BCS) during their annual production cycle if they are not supplemented to address nutrient deficiencies. Thus, producers require knowledge of forage nutritional value and animal nutrient requirements to manage economically for a desired level of productivity.

The productivity and profitability of cow/calf operations depends, in part, on how well their nutritional management plans meet the nutritional needs of the cow herd. Historically, when evaluating cow nutrient requirements, producers were concerned about maintaining/obtaining a desired cow BCS and/or specific intake of nutrients. However, a growing body of data suggests that current beef cow nutritional requirements (NASEM, 2016), especially during gestation, do not adequately account for subsequent offspring performance (Caton et al., 2019). Herein follows a brief discussion of factors for livestock managers to consider when developing nutritional management plans for gestating beef cows.

Forage Quality on Pacific Northwest Rangelands

Forage Species. Most rangeland grasses in the Pacific Northwest are cool-season (C3) due to climatic conditions (Roché et al., 2019). This is important because research has shown that low-quality forage intake and digestibility by non-supplemented ruminants depends, in part, on the cell wall structure and composition, with C3 forages being greater compared to warm-season (C4) forages with similar nutritional indices (Bohnert et al., 2011). Consequently, the response of ruminants to protein supplementation of low-quality forages appears to be dependent on forage type (Table 1).

Precipitation. After plant phenological stage (Angell et al., 1990; Clark et al., 1998; Arzani et al., 2004), the quantity and timing of precipitation has the most influence on quality of forage produced on western rangelands (Ganskopp and Bohnert, 2001; 2003). Ganskopp and Bohnert (2001; 2003) documented reduced forage quality (crude protein, digestibility, and mineral content) with above average, compared to below average, crop year precipitation for 7 common grass species on rangelands in SE Oregon (Figure 1). Thus, with abundant moisture during the growing season, forage quality rapidly deteriorates as plants progress through their reproductive stages of phenology. In contrast, fewer reproductive tillers develop with below average precipitation during the growing season, resulting in elevated forage quality and/or an extended period of adequate nutrition.

Cow Nutritional Requirements may not Account for Offspring Performance

Recent research has demonstrated that we do not have a good understanding of the nutrient requirements of gestating ruminants as they relate to the performance of the resulting progeny. A 2019 issue of the *Veterinary Clinics of North America: Food Animal Practice* (volume 35; issue 2) is dedicated to a comprehensive review of this topic, with articles covering multiple aspects of developmental programming in livestock production. In addition, this body of research has demonstrated effects of both nutrient restriction and provision of nutrients in excess of current requirements.

Protein and/or Energy. Many of the early studies designed to evaluate the effects of gestational nutrition on subsequent offspring in beef cattle focused on nutrient restriction of females at various stages of gestation (Corah et al., 1975; Stalker et al., 2006; Larson et al., 2009). Also, most revolved around supplementation of animals consuming low-quality forages with a protein supplement (Martin et al., 2007; Stalker et al., 2007; Bohnert et al., 2013). The supplements used in these studies also provided a source of energy, thereby making it difficult to determine if the observed responses were due to provision of supplemental protein, energy, or some combination. Briefly, these studies demonstrated that late-gestation supplementation of beef cows was an economical management practice due to improved cow pregnancy rate and performance of resulting progeny (greater weight gain; decreased calf morbidity and mortality). Interestingly, data also suggested that late-gestation supplementation of beef cows favorably influenced heifer progeny reproductive performance, by reducing age at puberty (Funston et al., 2010) and improving pregnancy rate (Martin et al., 2007). Recently, Caton et al. (2019) prepared an excellent review on the effects of gestational nutrition and developmental programming on the energy requirements of resultant progeny. Briefly, they concluded that the preponderance of data available with beef cattle suggests that epigenetic incidents occurring during fetal development alter the lifetime energy requirements of the subsequent offspring. Consequently, there is a need for research that directly assesses how maternal manipulation of nutrient supply alters protein and energy requirements of progeny.

Minerals. Mineral supplementation, specifically trace minerals, are essential for fetal development (Hostetler et al., 2003); however, little research is available related to gestational supplementation of beef cows on the performance and productivity of subsequent offspring. A recent study conducted by Marques et al. (2016a), provided above NRC (2000) requirements of Cu (200%), Co (2,160%), Mn (130%), and Zn (200%) from organic or inorganic sources to beef cows during the last third of gestation. They compared progeny performance with a control that received the same basal diet consumed by all cows, which met requirements for protein, energy, and macro minerals, trace minerals, and vitamins (NRC, 2000). They noted no treatment effects on calf birth weight; however, compared with the control, weaning weight was 24 kg greater for calves from cows receiving the organic source of minerals while the weaning weight of calves from cows receiving inorganic source was intermediate (Table 2). Similar tendencies were noted for calf weight at the end of both the growing lot and finishing lot feeding periods (112 and 153 d, respectively). The number of calves treated for bovine respiratory disease (BRD) in the growing lot was 60% less for calves born to cows supplemented with the organic source of minerals when compared to calves born to cows supplemented with the inorganic source of minerals or assigned to control treatment (Table 2). The results of this study suggest that strategic provision of minerals above current recommendations (NASEM, 2016) to gestating beef cows has developmental programming implications and requires additional research to evaluate potential ramifications on our current knowledge of cow mineral requirements – especially during gestation.

Fats/Lipids. In humans and livestock species, ω-3 and ω-6 polyunsaturated fatty acids (PUFA) are not synthesized by the body, yet play critical roles in several body functions (Hess et al., 2008). Consequently, they must be provided and consumed in the diet. In addition, dietary PUFA are transferred to the fetus during gestation from the dams' circulation via the placenta (Noble et al., 1978; Innis, 2005). In human nutrition, mothers are encouraged to consume supplemental PUFA for proper growth, nervous tissue response, immune function, and early-life development of the fetus/child (Greenberg et al., 2008). Consequently, recent research, albeit limited, has evaluated strategic supplementation of essential fatty acids to beef cows during gestation for effects on developmental programming. Marques et al. (2017) provided gestating beef cows in the last third of gestation with Ca salts of PUFA (ω-3 and ω-6) or an isolipidic amount of Ca salts of palmitic and oleic acids (control). They reported no effects on cow performance, calf birth weight, weaning weight, or health parameters; however, they did note that calves from PUFA supplemented cows had greater post-weaning ADG, which resulted in a greater body weight and hot carcass weight at slaughter compared with calves from control cows. Also, calves from PUFA supplemented cows had greater carcass marbling and the proportion of carcasses yielding choice tended to be greater compared with the calves from control cows. Additionally, in previous studies, cold tolerance and ability to respond to cold stress in calves was improved by providing essential fatty acids to gestating beef cows (Lammoglia et al., 1999a; 1999b). These data suggest that PUFA supplementation of gestating beef cows results in developmental programming effects in the subsequent offspring that could benefit their productivity, efficiency, and value. Consequently, further research is needed to elucidate the mechanisms underlying the observed responses. This is especially relevant, given that there is currently no defined requirement for essential fatty acids in ruminants (NASEM, 2016).

Consequences of Annual Variability in Cow Body Weight and BCS

Beef cows grazing rangelands in the Pacific Northwest typically gain and lose weight and BCS throughout the production year depending on their production state, forage quantity and quality, and environmental conditions. Figure 2 provides a typical weight/BCS cycle for an "average" spring calving cow. Given the narrow window of adequate forage quality noted for rangelands in the Pacific Northwest (Figure 1), cows often struggle to maintain BCS until weaning and/or until provided supplemental feed. Consequently, cows grazing rangelands in the Pacific Northwest face an inadequate or compromised nutritional environment during gestation. Our group evaluated the timing and effect of nutritional deficiencies and realimentation, throughout gestation, on the performance of the subsequent offspring (Marques et al., 2016b). Briefly, we classified cows at the beginning of gestation as adequate (BCS = 5.7) or inadequate (BCS = 4.5). Furthermore, within the inadequate group we randomly assigned cows to one of four groups that either maintained their BCS through gestation or gained 1.5 BCS during the first, second, or third trimester of gestation and maintained that BCS until calving. Following parturition, all cows were maintained in a common herd and managed similarly. We noted no differences in live calves at birth, birth weight, or live calves at weaning (Table 3); however, calf daily gain to weaning and weaning weight were affected by gestational nutrition. Calves from cows managed to gain BCS during the second and third trimesters had greater weight gains than the cows that maintained adequate and inadequate BCS throughout gestation. Interestingly, calf performance was similar for both cows that maintained adequate BCS and those that maintained inadequate BCS throughout gestation. In a similar study, Mulliniks et al. (2015) noted ADG of calves from cows that gained or maintained their BCS during the last third of gestation tended to be greater than that for calves from cows that lost BCS during the same period. Together, these data suggest that moderate nutrient restriction followed by realimentation of beef cows during mid- to late-gestation can be an acceptable management practice while maintaining or improving calf performance.

Management Recommendations for Beef Cows in the Pacific Northwest

Cow/calf producers in the Pacific Northwest require a knowledge of the highly variable climate and the probable responses of their livestock to the resulting variability in quantity and quality of forage produced each year. In addition, this necessitates a knowledge of cow nutrient requirements. Historically, beef cattle nutrient requirements have been studied, established, and incorporated by our industry. They have successfully allowed animals to be nutritionally managed for an expected level of performance. In addition, when economically evaluating a nutritional management plan, cattle producers have been primarily concerned with the cost of the nutritional inputs and the subsequent return from the performance of the animals being fed/managed. Developmental programming research has clearly shown that our current understanding of the nutritional requirements of gestating beef cows does not adequately account for the future performance of the resulting progeny. More importantly, cow/calf producers do not have the knowledge to assess accurately the full economic impact of nutritional management of gestating beef cows and the effects on the subsequent progeny. Box 1 provides some considerations related to developmental programming, based on recent research, for cow/calf producers to bear in mind when developing their nutritional plans.

Conclusion

Beef cattle grazing rangelands in the Pacific Northwest face a variety of environmental challenges ranging from annual variation in the amount, type, and timing of precipitation to challenges associated with forage quality and quality that can nutritionally restrict their performance. Consequently, gestating beef cattle face a much less controlled and managed production environment compared with other livestock species. They often go through cycles, within a year, in which they gain and lose weight depending on their production state and quality of the forage resources they are consuming. These challenges often interact to challenge the beef cattle manager who tries to balance animal performance with economic viability. Cattle operations have learned to deal with these nutritional challenges through management practices that have served our industry well (Cook and Harris, 1968; DelCurto et al., 2000; Olson, 2007); however, recent studies have highlighted additional challenges associated with the impact of gestational nutrition on offspring performance through what is commonly called developmental programming or fetal programming.

Gaps in our understanding of the consequences of gestational nutrition highlight the inadequacy of our current knowledge related to the nutrient requirements of gestating beef cows. In addition, the lack of consistency in progeny effects due to alterations in the nutritional management of beef cows demonstrates the need for more research around the timing and type of nutritional manipulation(s) applied to gestating beef cattle. Current and future research in the areas of gestational nutrition of beef cows should be designed to provide beef cattle producers with the knowledge and tools for development of nutritional management plans, and animal selection, that will help improve cattle production efficiency, predictability, and economic viability in an increasingly competitive industry.

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Table 1. Forage intake and nutrient[1] digestibility by steers consuming low-quality cool-season (C3) and warm-season (C4) grass hay with or without crude protein (CP) supplementation. Adapted from Bohnert et al. (2011).

	Treatment					*P*-Value[2]		
Item	C4	C4+CP	C3	C3+CP	SEM	CP vs No CP	C4 vs C3	Supp × Type
DM Intake, g/kg BW								
Forage	15.6	22.9	23.7	25.3	0.6	<0.01	<0.01	<0.01
Soybean meal	0.0	1.7	0.0	1.7				
Total	15.6	24.6	23.7	27.0	0.6	<0.01	<0.01	<0.01
Apparent digestibility, %								
DM	42.8	51.8	49.7	54.2	0.9	<0.01	<0.01	0.05
OM	45.6	54.6	53.6	58.5	0.9	<0.01	<0.01	0.05
N	28.4	54.5	37.5	55.2	3.5	<0.01	0.21	0.27
NDF	43.5	50.0	48.0	52.7	1.7	0.02	0.07	0.61

[1] DM = dry matter; OM – organic matter; N = nitrogen; NDF = neutral detergent fiber, [2] Supp = CP supplementation; Type = forage type.

Table 2. Effects of providing excess[1] Cu, Co, Mn, and Zn, from inorganic or organic sources, to late-gestation beef cows on performance of progeny from birth to slaughter. Adapted from Marques et al. (2016a).

Item	Control[2]	Inorganic	Organic	SEM	P-value
Birth wt., kg	42	42	41	1	0.63
Weaning wt., kg	212^{a}	223^{ab}	236^{c}	6	0.04
Growing lot performance[3]					
Treated for BRD, %[4]	42^{a}	59^{a}	20^{b}	10	0.02
Wt. at end of growing lot, kg	352^{a}	359^{ab}	374^{b}	8	0.09
Finishing lot performance[3]					
Treated for BRD, %[4]	0	5	4	4	0.37
Wt. at end of finishing lot, kg	649^{a}	663^{ab}	680^{b}	11	0.10
Hot carcass wt., kg	409^{a}	418^{ab}	428^{b}	7	0.10

[a,b,c] Within rows, means with different superscripts differ

[1] Trace minerals provided so the diet was above NRC (2000) requirements (200% for Cu & Zn; 2,160% for Co; 130% for Mn). Treatments were provided during the last third of gestation.

[2] No additional Cu, Co, Mn, or Zn provided; concentrations in diet were at or above requirements.

[3] Cattle were in the growing lot for 112 d, and then moved to an adjacent finishing lot where they remained for an average of 153 d until slaughter.

[4] Calves were classified as positive for BRD symptoms according to the DART system (Zoetis, Florham Park, NJ), and received medication according to the feedyard management criteria.

Table 3. Calving and weaning outcomes from cows that maintained inadequate (LBCS) or adequate (HBCS) body condition score throughout gestation, or cows that gained body condition score during the first (BCSG1), second (BCSG2), and third (BCSG3) trimester of gestation and maintained the resultant body condition score until calving. Adapted from Marques et al. (2016b).

Item	LBCS	BCSG1	BCSG2	BCSG3	HBCS	SEM	P-value
Calving							
Live calves, %	92	92	100	100	100	4.5	0.49
Birth wt., kg	44	43	44	42	42	1.4	0.73
Weaning							
Live calves, %	92	92	100	100	100	4.5	0.49
ADG to weaning, kg/d	1.07^{a}	1.10^{ab}	1.13^{b}	1.15^{b}	1.07^{a}	0.02	<0.01
Weaning wt., kg	249^{a}	256^{a}	265^{b}	262^{b}	248^{a}	4	<0.01

[ab] Means within a row with different superscripts differ ($P < 0.05$).

Box 1. Nutritional management considerations for beef cows in the Pacific Northwest with emphasis on developmental programming of subsequent offspring

- Providing mid- to late-gestation cows consuming low-quality forage with a protein/energy supplement
    - ➢ Increased weaning weight
    - ➢ Increased reproductive efficiency in heifers
    - ➢ Decreased morbidity and mortality

- Strategic supplementation of trace minerals, specifically Cu, Co, Mn, and Zn, during late-gestation
    - ➢ Increased weaning weight and weight at slaughter
    - ➢ Improved calf health

- Strategic supplementation of polyunsaturated fatty acids during late-gestation
    - ➢ Increased post-weaning performance
    - ➢ Improved carcass quality

- Allowing cows to have moderate nutrient restriction followed by realimentation during mid- to late-gestation
    - ➢ Increased weaning weight

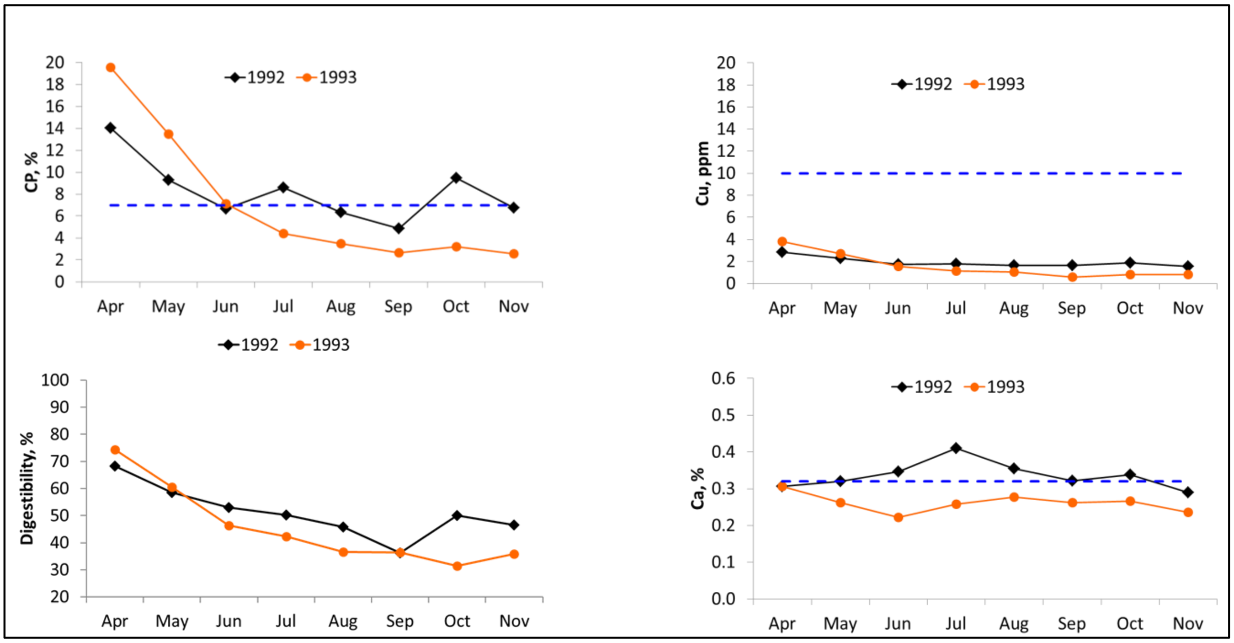

Figure 1. The effects of crop year precipitation on forage quality indices; averaged for 7 common grass species. Crop year precipitation in 1992 (black markers and line) was 86% of long-term average; 1993 (orange markers and line) was 167% of long-term average. The observed response for forage Zn concentration was similar to that reported for Cu while Mg, P, K, and Mn were similar to that observed for Ca. The dotted, blue horizontal lines indicate the estimated nutrient concentration necessary to meet the requirements of a 5 year old, 454 kg Angus x Hereford cow that has a body condition score 5, is 60 days pregnant, 120 days in milk, and consuming 11.4 kg of forage dry matter per day (NRC, 1996). Adapted from Ganskopp and Bohnert (2001; 2003).

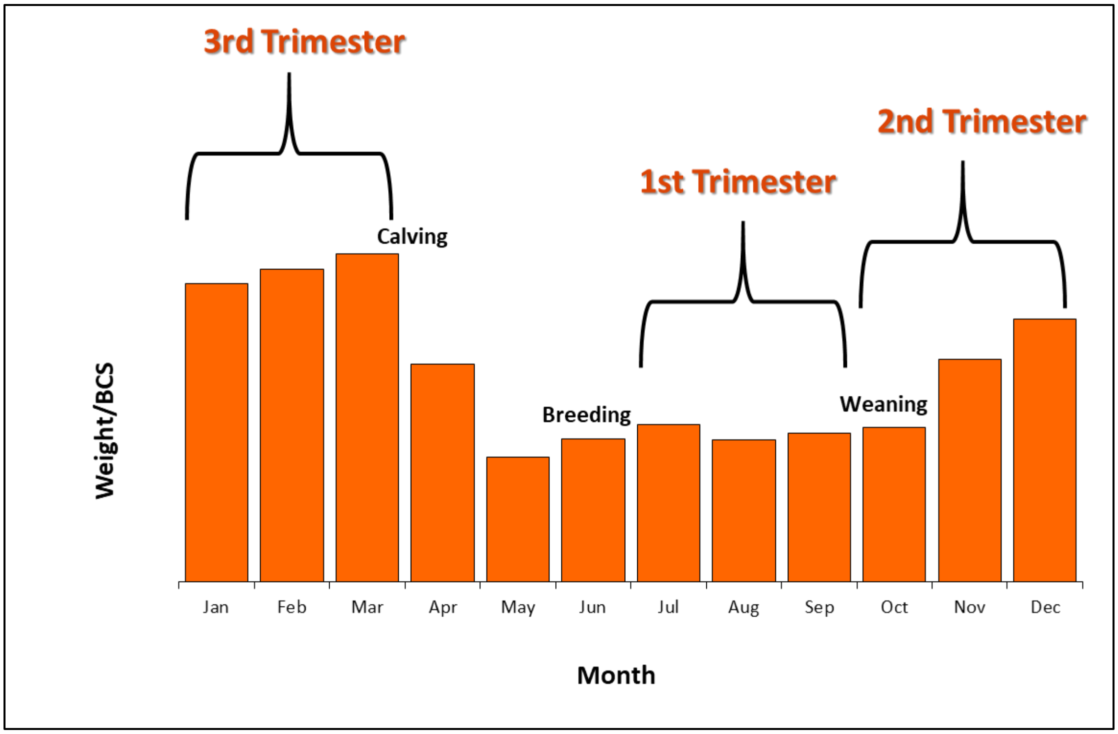

Figure 2. Typical weight/Body Condition Score (BCS) cycle for spring calving beef cows in the Pacific Northwest.

Feedlot Trilogy: Acidosis prevention, cereal grains and grain processing, and liver abscess control strategies

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Introduction

Feedlot production systems in North America historically have been very technologically driven, drawing from a wide range of resources to capture efficiencies of production. An evolving regulatory climate, changes in consumer preferences, and access to new technologies are important factors that impact the business landscape, and thus the need for research. Our research program, as is the case with many others, is thus multi-faceted. This paper provides an overview of several research topics that have been emphasized in our program in the past several years, including utilization of novel types of cereal grains, acidosis prevention with lactic-acid utilizing bacterium, and strategies for decreasing usage of in-feed antibiotics in feedlot cattle.

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A New Stategy for Controlling Acidosis

In modern systems of beef and dairy cattle production, energy-dense feeds such as cereal grains, cereal grain co-products, syrups, whey, and other products often are fed as energy sources to improve growth rate, milk production, and efficiency of feed utilization. The inherently rapid rates of fermentation of these ingredients can result in the overproduction and accumulation of organic acids in unadapted animals, or in adapted animals that overconsume, thereby decreasing ruminal pH. These conditions impede productivity and can compromise growth performance or animal health and well-being, often with severely debilitating or life-threatening outcomes. Feeding diets that are rich in non-structural carbohydrates predisposes ruminants to digestive disturbances, and gradual adaptation to highly fermentable diets and subsequent regularity in feeding practices are regarded as essential in minimizing the occurrence of ruminal acidosis and related maladies, such as bloat, liver abscess, endotoxemia, respiratory disease, and laminitis. Gradually shifting the proportion of rapidly fermentable dietary substrate allows ample time for establishment of key microbial populations, including *Megasphaera elsdenii*, that otherwise are represent only a small fraction of the microbial population.

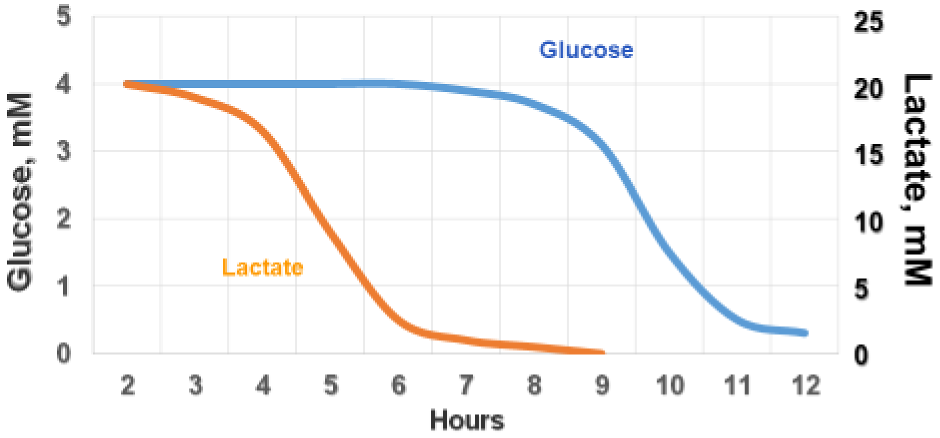
Herein an alternative approach to diet transition is described in which animals are inoculated with live cultures of the probiotic bacterium, *Megasphaera elsdenii* (Lactipro; MS Biotec). Most commonly the transition to concentrates in the absence of exogenous *Megasphaera* is accomplished by feeding a series of 3 to 5 step-up diets for which the roughage component is progressively decreased and replaced by increasing proportions of highly fermentable carbohydrate sources, such as cereal grains or grain byproducts. As animals progress through the diets, the populations of flora and fauna in the rumen shift from those that are adept at digesting cellulosic materials to those that digest non-structural carbohydrates. Key in this process is maintaining a balance between organic acid production and organic acid absorption and utilization, thus avoiding major shifts in ruminal pH. The step-up process most commonly is implemented over a period of three weeks, starting with 40 to 50% roughage and ending with a final finishing diet containing 6-8% roughage. Other step-up strategies have been developed, such as the two-ration feeding system, in which cattle initially are fed 3 meals per day of a diet containing 50-60% concentrate upon arrival in the feedlot. After 6 to 7 days, cattle are then fed the high-roughage diet (i.e., 40-50% roughage) for the first and second feedings, and then fed the finisher diet (6-8% roughage) at the third feeding. This is continued for 6-7 days or more, after which cattle are fed the high-roughage for the first meal, and the finish diet for the second and third meals. This is repeated for 6-7 days or more, after which cattle receive the finish diet at all three feedings, which normally begins on or around day 21 after arrival in the feedlot. Numerous variations of this this method are used, but all attempt to satiate cattle at early feedings using a diet that contains more roughage, followed by higher concentrate levels in subsequent feedings when cattle are less likely to exhibit overeating behaviors. Proportions of concentrate consumed over a 24-hour period are thus changed gradually, just as with the multi-ration step-up system. Regardless of the method used, common to all step-up strategies is the goal of increasing proportions of concentrate gradually to allow for changes in the microbial population, as well as changes in the animals' capacity to absorb and metabolize increasing amounts of fermentative end-products.

Abrupt changes in the proportion of concentrates in finishing cattle diets can have dire consequences for finishing cattle. Opportunistic, highly prolific bacteria, such as *Streptococcus bovis* and *Lactobacillus sp*. colonize the rumen rapidly in the presence of these substrates, metabolizing the carbohydrates to yield substantial amounts of D,L-lactate. *Streptococcus bovis* is regarded as a key microorganism in the manifestation of acidosis, as it metabolizes starches and sugars to produce large amounts of lactic acid as an end-product of its metabolism. Lactic acid is a relatively strong acid (pKa=3.8) in comparison to the major volatile fatty acids (pKa=4.8), and its production by *S.bovis* frequently is associated with the initial decline in ruminal pH (Nagaraja and Titgemeyer, 2007). The resulting decrease in ruminal pH favors growth and activity of other lactate producers, such as *Lactobacillus* sp., further exacerbating the imbalance between lactate production and lactate utilization. The imbalance is greatest during acute bouts of acidosis (Chen, et al, 2019), where ruminal pH can drop below 5 and jeopardize well-being of the animal.

Lactate-utilizing species of microorganisms normally are present in relatively low numbers in forage-fed animals. For example, we measured populations of *Megasphaera elsdenii* in steers fed a diet consisting of alfalfa hay using a quantitative RT-PCR assay (McDaniel, 2009) and determined that baseline populations were approximately 10^4 genomes per mL of ruminal contents, thus comprising a relatively small proportion of the overall bacterial population of 10^{11} cells per gram of ruminal contents reported by Ji et al. (2017). Given the propensity for rapid colonization of the rumen by lactate producers and the relatively small starting populations of lactate utilizers, cattle are very susceptible to imbalance between production and utilization of lactate during the initial stages of feedlot finishing. Excess production of organic acids can alter the rumen environment to favor growth of lactate producers, creating conditions that can spiral into subacute or acute ruminal acidosis. Gradual adaptation to high-concentrate aims to avoid this imbalance by matching organic acid production with absorption metabolism of organic acids, thereby reducing the occurrence of ruminal acidosis.

Megasphaera elsdenii has been identified as the predominant utilizer of lactic acid in grain-adapted animals (Counotte et al., 1981), and is believed to play a central role in maintaining a healthy, productive ruminal environment. A key feature of *Megasphaera elsdenii* is its preferential use of lactic acid as a carbon source, as illustrated in Figure 1. This is key to the success of *Megasphaera* in attenuating ruminal pH depression, as it converts lactate to less potent organic acids, including butyrate, propionate, and acetate, rather than synthesizing organic acids from glucose, as is the case with other organisms. When lactate is depleted the organism immediately begins to metabolize other substrates, thus ensuring its survival under conditions of low lactate availability.

Figure 1. Substrate disappearance from *Megasphaera elsdenii* cultures containing a mixture of glucose and lactate as carbon sources. Lactate is utilized almost entirely before organisms begin to metabolize glucose. Adapted from Hino et al, 1994, Applied and Environmental Microbiology, V60:1827-1831.

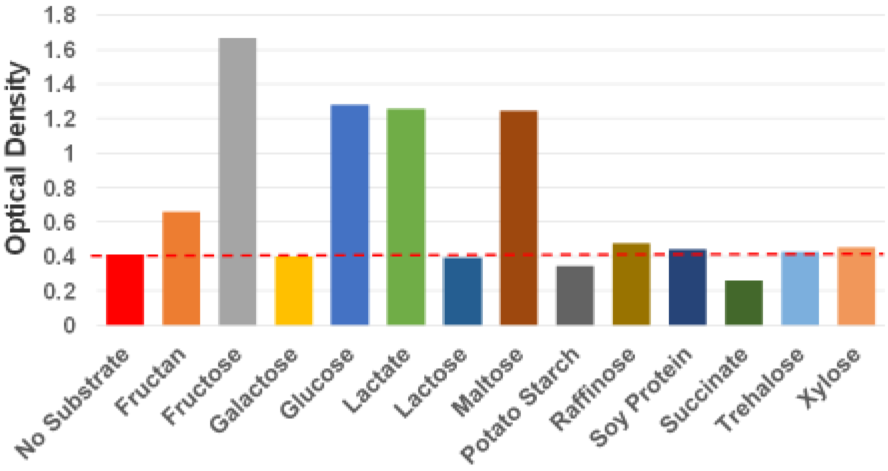


The potential for use of *Megasphaera elsdenii* as a probiotic bacterium was recognized relatively soon after its initial isolation. Its use for prevention of lactic acidosis has been the subject of several patents, the first of which was granted as early as 1976 (Hahn and Abdo, 1976; Das, 1979; Leedle et al., 1995). Early attempts to commercialize strains of *Megasphaera elsdenii* were hindered by several factors, including one or more of the following: insufficient replication rate within industrial fermenters; inhibition by ionophores within the rumen; poor replication at low ruminal pH; failure to preferentially use lactic acid as substrate; or inability to utilize substrates other than lactic acid (Meissner et al., 2010). Variance among isolates with respect to these characteristics is substantial, suggesting it is feasible to overcome these limitations. We examined nearly 300 isolates of *Megasphaera elsdenii* obtained from the ceca of horses fed diets consisting predominantly of roughage or a concentrate-roughage mixture, and observed more than 10-fold differences in growth rate and capacity for lactic acid utilization (unpublished data) among isolates. Moreover, some isolates grew poorly at low pH, and thus were not affective in attenuating lactic acid accumulation, while others had generation intervals of less than 50 minutes and aggressively metabolized lactate.

The first successful commercialization of *Megasphaera elsdenii* did not occur until the mid-2000s with an initial appearance in South Africa, followed several years later by regulatory approval and introduction into the U.S. market in 2010. The commercialized strain, NCIMB 41125 (sold under the trade name, Lactipro), was developed in South Africa, and is the product of a very rigorous selection process aimed at overcoming limitations encountered in previous commercialization attempts. In this process, candidate strains were selected only from grain-fed animals and were isolated under conditions that favored capacity for rapid replication at low pH and in the presence of relatively high concentrations of lactic acid. Consequently, NCIMB 41125 has remarkable capacity for lactic acid utilization and replicates quickly, and thus is well-suited to production in commercial fermenters. Additionally, the strain maintains vibrant growth and metabolic activity in the presence of ionophores, antibiotics, beta agonists, anthelmintics, and other feed additive compounds. Cultures also are substantially aerotolerant, withstanding oxygen presence for up to several hours without appreciable loss of viability. The organism also has the ability to utilize carbon sources other than lactate, which is an important feature if the organism is to persist within the gut in the absence of large quantities of lactic acid. Figure 2 summarizes a laboratory experiment we conducted (Mobiglia et al, 2016) to assess relative growth of NCIMB 41125 when cultivated with different carbon sources. This and other experiments have revealed that the organism grows exceptionally well with maltose, glucose, fructose, and sucrose (not shown) as substrates, and to a lesser extent with fructooligosaccharide (fructan). When presented combinations of substrates, however, the strain preferentially utilizes lactic acid over other carbon sources. These factors are crucially important, recognizing that the objective in using *Megasphaera* as a probiotic is to ensure that a sufficient population of lactate-utilizing bacteria are present *prior* to a dietary challenge. The organism must therefore be capable of colonizing the rumen utilizing substrates other than lactate (i.e., prior to a dietary challenge) in the absence of appreciable amounts of lactate, to ensure its presence is maintained when ruminal metabolism shifts to yield more lactate. As readily fermentable carbohydrates are added to the diet in greater proportions, more lactic acid is generated, and the presence of substantial numbers of

Megasphaera ensures that a metabolic insult is avoided by metabolizing the acid to yield volatile fatty acids.

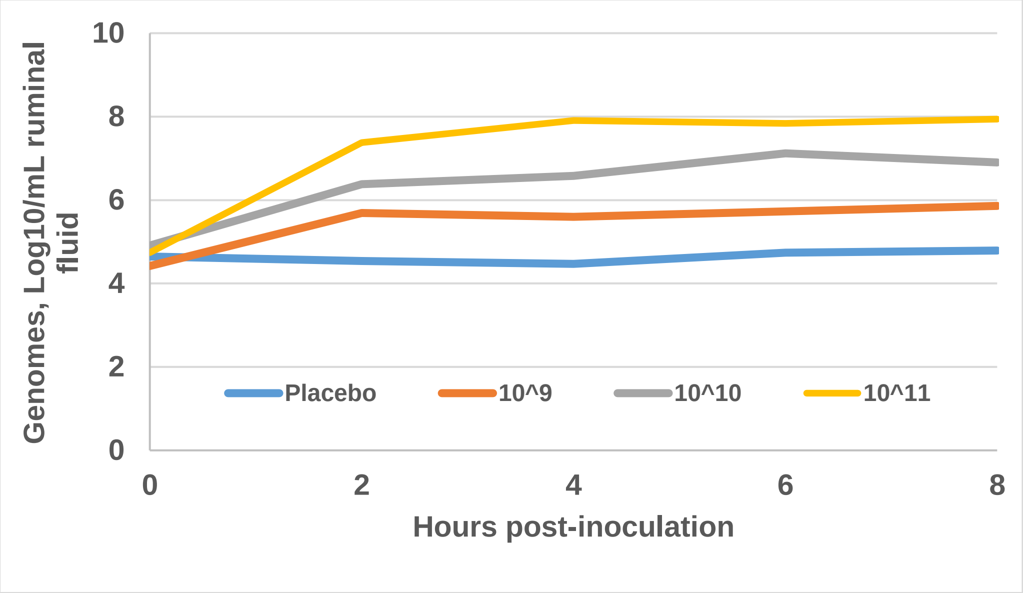
Figure 2. Substrate utilization by *Megasphaera elsdenii*. Cultures were grown on media containing yeast extract, peptone, minerals, vitamins, and the respective carbon source. The assay measures change in optical density after 24 hours. Bars above the dashed red line (i.e., control) are presumed to indicate capacity to utilize the respective substrate. Bars at or below the dashed line indicate no capacity or limited capacity to utilize that substrate. Adapted from Mobiglia et al., 2016.



Acidosis remains as a major challenge for beef and dairy producers that use significant proportions of grains and grain byproducts in cattle diets. Adapting cattle to these diets is both time-consuming and costly. Roughages used during the transition period generally have a high cost per unit of energy. Additionally, they contribute disproportionately to manure accumulation due to their low digestibility. Use of multiple step-up diets also increases complexity of the feeding system, and decrease efficiency of the diet fabrication process, thus adding cost to the production system. Alternative strategies are thus needed to minimize occurrence of acidosis while avoiding unnecessary costs.

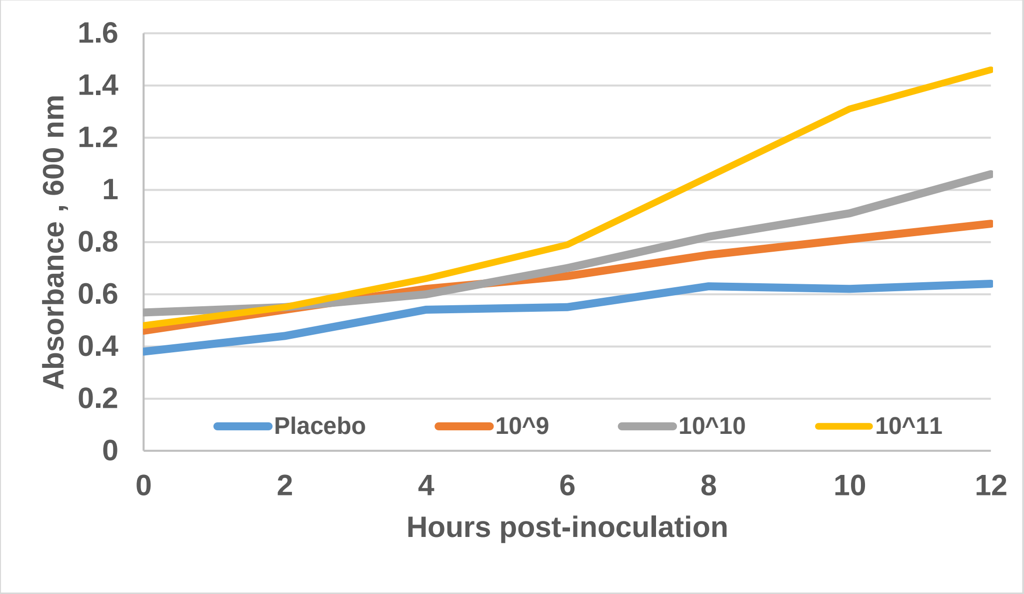
Armed with knowledge of the capacity for *Megasphaera* to utilize lactate preferentially, the objective of our first animal experiments was to determine the dosage (i.e., number of colony forming units) required to prevent acidosis when animals were subjected to a carbohydrate challenge. The key was in finding the minimum inoculation rate that would result in effective ruminal colonization in an effort to contain costs of inoculating animals. Twenty ruminally-cannulated beef steers were used in this experiment (McDaniel, 2009). The cattle were maintained on a diet of alfalfa hay and salt for two weeks. Following the acclimation period, feed and water were withheld for 24 hours, and animals were then dosed with 0, 1 billion, 10 billion, or 100 billion colony-forming units of *Megasphaera elsdenii* via the ruminal cannula. Animals were immediately fed a diet consisting of 65% finely ground wheat grain (a diet know to provoke acidosis). Samples of ruminal contents were obtained via the ruminal cannula before and at several points after inoculation to assess changes in microbial populations and organic acid profiles. *Megasphaera* populations in the rumen were ≤10^5 genomes/mL of ruminal fluid for all treatments prior to inoculation, and lactic acid concentrations were undetectable. Figure 3 illustrates changes in populations of *Megasphaera elsdenii* after inoculation and introduction of the concentrate-based diet. Ruminal concentrations of *Megasphaera* in the control group remained below 5 logs 8 hours after inoculation, while inoculated cattle achieved concentrations of 6, 7, and 8 logs per mL of ruminal contents, thus increasing in a dose dependent manner.

Figure 3. Ruminal proliferation of *Megasphaera elsdenii* following inoculation with 0, 1 billion, 10 billion, or 100 billion colony-forming units of a liquid culture (Lactipro). Treatment by time interaction, P < 0.05; Linear effect of dosage size, P < 0.05. Placebo versus *Megasphaera* groups, P < 0.01. SEM = 0.44. Adapted from McDaniel, 2009.



To assess changes in capacity of ruminal flora to metabolize lactic acid, we extracted ruminal contents from steers 8 hours after inoculation and transferred a small amount (1% inoculation rate) into test tubes containing medium with lactate as the predominant carbon source. Changes in absorbance (600 nm) were used as an indicator of bacterial proliferation in lactate medium. Tubes were incubated at 39C for 12 hours, and changes in absorbance were measured at 2-hour intervals post-inoculation. The results of this assay, shown in Figure 3, illustrate that growth of cultures on lactate medium was minimal for tubes inoculated with ruminal fluid extracted from control steers, but increased in a dose-dependent manner for ruminal contents of steers inoculated with 1, 10, and 100 billion colony-forming units of *Megasphaera elsdenii*.

Figure 4. Microbial growth (estimated as changes in optical density) in lactate media inoculated with ruminal contents extracted from steers dosed with 0, 1 billion, 10 billion, or 100 billion colony forming units of *Megasphaera elsdenii.* Treatment by time interaction, P < 0.05; Linear effect of dosage size, P < 0.05. Placebo versus *Megasphaera* groups, P < 0.01. SEM = 0.04. Adapted from McDaniel, 2009.



More direct evidence of changes in capacity for lactate metabolism are shown in Table 1, which summarizes ruminal concentrations of lactate and major VFAs eight hours after steers were inoculated with 0, 1, 10, or 100 billion CFUs of *Megasphaera*. These results reveal a partial amelioration of lactate accumulation with the lowest dosing rate (1 billion CFU), as lactate concentration 8 hours post-inoculation was essentially half that of controls. Administering 10 or 100 billion CFUs of *Megasphaera* resulted in near complete metabolism of lactate. Moreover, acetate and butyrate increased linearly with increasing dosage of *Megasphaera*. Based on results of this and similar experiments, our subsequent studies have used a targeted dosage of 10 billion colony forming units, as this appears to provide sufficient protection against lactate accumulation within the rumen.

Table 1. Ruminal organic acid concentrations 24 hours post-challenge for steers dosed with 0, 1 billion, 10 billion, or 100 billion colony forming units of *Megasphaera elsdenii*. Adapted from McDaniel, 2009.

	Dosage size, CFU/animal					
Concentration, mM	**0**	**10^9**	**10^{10}**	**10^{11}**	**SEM**	**Contrasts[a]**
Acetate	29.3	34.0	32.6	40.3	3.91	L, Q
Propionate	17.4	17.1	28.1	19.7	2.39	-
Butyrate	13.3	9.6	16.0	19.3	2.99	L, Q
Lactate	49.8	24.6	3.5	3.0	4.47	M, L

[a] Significant at $P < 0.05$.

M = Placebos versus average of *Megasphaera* treatments
L = Linear effect of dose size
Q = Quadratic effect of dose size

Feedlot cattle normally are managed in ways that are designed to minimize the occurrence of acidosis. Consequently, probiotic strategies aimed at alleviating acidosis logically would be efficacious only for those animals in the population that are more vulnerable to acidosis. It is feasible, however, to modify feeding systems to more fully exploit the benefits of *Megasphaera*. This was the goal of one of our very early performance studies conducted in 2003. Yearling steers were transitioned to diets containing 94% concentrate and 6% roughage in either 8 or 17 days. The 17-day transition regimen used four step-up diets containing 55, 65, 75, and 85% concentrate, with each diet being fed for 4 days, followed by the finishing diet with 94% concentrate, which was introduced on day 17. This normally would be regarded as an aggressive step-up strategy for USA feedlots, as transition programs are more commonly 21 days or more in duration. We also included a far more aggressive step-up regimen in which cattle were fed the diet containing 55% concentrate for three days and the diet with 75% concentrate for four days, followed by introduction of the 94% concentrate finishing diet on day 8. Cattle within each group received 0 or 10 billion colony-forming units of *Megasphaera elsdenii* as a fresh liquid culture prior to initiating the step-up program. Results of the study are shown in Table 2. Improvements in performance associated with *Megasphaera* administration were relatively small when included as part of the les aggressive (17-day) step-up regimen. The *Megasphaera* cattle gained slightly more, and were moderately more efficient. The aggressive, 8-day transition regimen clearly overwhelmed the control cattle, whereas those that received *Megasphaera* were able to cope with the rapid transition to concentrates, yielding carcass weights comparable to those of control cattle fed the more traditional step-up regimen. This strategy has gained favor among nutritionists and feedlot operators in the USA, South Africa, and Australia, as it: 1) provides for modest improvements in animal efficiency and carcass quality; 2) decreases complexity of the feed milling operation by reducing number of diets that must be fed; 3) decreases manure production as a result of decreased consumption of poorly digested feedstuffs.

Table 2. Feedlot performance and carcass traits of steers transitioned to 94% concentrate diets using a 17-day program with 4 step-up diets each fed 4 days; or an 8-day regimen (3 diets) with or without oral dosing of *Megasphaera*

	17-day step-up		*8-day step-up*			
Item	**Control**	**Mega**	**Control**	***Mega***	**SEM**	**P-value**[1]
Dry matter intake, kg/day[a]	7.87	8.19	7.66	7.64	0.22	0.25
Feed intake variation[b]	117	102	119	84	14	--
Average daily gain, kg	1.49	1.52	1.34	1.48	0.07	0.20
Carcass-adjusted ADG, kg	1.25	1.31	1.08	1.25	0.07	0.09
Liver abscesses, %	4.7	5.5	20.1	9.7	6.6	0.47
Carcass weight, kg	334	338	324	335	9.7	0.10

[1] Main effect of *Megasphaera elsdenii*. [a] Feed intake measured daily for individual animals over the initial 28 days on feed. [b] Coefficient of variation for changes in feed intake during the first 3 days on feed.

In a follow-up experiment we further evaluated this concept, but to greater extreme. Our control program consisted of 3 step-up diets, each fed for 6 days, with introduction of a finishing diet (10% corn silage as the roughage source) on day 19. The *Megasphaera* treatment consisted of a single oral dose of approximately 1 billion CFU of *Megasphaera elsdenii*, and direct placement of cattle onto the finishing diet with no step-up program. Fecal output was measured for the first 24 days on feed to assess impact of feeding less forage. Results of the study are shown in Table 3. Cattle placed directly onto the finishing diet with *Megasphaera* consumed 40 kg less roughage per animal for the 115-day feeding period, and fecal output during the first 24 days alone was decreased by approximately 16 kg of dry matter per animal. Cattle in the *Megasphaera* treatment displayed no clinical symptoms of acidosis in spite of being placed directly onto the finishing diet. Additionally, gain of the *Megasphaera* cattle was similar to that of controls, but feed intake was marginally less and feed efficiency tended to improve (~1.4% improvement relative to the control group). Liver abscess rates did not differ between treatments, suggesting that the occurrence of acidosis was no greater in the *Megasphaera* group than in the control group in spite of the abrupt diet change. Finally, as has been noted in many experiments with *Megasphaera* administration, cattle that received *Megasphaera* at initial processing tended to have improved marbling, resulting in an increase in the percentage of carcasses that received market premiums for high quality grades. The modest improvements in quality grade and efficiency are overshadowed by the logistic benefits associated with a greatly simplified feeding management system, however.

Table 3. Performance and health of high-risk calves[a] orally drenched with *Megasphaera elsdenii* at initial processing.

Item	Control	*Megasphaera*	SEM	P-value
No. of cattle	221	222	--	--
Days on feed	115	115	--	--
Initial weight, kg	402	399	2.44	0.12
Dry matter intake, kg	12.8	12.6	0.12	0.07
Silage DM intake, kg/steer	176.3	146.2	1.34	< 0.01
Fecal output, kg/d	2.3	1.7	0.06	< 0.01
ADG, kg	2.26	2.25	0.034	0.65
Feed:gain	5.68	5.60	0.051	0.14
Liver abscess, %	11.8	10.8	2.14	0.75
Choice + Prime	81.5	87.0	2.54	0.07

Accelerated studies have since been completed in commercial feedyards with thousands of animals, and these studies have yielded benefits comparable or superior to those shown in our experiments. Table 4 summarizes results of a feedlot study with 4,950 yearling cattle with average initial body weight of 372 kg. Cattle were assigned to one of three treatments: A **Control**, consisting of a conventional program of four step-up diets fed for 5 days each, with the finishing diet introduced on day 21; an **Accelerated** treatment for which cattle were dosed with *Megasphaera*, fed the step 3 diet for 5 days, then placed onto the finishing diet on day 6; and the **Direct Finish** treatment for which cattle were dosed with *Megasphaera* at initial processing and placed directly onto the finish diet on day 1. Cattle in the Accelerated and Direct Finish treatments gained more weight but ate similar amounts of feed compared to controls, and thus were approximately 4% more efficient than cattle fed the conventional regimen. Carcass value increased by $9-12/animal for the *Megasphaera* groups as well. According to the proprietors, logistical efficiencies associated with diet manufacturing, feed delivery, and manure removal were of even greater magnitude, allowing them to reduce staff hours and the number of feeding vehicles.

Table 4. Feedlot performance and carcass traits of commercial feedlot steers (4,950 head) transitioned to high-concentrate diets using a conventional 21-day program with 4 step-up diets; an accelerated regimen with *Megasphaera* followed by 5 days on the step 3 diet and then placed onto the finish diet; or a *Megasphaera* treatment for which cattle were placed directly onto the finish diet with no step-up diets. Sixteen replicates were fed for an average of 142 days.

Item	Control	Accelerated	*Direct finish*	SEM	P-value
Initial weight, kg	372	372	371	7.3	0.75
Average daily gain, kg	1.55^{a}	1.59^{ab}	1.61^{b}	0.090	0.07
Dry matter intake, kg/day	9.58^{x}	9.48^{y}	9.53^{xy}	0.367	0.19
Feed:gain	6.24^{a}	6.01^{b}	5.97^{b}	0.132	<0.01
Carcass weight, kg	385.1	387.4	386.9	5.76	0.21
Carcass value, $	1,589	1,598	1,601	20.85	0.05

[a] Crossbred calves (504 bulls, 141 steers; initial body weight = 221 ± 4.9 kg) were received from Texas over a 2-week period in January (2 loads per day; on the 14th, 19th, and 26th).

In U.S. feedlot production systems, approximately 40% of cattle enter feedlots as weaned calves, with the remaining 60% being fed as yearlings. Many of these calves originate in the Southeast U.S., and are transported 1,000 to 2,000 kilometers or more to feedlot destinations in the Central and Southern Plains. Throughout this process the calves are subjected to a wide range of stressors, including separation from dams and herd mates, feed and water deprivation, commingling with unfamiliar animals, exposure to novel pathogens, dramatic climate changes, and transportation over long distances. Cattle subjected to the conditions frequently are viewed as being at “high-risk” for development of bovine respiratory disease (BRD). We have studied BRD for a couple of decades, and have come to the conclusion that symptoms of BRD are not readily distinguishable from symptoms of acute acidosis. Moreover, response to antibiotic therapy often is poor, leading to multiple therapeutic treatments at significant cost to producers. Similarities in clinical symptoms and treatment response failures led us to hypothesize that some of the cattle might actually be afflicted with acute acidosis rather than BRD. We completed two studies to evaluate this hypothesis, once of which is summarized Table 5 (Miller, 2013). High-risk calves were purchased from the Southeastern U.S. and transported to Manhattan, Kansas. At initial processing half of the cattle received an oral drench consisting of 1 billion colony-forming units of *Megasphaera elsdenii*. Cattle were placed into pens a fed the same receiving diet consisting of 45% corn silage and 55% concentrate. Calves dosed with *Megasphaera* ate 6.6% more feed, gained 24% more weight per day, and were 15% more efficient than their counterparts in the control group. Moreover, morbidity rate decreased by 30% and death loss decreased from 4.9% to 3.8%.

Table 5. Performance and health of high-risk calves[a] orally drenched with *Megasphaera elsdenii* at initial processing.

Item	Control	*Megasphaera*	SEM	P-value
Initial weight, kg	200	203	4.9	0.23
Average daily gain, kg	0.644	0.798	0.073	0.02
Dry matter intake, kg/day	4.32	4.61	0.167	0.01
Feed:gain	6.80	5.75	0.59	0.05
Morbidity, % of population				
Total morbidity	37.7	26.4	4.81	0.02
1st antibiotic therapy	32.0	22.0	4.13	0.02
2nd antibiotic therapy	17.4	11.5	2.09	0.03
3rd antibiotic therapy	5.9	4.4	1.22	0.36
Mortality, %	4.9	3.8	1.13	0.50
Medication cost, $/head	19.70	17.06	0.98	0.01

[a] Crossbred calves (504 bulls, 141 steers; initial body weight = 221 ± 4.9 kg) were received from Texas over a 2-week period in January (2 loads per day; on the 14th, 19th, and 26th).

Cereal Grains and Grain Processing

Cereals grains typically represent a major proportion of finishing cattle diets, and optimizing digestion of grains thus is key to maximizing profitability of feedlot operations. Grinding, rolling, high-moisture ensiling, and steam flaking all are common practices employed by feeders to enhance digestibility of grains. These processes vary markedly with respect to capital investments, operating costs, and expected improvements in efficiency of feed utilization. Cereal grain hybrids vary markedly with respect to endosperm structure or other attributes, and it is becoming increasingly evident that these factors can have important implications for cattle performance. Our research efforts on this front have focused mostly on use of corn hybrids that have been genetically modified for high expression of amylase, or through screening of sorghum hybrids characterized by a wide range of genetic diversity.

Enogen® Feed Corn

Enogen® Feed Corn (Syngenta Crop Protection; hereafter referred to as Enogen or EFC) was developed through genetic modification to produce grains containing relatively high concentrations of amylase, an enzyme that plays a key role in digestion of starch. Initially developed for use by the fuel ethanol industry, incorporating as little as 10-15% Enogen into fermenters made it feasible to eliminate the need for use of exogenous amylases. More recently, the focus has shifted to use of Enogen Feed Corn in livestock and poultry production. The potential value of a high-amylase hybrid in feedlot production seemed reasonable to us, recognizing that capacity for secretion of pancreatic amylase is limited in cattle. In ruminants, total tract digestion of starch relies heavily on extensive digestion within the rumen. This is readily evident in the comparison of cattle fed grains processed by dry rolling, high-moisture ensiling, and steam flaking, whereby the proportions of starch appearing in feces decrease progressively with more rigorous methods of grain processing. Our first investigation into the use of Enogen Feed Corn was with steam flaking, in which we evaluated flaking characteristics of Enogen compared to a mill-run variety of corn. In our initial attempts to flake Enogen grain we utilized our standardized processing conditions, which include final moisture content of 21%, steam conditioning time of 40 minutes, and final bulk density of 28 lb/bu (360 g/L). Enogen grain rapidly imbibed moisture, leading to swelling and bridging in the steam chest. Enogen also was characterized by extensive starch hydrolysis, as indicated by high starch availabilities and a strong aroma of maltose. In subsequent attempts we decreased conditioning time and increased bulk density to 30.5 lb/bu (393 g/L), effectively increasing throughput from 6 tons per hour to 9 tons per hour. In vitro gas production, which we regard as an excellent indicator of ruminal starch availability, is shown in Figure 3 for commodity grain flaked to a density of 28 lb/bu and Enogen flaked to a density of 30.5 lb/bu. In spite of being processed to a lesser degree, Enogen maintained a greater degree of gelatinization.

In the process of steam flaking, moisture often is added to grain and allowed to temper prior to steam conditioning. This provides means for maintaining consistent moisture concentrations in the final flaked grain, but also facilitates starch gelatinization. Targeted moisture concentrations in the final flaked grain products vary among feedyards, but typically range from 19 to 23%. Steam conditioning times also vary, and are a key determinant of mill throughput, as steam chests are of fixed volume. We evaluated impact of tempering moisture (0, 3, or 6% added water) and steam conditioning times (15, 30, or 45 minutes) across a range of mixtures of Enogen and commodity corn (0, 25, 50, 75, or 100% Enogen as proportion of mixture). There were no interactions between moisture level and grain type, or between conditioning time and grain type. The amount of moisture added during the tempering process increased starch availability linearly (47.2, 49.5, and 51.2 for 0, 3, and 6%, respectively; $P < 0.01$), and tended to improve 14-hour in situ dry matter disappearance of flaked grains (40.3, 40.7, and 42.0 for 0, 3, and 6% added moisture; $P = 0.06$). Not surprisingly, presence of moisture is a key factor in gelatinization of starch. With respect to tempering times, there also were no interactions. Table 6 summarizes effects of steam conditioning times on starch availability, in situ dry matter disappearance, in vitro gas production, and production of volatile fatty acids during in vitro incubations of flaked grains. The results clearly demonstrate a quadratic response to conditioning time, suggesting that grains can be underconditioned or overconditioned. We have observed similar effects in other studies, and previously have noted that availability of both starch and protein decrease when grains are overconditioned, which may be due to formation of early stage Maillard reaction products that are poorly digested. Conditioning times in our study likely cannot be extrapolated directly to commercial flaking systems, nevertheless the potential for overconditioning exists within any system, and given the increased processing costs associated with extended conditioning times, there is financial incentive to identify optimum conditioning times for grains.

Figure 3. Time course for in vitro digestion of steam-flaked grains. Mill-run corn was flaked to a density of 360 g/L (28 lb/bu) and Enogen was flaked to a density of 393 g/L (30.5 lb/bu). Grain type by time interaction, P < 0.0001; Effect of grain type, P <0.001.

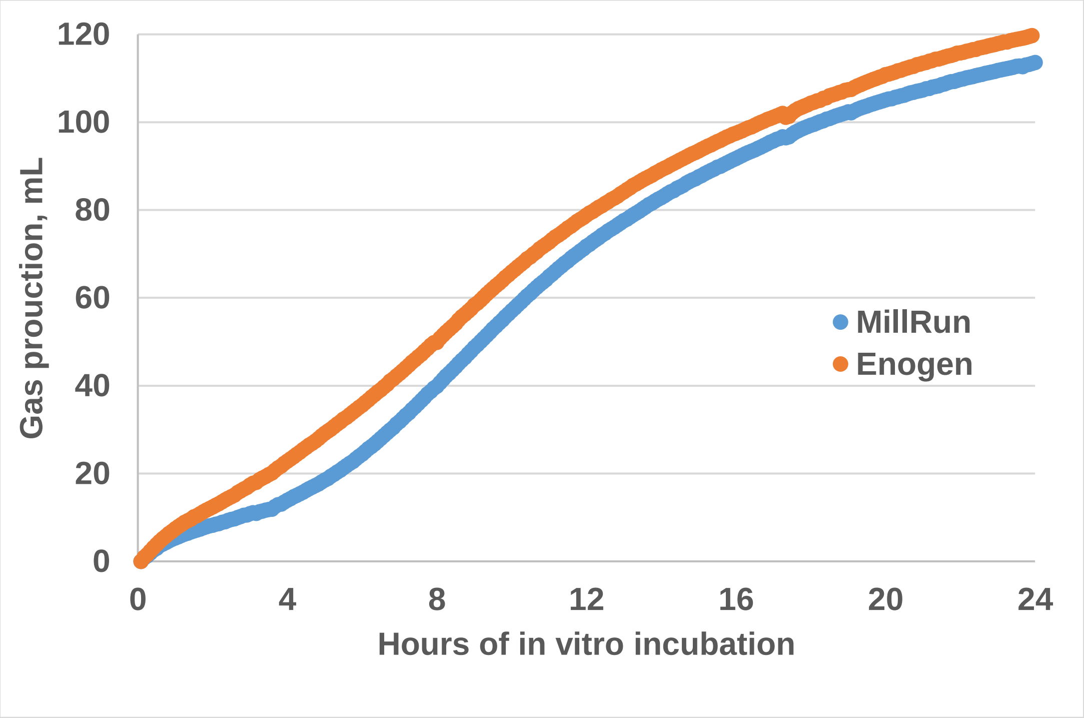


Table 6. Effects of steam conditioning time for flaked Enogen Feed Corn on starch availability, *in situ* dry matter disappearance (ISDMD), and production of fermentative gasses by *in vitro* cultures of mixed ruminal microbes (IVGP)

	Conditioning time, min				*P*-value	
Item	15	30	45	SEM	Linear	Quadratic
Starch availability[1], %	48.5	51.5	47.9	0.60	0.52	< 0.01
ISDMD[2], %	36.6	44.4	42.0	2.66	0.15	0.12
IVGP[3], mL	102.3	121.3	104.9	7.33	0.57	< 0.01
Total VFA[4], mmoles	3.88	4.32	3.78	0.082	0.25	<0.01

[1] Measured using refractive index method (Sindt et al., 2006b) once per sample (90 observations, 30/conditioning time) shortly after flaking.

[2] Measured over 3 d in triplicate by incubating 2.5 g (dry matter; DM) corn flakes in Dacron bags ruminally for 14-h (270 observations, 90/treatment).

[3] Mean gas production during 24-h *in vitro* incubation period. Measured by incubating 3 g (DM) flaked corn as substrate, with 10 mL ruminal fluid inoculum, and 140 mL McDougall's Buffer at 39ºC. Repeated with 2 replicates per sample (90 samples, 180 observations, 60/treatment).

[4] Volatile fatty acids are expressed as mmoles produced per gram of substrate dry matter and were measured by gas chromatography following 24-h incubation of cultures containing 3 g (DM) flaked corn as substrate, 10 mL ruminal fluid inoculum, and 140 mL McDougall's Buffer at 39ºC. Initial incubation was repeated with 2 replicates per sample (90 samples, 180 observations, 60 per conditioning time).

As previously mentioned, the development of Enogen grain targeted the fuel ethanol industry, as the production of ethanol from starch requires conversion of starch to glucose by starch degrading enzymes. Replacement of exogenous enzymes could be accomplished by incorporating as little as 10 to 15% Enogen grain into the process. Consequently, one of our objectives was to determine if Enogen could be used in combination with other grains to enhance digestion of starch from all sources in the diet. Grains were blended, flaked to a density of 28 lb/bu (360 g/L), and then subjected to *in vitro* and *in situ* evaluations. The results, shown in Table 7, indicate that increases in starch availability and *in situ* disappearance were in direct proportion to changes in Enogen content with no indications of a plateau effect. We interpret these observations to suggest that effects of the enzymes are localized, and that there is little migration of enzyme from one grain to the other. It is conceivable that results *in vivo* could be different due to mastication, extensive hydration, and commingling of grains within the rumen, which may more closely emulate conditions in an ethanol fermenter that consists of finely ground grains in a mash form.

Table 7. Effects of increasing proportion of steam-flaked high-amylase corn (EFC) in grain mixtures on starch availability and *in situ* dry matter disappearance (ISDMD)

	Proportion of grain mixture as EFC, %						*P*-value	
Item	0	25	50	75	100	SEM	Linear	Quadratic
Starch availability[1], %	45.0	47.7	50.5	50.7	52.7	0.77	< 0.01	0.16
ISDMD[2], %	34.8	39.5	40.8	43.8	46.0	1.70	< 0.01	0.27

[1] Measured using refractive index method (Sindt et al., 2006b) once per sample (90 observations, 18/treatment) shortly after flaking.

[2] Measured over 3 d in triplicate by incubating 2.5 g (dry matter) corn flakes in Dacron bags ruminally for 14-h (270 observations, 54/treatment).

In vitro and *in situ* evaluations are very useful as screening tools, and essentially allowed us to identify logical targets for processing of Enogen in a feeding study. We followed these experiments with a cattle finishing study involving 700 crossbred beef heifers. Cattle were fed finishing diets with 7% alfalfa hay as roughage, 33 g/ton monensin, no tylosin, and 85.4% flaked corn, either as a commodity grain of unknown hybrid (i.e., mill run) or as Enogen Feed Corn. Mill run corn was flaked to a density of 28 lb/bushel (360 g/L), and Enogen was flaked to a density of 30.5 lb/bu (393 g/L), providing grain throughput of 6 tones per hour for mill-run corn and 9 tones per hour for Enogen. Cattle were fed for a total of 136 days. Dry matter intake did not differ between groups, as presented in Table 8, but gain, final live weight, feed efficiency, and carcass weight all were improved for cattle fed the Enogen-based diet (P < 0.01). Interestingly, liver abscesses were less prevalent in heifers fed Enogen (Table 9). Total tract digestibility of starch in cattle fed flaked grains typically is 99% or more, leading us to question the mechanism by which the cattle achieved a 5% improvement in feed efficiency. *In vitro* experiments indicate increases in propionate in proportion to acetate, which is energetically more favorable. We recently have initiated a study to determine site and extent of digestion, as well as fermentative end products, including volatile fatty acids, lactate, and methane. It is our hope that these data will provide insight relative to the underlying mechanism of efficiency improvements with Enogen corn.

Table 8. Feedlot performance and **c**arcass characteristics of heifers fed diets containing steam-flaked mill-run corn (CON) or steam-flaked high-amylase corn (EFC)[1]

Item	CON	EFC	SEM	*P*-value
Initial body weight, kg	395	394	8.6	0.52
Final body weight, kg	588	599	10.7	< 0.01
Dry matter intake, kg/d	10.00	10.07	0.196	0.78
Average daily gain, kg	1.60	1.69	0.028	< 0.01
Feed:gain	6.25	5.95	0.074	< 0.01
Hot carcass weight, kg	366	372	6.41	< 0.01
Longissimus muscle area, cm^2	94.7	94.6	1.02	0.89
12th rib fat thickness, cm	1.16	1.19	0.045	0.21
Marbling score[†]	605	589	10	0.04
USDA Prime, %	6.6	4.9	1.68	0.33
USDA Choice, %	68.7	70.4	4.44	0.62
USDA Select, %	10.7	11.4	2.58	0.79
USDA sub-Select[2], %	9.0	9.3	2.61	0.68
USDA Yield Grade	2.07	2.15	0.069	0.13
Yield Grade 1, %	23.2	19.5	3.77	0.22
Yield Grade 2, %	49.4	47.7	3.01	0.64
Yield Grade 3, %	25.1	30.9	3.07	0.09
Yield Grade 4, %	2.0	2.0	0.76	1.00
Yield Grade 5, %	0.3	0.0	0.20	0.32

[†] 500 to 599 = Small degree of marbling; 600 to 699 = Modest degree of marbling.

[1] Trial utilized 700 beef heifers in a randomized complete block design, with 25 animals/pen, 14 pens/treatment, and fed 136 d prior to transport to a commercial abattoir wherein carcass data were collected.

[2] Carcasses graded as USDA Standard, Commercial, Utility, or Cutter.

Table 9. Liver abscess prevalence and severity[1] in heifers fed diets containing steam-flaked mill-run corn (CON) or steam-flaked high-amylase corn (EFC)[2]

Item	CON	EFC	SEM	*P*-value
Total liver abscesses, %	34.4	26.6	2.47	0.03
Mild, %	11.9	12.7	1.80	0.73
Moderate, %	14.7	9.2	1.74	0.03
Severe, %	7.5	4.6	1.40	0.11

[1] Severity measured using Elanco scoring system (Liver Abscess Technical Information AI 6288; Elanco Animal Health, Greenfield, IN). [2] Trial utilized 700 beef heifers in a randomized complete block design, with 25 animals/pen, 14 pens/treatment, and fed 136 d prior to transport to a commercial abattoir wherein livers from each carcass were scored.

Identification of Sorghum Cultivars for Cattle Feeding

Water scarcity in the High Plains cattle feeding region is of growing concern, and efforts to decrease water consumption are deemed essential as a means of preserving the long-term economical livelihood of agricultural-based industries in this region. Kansas is the country's leading producer of grain sorghum, a cereal that is well-adapted to production in arid climates. Nevertheless, relatively little sorghum is used in commercial feedlots, as it is perceived to be nutritionally inferior to corn, and also is more costly to process. We have embarked on a long-term project to evaluate a broad range of sorghum cultivars—both parent lines and hybrids, in an attempt to identify cultivars that can be competitive with corn as energy sources for cattle. *In vitro* screening of more than 2 dozen sorghum cultivars was utilized to identify grains with increased susceptibility to digestion by ruminal microbes, as we regard ruminal disappearance as being the most important contributor to total tract starch digestion. Cultivars with superior *in vitro* digestion characteristics that could be sourced in ample quantities were then used in a feeding study.

For our in vitro screening experiments grains were ground through a 1-mm screen and 3grams of each grain were added to culture flasks containing 125 mL of buffer and 25 mL of strained ruminal fluid. Bottles were capped with an Ankom RF gas pressure monitoring apparatus, and incubated under continuous agitation at 39 Celsius. In vitro gas production, dry matter disappearance, and VFA profiles were used as indicators of susceptibility of grains to microbial digestion. In vitro gas production by microbial cultures in two experiments are summarized in Figures 4 and 5. Sorghum cultivars encompassed a broad range of *in vitro* digestion, as indicated by production of fermentative gasses, some of which were far less than that of the corn control that was used as a benchmark, while others were substantially greater than corn. Profiles of volatile fatty acids (data not shown) also revealed broad divergence among cultivars, with a range of 0.64 to 1.69 for acetate:propionate ratio.

Figure 4. Gas production (experiment 1) by *in vitro* cultures using ground grains as substrate and ruminal fluid as microbial inoculum. SEM = 1.2. Effect of cultivar, P < 0.0001.

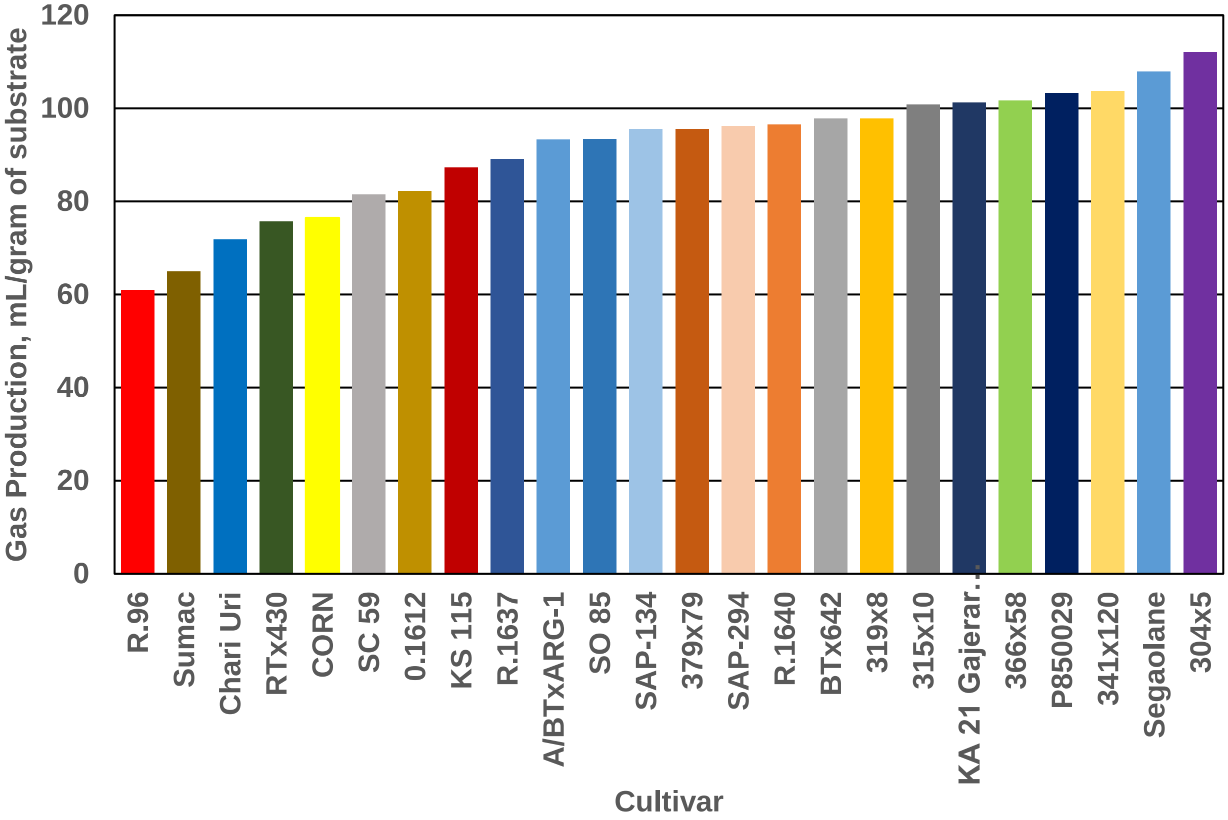
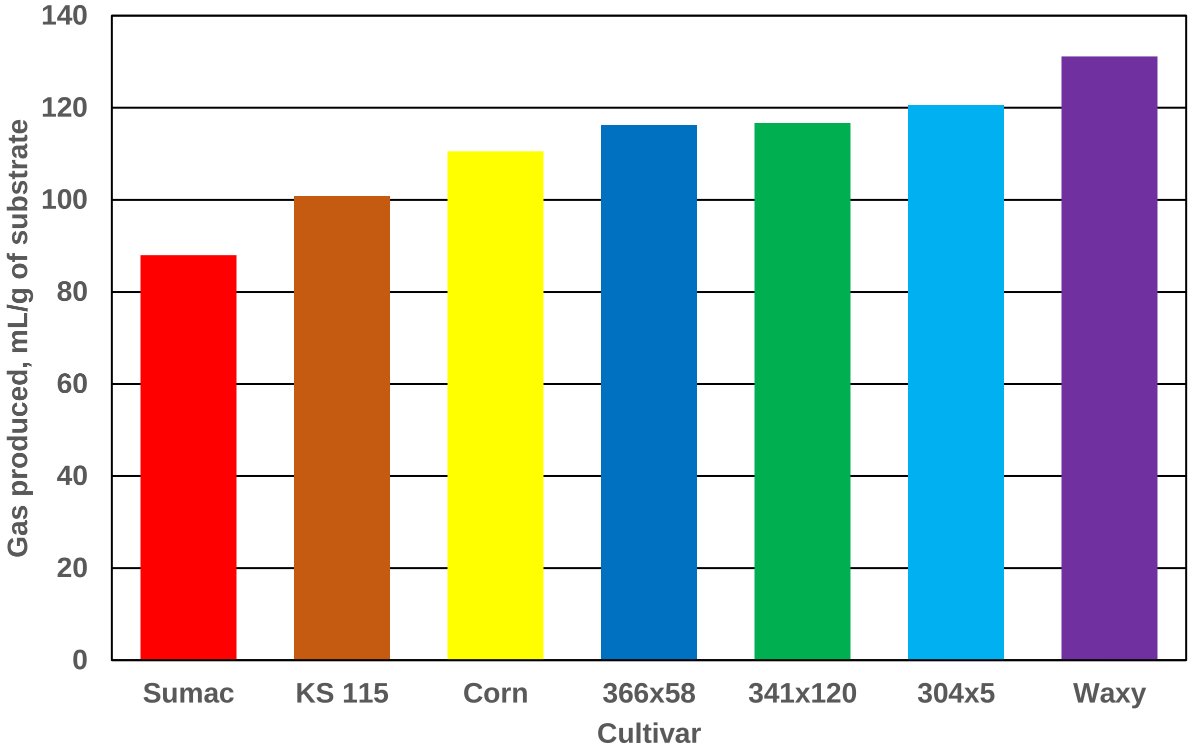


Figure 5. Gas production (experiment 2) by *in vitro* cultures using ground grains as substrate and ruminal fluid as microbial inoculum. SEM = 1.2. Effect of cultivar, P < 0.0001.



The *in vitro* screening data provided guidance in the selection of candidate cultivars for use in a cattle feeding study. Not all cultivars used in the screening work were available in quantities sufficient to perform a feeding trial, but two from the upper range of *in vitro* screening tests were identified-- a waxy sorghum and the 341x120 hybrid. The products were included in backgrounding diets consisting of 40% dry-processed grain, 30% alfalfa hay, 26% corn silage, and 4% supplement and fed to crossbreed beef steers (n=120) for 60 days using the Insentec feeding system that allowed for measurement of individual daily feed intakes. Results of the study are shown in Table 10. Feed intake was greater for cattle fed 341x120 sorghum compared to corn, and was intermediate for cattle fed wax sorghum, yielding greater average daily gain for both groups of cattle fed sorghum compared to those fed the corn-based diet. Feed efficiencies of sorghum-fed cattle were numerically improved, but differences were not significant. The study provides evidence that sorghum can be competitive with corn.

Table 10. Cattle performance during a 60-day backgrounding period when fed diets containing 40% dry-processed corn, 241x120 hybrid sorghum, or waxy sorghum.

	Grain Source				
Item	**Corn**	**341x120**	**Waxy**	***P*** **<**	**SEM**
No. animals	39	40	40	-	-
Initial BW, kg	272^{a}	271^{a}	276^{a}	0.2399	12.8
Final BW, kg	355^{a}	362^{ab}	369^{b}	0.0141	13.7
DMI, kg/d	8.79^{a}	9.48^{b}	9.30^{ab}	0.0397	0.245
ADG, kg	1.37^{a}	1.52^{b}	1.55^{b}	0.0055	0.041
Feed:gain	6.29	6.15	5.88	0.3585	0.220

[a,b]Means within a row without a common superscript letter are different, $P < 0.05$.

Strategies for Control of Liver Abscesses

Cattle fed diets containing large proportions of highly fermentable carbohydrates are predisposed to development of liver abscesses. For more than four decades tylosin has been used as an effective strategy to reduce incidence of liver abscessed in feedlot cattle. There is increasing pressure to reduce antibiotic usage in livestock production systems, owing to concerns over development of antibiotic resistance. We have conducted a series of experiments aimed at reducing the use of in-feed antibiotics for liver abscess control. In the first of these experiments, crossbred steers (n=336) were fed diets with no tylosin, tylosin continuously throughout the 115-day trial, or tylosin fed only the final 34 days on feed. Feed intakes were greater for the two tylosin groups compared to the negative control (Table 11), but neither gain nor efficiency were different among treatments. Overall, abscess rate was relatively low, and was not different among groups. The continuous tylosin group had numerically fewer abscesses compared to the control, but this was not the case for the group fed tylosin only for the final 34 days on feed, suggesting exposure throughout the finishing phase may be necessary to reduce overall incidence.

Table 11. Finishing performance of steers (n=336) fed diets with no tylosin, continuous use of tylosin, or fed tylosin only during the final 34 days of feed.

Item	No tylosin	Tylosin fed Continuously[1]	Tylosin fed final 34 days[1]	SEM	*P* <
Initial BW, kg	456	460	458	6.4	0.79
Final BW, kg	627	631	625	7.9	0.76
DMI, kg/d	10.61^a	10.94^b	10.98^b	0.116	0.01
ADG, kg	1.49	1.48	1.45	0.045	0.68
Feed:gain	7.14	7.41	7.58	0.233	0.19
Carcass weight, kg	392.7	394.4	388.8	4.13	0.23
Liver abscess, %	14.5	12.6	17.0	4.77	0.66
A-	9.2	6.2	9.8	3.72	0.51
A^0	1.8	3.6	2.7	2.20	0.72
A+	3.5	2.8	4.5	2.52	0.80

[1] Tylosin phosphate included in diets at 8 mg/kg dry matter.

[a,b] Means within a row without a common superscript letter are different, $P < 0.05$.

Our second experiment utilized 312 cross bred steers fed for 119 days. Steers were fed diets with no tylosin, tylosin fed continuously at the rate of 8 mg/kg diet dry matter, or tylosin fed in a predetermined intermittent use pattern. Cattle in the intermittent group received tylosin at 8 mg/kg diet dry matter throughout the 3-week step-up phase, and for the balance of the study tylosin was used in an off/on rotation in which it was removed from the diet for two weeks and included for one week. Overall, tylosin use for the intermittent group 60% less than that of the continuously-fed group. Feedlot performance, carcass weights, and liver abscess rates are shown in Table 12. Feed intake, average daily gain, efficiency, and carcass weights were similar among treatments. Cattle fed tylosin continuously or intermittently yielded similar improvements in reduction of liver abscesses compared to cattle in the control group.

Table 12. Finishing performance of steers (n=312) fed diets with no tylosin, continuous use of tylosin, or tylosin fed intermittently in a 1 week on two week off rotation for the duration of the finishing period.

Item	No tylosin	Tylosin fed Continuously[1]	Tylosin fed intermittently[2]	SEM	*P* <
Initial BW, kg	410	411	411	6.7	0.40
Final BW, kg	628	635	626	4.9	0.23
DMI, kg/d	10.89	11.23	10.86	0.235	0.28
ADG, kg	1.83	1.87	1.80	0.036	0.21
Feed:gain	5.96	6.01	6.04	0.097	0.75
Carcass weight, kg	380	383	380	6.1	0.51
Liver abscess, %	21.36^a	7.84^b	9.62^b	4.655	0.01
A-	12.62	6.86	5.77	4.011	0.19
A^0	6.88	0.98	2.88	2.589	0.07
A+	1.94	0.00	0.96	1.378	0.37

[1] Tylosin phosphate included in diets at 8 mg/kg dry matter.

[2] For the intermittent treatment, tylosin was included in diets at 8 mg/kg dry matter during the step-up phase (initial three weeks on feed) and subsequently in rotations of 1 week on and 2 weeks off.

[a,b] Means within a row without a common superscript letter are different, $P < 0.05$.

Conclusions

- The commercialized strain of *Megasphaera elsdenii* can be used effectively to accelerate adaptation to highly fermentable diets. Benefits of the organism manifest in the form of extensive lactic acid metabolism, decreased incidence of acidosis, improved cattle health, increased gain, improvements in efficiency, improvements in carcass quality, and increased carcass value. Additionally, *Megasphaera* can be exploited as a tool to simplify feeding management, making it possible to decrease the number of diets used in the transition phase, decreased time to achieve top finishing rations, and decreasing manure output.
- High-amylase corn (Enogen Feed Corn) is promising as an energy source for cattle, improving feed efficiency while also reducing costs associated with grain processing.
- Sorghum grain cultivars are highly variable with respect to susceptibility to digestion by ruminal microorganisms. Cultivars are available that are competitive with corn and well-suited to use in cattle feeding.
- Tylosin is effective in controlling liver abscess incidence when fed in a discontinuous manner that results in substantial decrease in antibiotic usage.

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Beef sustainability: Beyond the headlines and towards the facts

Sara Place,
Elanco Animal Health

BEEF SUSTAINABILITY

EXECUTIVE SUMMARY

U.S. Cattle Production Sustainability Overview

GREENHOUSE GAS EMISSIONS BREAKDOWN IN THE U.S.

According to the U.S. EPA's greenhouse gas (GHG) emissions inventory, ***2% of U.S. emissions come directly from beef cattle***[1] (methane from cattle belches, methane and nitrous oxide from manure). Total direct emissions from all agricultural production, crops and livestock collectively, were 8.4% of U.S. emissions in 2017. Agriculture, land use, land use change, and forestry combined in the United States are a net sink of CO_2 equivalent (CO_2e) emissions, meaning they removed 172 million metric tons of CO_2e from the atmosphere in 2017.

Table 1. 2017 U.S. Greenhouse Gas Emissions Sources and Sinks[1]

Item	Million metric tons CO_2e	% of US total GHG emissions
Beef cattle	138.3	2%
Other animal ag	117.5	2%
Crop agriculture	286.3	4%
Agriculture total	542.1	8%
Transportation	1800.6	28%
Electricity	1732	27%
All other human-caused GHG emissions	2382	37%
U.S. total GHG emissions	6456.7	100%
Land use, land use change, forestry	-714.1	
Agriculture, land use, land use change, forestry	-172	

WHAT'S THE GLOBAL SITUATION LOOK LIKE

Large disparities in emissions intensities, or GHG emissions per lb of beef produced, exist across regions of the world. The U.S. has one of the lowest beef GHG emissions intensities: 10 – 50 times[2] lower than other parts of the world. Most of this variation is driven by the number of cattle required to produce beef. For example, the U.S. produces around 18% of the world's beef with 8% of the world's cattle herd.[3] Fewer cattle required for a given amount of beef produced means fewer GHG emissions and fewer natural resources required to produce human nourishment. The U.S. is a leader in beef production efficiency because of scientific advancements in beef cattle genetics, nutrition, husbandry practices, and biotechnologies.

CORRECTING THE MISINFORMATION

A quick Google search of beef and GHG emissions will result in a wide range of statistics. Unfortunately, two types of conflation typically occur that muddy the waters. First, globally-relevant statistics are often conflated with U.S. emissions, and second all emissions from livestock production are often ascribed to beef.

Globally, life cycle emissions from livestock production (emissions from feed production to consumer) are 14.5% of GHG emissions. ***Global beef life cycle emissions are 6% of the world's GHG emissions.***[4] The disparity between these two percentages is due to the other forms of livestock agriculture accounted for in the 14.5% figure, such as poultry, pork, and dairy production. In the United States, ***beef cattle production produces 3.7% of U.S. GHG emissions from a life cycle perspective***[5] (adding in feed production, fuel and electricity use, etc. to the 2% estimation from the EPA inventory). The GHG emissions produced by U.S. beef cattle contribute only a fraction of the GHG emissions attributed to global beef production, as most cattle in the world are located outside U.S. borders. ***U.S. beef cattle emissions are less than ½ percent of the world's GHG emissions.***[6]

Figure 1. *U.S. beef cattle production emissions in the context of total global GHG emissions*

Upcycling is the Ruminant Advantage

Cattle are ruminants. This means they have a symbiotic relationship with the microorganisms that live within their specialized stomach compartments that provides them their upcycling superpower. Upcycling is converting something of little to no value to a higher value product. Cattle upcycle every day, converting solar energy in plants that's inaccessible to humans to high-quality protein, micronutrients, and ancillary products such as leather and pharmaceuticals. The U.S. beef cattle industry provides more than two times the high-quality protein (accounting for amino acid profile and bioavailability)[7] to the U.S. food supply than cattle consume: cattle directly contribute to food security. Additionally, beef is rich in micronutrients such as Zinc, Iron, Selenium, Choline, Niacin, Riboflavin, Vitamin B_{12} and Vitamin B_6.

Cattle Provide Far More than Beef

Cattle production results in more benefits to society than just the excellent nutrient package that is beef. Cattle are a source of fiber (leather), fertilizer, fuel, and wealth. Beef cattle operations represent over 1/3 of U.S. farms and ranches[8] – the single largest segment of U.S. agriculture. Cattle production preserves and enhances grassland ecosystems. Cattle grazing can help mitigate the risk of catastrophic wildfires.[9] Cattle grazing lands help regulate and purify the water supplies for major municipalities in the United States.[10] Conservatively, the ecosystem services of cattle ranching and farming provide $14.8 billion of societal value in the U.S.[11] In short, cattle production is a key part of the social fabric of America, from cultural contributions of cowboy Americana to provisioning of heart valves to people. Cattle are a self-replicating, solar-powered plant-based protein source with numerous unmatched co-benefits. Humanity has depended upon cattle production for the whole of civilization and will continue to do so far into the future: beef cattle production is sustainable.

Beef cattle production is always getting better

Despite having a highly resilient and efficient beef production system in the USA currently, cattle producers are always looking for ways to get better. Compared to 1975, it takes 36% fewer cattle[12] to produce the same amount of beef today. This dramatic improvement in efficiency has been driven by improvements in beef cattle genetics, nutrition, biotechnologies, and husbandry practices that result in improved animal well-being. Research and extension and adoption of new knowledge is a continuous process that delivers on incremental improvements in reducing beef cattle production's resource use and environmental impacts. Advancements in grazing land management, genomically-enhanced expected progeny differences (EPDs), methane-inhibitors, integrated crop-livestock systems, water recycling technology, and manure composting are just a few of the examples of new technologies being deployed and tested that will further enhance the sustainability of U.S. beef production. Ultimately, the U.S. beef industry is resilient and well-positioned to continue to provide U.S. and international consumers a superior animal source food in a socially and environmentally responsible manner for decades to come.

For more information, go to www.beefresearch.org/beefsustainability.aspx

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Graduate Student Abstracts

In Vitro Fermentation Characteristics, Feeding Behavior, and Preference of Growing Holstein Dairy Heifers to a Modified Lignin Product

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Five feeds were prepared to meet the nutrient requirements of growing Holstein heifers using two different binders in a pelleted form: a negative control that contained neither molasses nor the new product (treatment 1), a positive control that contained molasses (2% DMB, treatment 2), and three pelleted feeds that contained a new binder in varying concentrations – low (1.0% DMB, treatment 3), medium (2.5% DMB, treatment 4), high (4.0% DMB, treatment 5) – plus molasses (2% DMB). Feeding behavior was recorded via placing heifers (n=8 per treatment) into individual pens (3 × 5 m) with one pelleted feed offered at a time over 60 min period to evaluate feed acceptance. Feeding preference was determined by offering two feed bunks at a time: one containing a reference diet (negative control) and the other containing one of the test diets (one of five treatments). Intake was measured and preference was calculated using the following formula: Preference % = (Test diet) / (Test diet + Reference diet) × 100%. The five feeds were then placed in the rumen fluid for 3, 6, 9, 12, 18, 24, and 48 hours *in vitro*. After incubation, the dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) degradation were determined. Data were analyzed using the Proc Glimmix of SAS (v. 9.4) with animals as the random effect. Significance was declared at $P \leq 0.05$ and trends at $P < 0.1$. Results showed that the animals accepted the pelleted feed containing the high inclusion modified lignin product more than the other feeds ($P < 0.05$). Heifers spent more time ruminating ($P < 0.05$) and eating ($P < 0.05$) on negative control compared with that of other feeds except for the low inclusion. Results also showed that there was a significant effect of treatment on preference (68.0, 69.2, 68.4, 77.8, and 79.0% ± 3%, treatment 1 to 5 respectively, $P < 0.05$) among treatments with treatments 4 and 5 being more preferred over control feeds. There was a significant effect of treatment on *in vitro* DM degradation (32.7, 32.9, 33.9, 35.8, and 36.1 ± 0.53%, treatment 1 to 5, respectively; $P < 0.05$) among treatments. The degree of DM degradation for treatment 4 and 5 was greater than treatment 1, 2 and 3. Significant effect of treatment on *in vitro* OM degradation was observed (29.8, 32.5, 35.2, 36.1, and 38.9 ± 0.77%, treatment 1 to 5, respectively; $P < 0.05$). *In vitro* OM degradation of treatment 5 showed the greatest value among treatments. *In vitro* NDF degradation at 48 hours did not however differ significantly among treatments (23.3, 11.78, 15.6, 17.47, and 23.38 ± 3.8%, treatment 1 to 5, respectively; $P = 0.14$). There was a trend of *in vitro* degradation of ADF at 48 hours over five treatments ($P < 0.1$). Research is needed to evaluate nutritional value of modified lignin products in animal production settings.

Keywords: feeding behavior, feed binder, pelleted feed, growing heifer

Intake and *in Vito* Fermentation Characteristics of Pelleted Feeds Containing Different Binders in Growing Primiparous Holstein Cows Diets

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Five pelleted feeds were prepared to meet the nutrient requirements of growing dairy Holstein heifers. They included a negative control containing neither molasses nor the new product (CTRL-N; negative control), a positive control that contained Ameri-Bond 2X at 2.1% dry matter basis (DMB, CTRL-P; positive control). Three pelleted feeds that contained a new binder in varying concentrations were low (1.6% DMB, DMB-L), medium (3.2% DMB, DMB-M), high (4.8% DMB, DMB-H) inclusion rates. Ten Holstein heifers were placed in individual stalls, and given one feed for one hour to determine the acceptance and intake of each feed. The five treatments were then placed in the rumen fluid for different lengths of time – 0, 4, 8, 12, 18, 24, 48 and 72 hours. Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash were analyzed after incubation. Data were analyzed using the PROC GLIMMIX of SAS (v. 9.4). Significance was declared at $P \leq 0.05$. Preliminary results showed a significant effect of treatment on DM intake (3.72 ± 0.28, 4.41 ± 0.29, 5.11 ± 0.28, 3.62 ± 0.28, and 4.00 ± 0.28 kg, CTRL-N to DMB-H, respectively, $P = 0.001$). Intake of DMB-L (low inclusion) was the highest among all treatments while intake of CTRL-P (positive control) did not differ from that for CTRL-N (negative control), DMB-M (medium) or DMB-H (high). Preliminary results showed that there was a significant effect on overall DM *in vitro* degradation (32.0, 34.4, 35.2, 34.0, and 33.4%± 0.52%, CTRL-N to DMB-H, respectively, $P = 0.0004$) among treatments. The degree of DM degradation for CTRL-P (positive control), DMB-L (low), and DMB-M (medium) were greater than that for CTRL-N (negative control) and DMB-H (high). Overtime organic matter *in vitro* degradation of CTRL-P- through DMB-H were all greater than CTRL-N. Overtime *in vitro* NDF degradation differed among treatments ($P = 0.002$). While NDF degradation did not differ at 0, 24 or 72 h, they differed at 48 h with CTRL-N, DMB-L, and DMB-H not differing from each other but greater than CTRL-P and DMB-M. There was a significant difference in ADF *in vitro* degradation by treatment ($P =0.005$). Overall i*n vitro* ADF degradation of CTRL-N was greater than that of other treatments while difference at 48 h and 72 h showed that DMB-L and DMB-H did not differ from CTRL-N (negative control). Research is continuing to determine the preference of pelleted feeds containing various feed binders.

Keywords: feeding behavior, feed binder, pelleted feed, growing heifer

Effect of feeding supplemental zeolite on measures of nitrogen utilization in backgrounding cattle

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Although it could potentially reduce the loss of ammonia-N from the rumen into blood as a result of its high ion exchange affinity for cations including ammonium ions, the impact of feeding zeolite on nitrogen (N) excretion and reactive N emissions remain to be determined in cattle. Therefore, the objective of this study was to evaluate the effects of feeding supplemental zeolite with a particle size of either 30 microns or 400 microns (US 40 mesh) on ruminal ammonia-N and plasma urea-N concentrations, and N excretion in backgrounding cattle. A total of 6 cannulated beef heifers were used in a replicated 3 × 3 Latin square design with 21-d periods. The dietary treatments were: 1) a typical forage-based backgrounding TMR with no supplement (CON), 2) CON + 30-micron zeolite (ZEO-30), and 3) CON + 400-micron zeolite (ZEO-400). The supplementation rate was 2.5% of diet DM, with the zeolite top-dressed during morning feeding (1100 h). Ruminal fluid (d 19 at 2 h post-feeding), blood (d 21 at 3 h post-feeding), and spot urine samples (d 19 at 1400 and 1900; d 20 at 0300, 0700, 1500, and 2300 h; d 21 at 0500 and 1100 h) were collected and analyzed for metabolites. All data were analyzed using the MIXED procedure of SAS. Dry matter intake was lower ($P = 0.047$) for heifers fed the ZEO-30 diet compared to the CON and ZEO-400 diets (16.3, 15.1, and 15.6 kg/d for CON, ZEO-30, and ZEO-400, respectively). However, there was no diet effect ($P \geq 0.19$) on ruminal ammonia-N concentration (14.6, 14.0, and 12.8 mg/dL for the CON, ZEO-30, and ZEO-400 diets, respectively). Similarly, plasma urea-N concentration did not differ ($P = 0.91$) across diets. Urine output (12.5, 11.2, and 11.1 L/d for the CON, ZEO-30, and ZEO-400 diets, respectively) and urine urea-N output (104, 96, and 93 g/d for the CON, ZEO-30, and ZEO-400 diets, respectively) also did not differ ($P \geq 0.39$) across diets. In summary, although feeding ZEO-30 to beef heifers resulted in an undesirable decrease in DMI, both ZEO-30 and ZEO-400 did not result in changes in measures of N utilization.

Keywords: ruminal nitrogen metabolism, nitrogen excretion, zeolite

In vitro rumen fermentation characteristics of high-grade crystalline vs. low-grade liquid betaine products

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Betaine, a co-product of sugar-beet processing, can be used to feed cattle. Because high-grade betaine (> 90% pure; dry matter (DM) basis) is expensive, feed-grade products with lower betaine concentration are typically used in cattle rations. However, there is limited information on the impact of feeding the feed-grade betaine products on rumen fermentation characteristics. Therefore, our objective was to compare *in vitro* rumen fermentation characteristics of a high-grade betaine (97% purity) to a feed-grade betaine product (32% purity). The ANKOM gas production system was used (ANKOM Technologies, Macedon, New York, USA) to determine the *in vitro* fermentation characteristics of both products at the same inclusion level. Three dietary treatments were used: control (CON) with no betaine added, high-grade crystalline betaine (CRYS), and feed-grade liquid betaine (LB50) at 0.50% of diet DM. The study was a completely randomized design and each treatment was added to 2 ANKOM modules, which contained 1.5 grams of total mixed ration, 15 mL rumen fluid, and 45 mL McDougall's buffer. Two ANKOM modules were also used as blank/run. A total of 3 runs were conducted, each run lasted for 24-hours. Data were analyzed using the mixed procedure of SAS. Crystalline betaine had a greater crude protein content compared to the liquid betaine (72.8 vs 56.7 % DM). Total volatile fatty acid production tended to be greater in LB50 vs CRYS (140.23 vs. 109.14 mM respectively, $P = 0.09$) while no differences ($P > 0.1$) were detected in the molar proportions of acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and caproate, which averaged 49.15 ± 0.81, 29.67 ± 0.69, 13.78 ± 0.51, 1.27 ± 0.04, 3.50 ± 0.12, 2.06 ± 0.09, and 0.58 ± 0.07 % respectively. Final pH did not differ ($P = 0.27$) among treatments and averaged 6.20 ± 0.02. Similarly, *in vitro* true DM digestibility and methane production did not differ ($P \geq 0.15$) among treatments. In summary, the lack of differences in *in vitro* fermentation characteristics between an expensive high-grade and a lower-grade betaine product suggests a similar feeding value when fed at the same dietary inclusion rate.

Keywords: *In-vitro* fermentation, VFA, betaine

In vitro fermentation characteristics of Cheatgrass (*Bromus tectorum* L.) and Medusahead (*Taeniatherum caputmedusae* L.) harvested on Idaho rangeland in different seasons

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Grazing could be used to control invasive grass species on rangeland. However, forage digestibility, which can change as plants mature, influences the amount that cattle can graze. Therefore, the objective of this study was to determine *in vitro* fermentation characteristics of Cheatgrass (*Bromus tectorum* L.) and Medusahead (*Taeniatherum caputmedusae* L.) harvested on Idaho rangeland in summer (June 2018), fall (September 2018) and winter (January 2019). Forage samples were collected from the Reynold Creek watershed (Owyhee county, ID). A batch culture system was then used to evaluate volatile fatty acid (VFA) production (6 h), and 24 h *in vitro* dry matter (IVDMD) and NDF digestibility (IVNDFD). All data was analyzed using the MIXED procedure of SAS. Although the crude protein (CP) content was greater for Cheatgrass than Medusahead in summer (14.1 vs. 10.6%; DM basis), it did not differ in fall and winter. However, for both grasses, there was a dramatic decrease in the CP content in fall and winter (average of 3.9% for both seasons). The TDN content was also greater for Cheatgrass than Medusahead in summer (66% vs. 55%), with the content decreasing dramatically in both grasses in fall and winter. However, the NDF content of both grasses increased with advancing maturity. There was no season or grass species effect ($P \geq 0.24$) on total VFA concentration and the molar proportion of acetate. However, there was a season × grass species interaction ($P = 0.04$) for the molar proportion of propionate; it was greater for Cheatgrass than Medusahead in summer, but not in winter and fall. There was a season × grass species interaction ($P < 0.01$) for 24 h IVDMD and IVNDFD; both IVDMD and IVNDFD were greater ($P < 0.01$) for Cheatgrass than Medusahead when harvested in summer (62.9% vs. 51.3% and 49.7% vs. 44.9%, respectively), with no differences across grass species in winter and fall. However, IVDMD and IVNDFD for both grasses decreased by 45 to 50% and 47 to 59%, respectively, as the season advanced from summer to fall and winter. In summary, forage quality was greater for Cheatgrass than Medusahead in summer; however, there was a dramatic and comparable decrease in DM and fiber digestibility for both grasses beyond summer, which possibly could compromise forage intake, thus, limiting the effectiveness of grazing as a tool to control their spread on rangeland.

Keywords: *in vitro* fermentation, invasive grass species, Idaho rangeland

Impacts of heifer post-weaning residual feed intake classification on reproductive and performance measurements of first, second and third parity Black Angus females

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The objectives of this study were to evaluate heifer post-weaning residual feed intake (RFI) classification on reproductive and performance measurements of first, second and third parity Black Angus females. We analyzed the annual as well as cumulative production of 347 Angus females from birth through weaning of their 3rd calf. Heifer post-weaning RFI was calculated as the actual dry matter intake minus the predicted dry matter intake based on the average daily gain of the contemporary group on an annual basis and ranged from -1.99 to +1.71 kg as fed d^{-1} (SD = 0.55). Heifers were categorized as either low (< -0.50 SD from mean), or average (+\- 0.50 SD from mean) or high (> +0.50 SD from the mean) within year. Julian birth day of heifers was influenced by RFI classification ($P < 0.01$) and displayed both linear ($P = 0.05$) and quadratic ($P = 0.02$) effects with high RFI calves being born earlier in the calving season than average or low RFI calves (71.2 vs 75.3 days). Cow birth weight, weaning weight, as well as yearling weight and body condition were not influenced by RFI classification ($P > .05$). Parity number differed related to weight with third parity pregnancy weights being heavier than second and first, and second parity pregnancy weights being heavier than first but lighter than third pregnancy ($P < 0.01$) with no RFI or RFI*parity interaction ($P > 0.05$). Calf birth weights differed by RFI classification ($P < 0.03$) and parity ($P = 0.01$) with second and third parity calves having heavier birth weights than first parity calves, however, no RFI*parity interaction was observed ($P > 0.05$). Calf 205 day weaning weights and weaning weight ratio was influenced by parity ($P < 0.05$) with increasing weaning weights and decreasing weaning weight ratio for the first calf to the 3rd calf. In contrasts, RFI classification had no effect on weaning weights or weaning weight ratios ($P > 0.05$). Cow conception probability differed by year of pregnancy ($P < 0.05$) and a RFI*pregnancy interaction ($P = 0.02$) was observed with younger low RFI cows tending to have lower conception rates. Cow AI conception probability differed by year of pregnancy ($P < 0.01$) but not RFI classification ($P > 0.05$). In summary, heifer post-weaning RFI classification had minimal effects on beef cattle production and reproductive efficiency.

Key words: beef cattle, heifer, parity, production, reproduction, residual feed intake (RFI)

Effect of feeding ensiled or dried grape pomace on measures of nitrogen utilization in backgrounding cattle

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Because of its content of polyphenolic compounds, feeding grape pomace could alter ruminal and whole-body nitrogen (N) utilization in cattle in a manner that reduces N wastage. However, the preservation method used for grape pomace, whose shelf-life is limited when fresh, could potentially cause changes in the bioactivity of the polyphenolic compounds. Therefore, the objective of this study was to evaluate the effects of feeding either ensiled or sun-dried grape pomace on ruminal ammonia-N and plasma urea-N concentrations, and N excretion in backgrounding cattle. A total of 6 cannulated beef heifers were used in a replicated 3 × 3 Latin square design with 21-d periods. The dietary treatments were (DM basis): 1) a typical backgrounding diet (CON), 2) CON + 15% ensiled grape pomace (ENS), and 3) CON + 15% sun-dried grape pomace (DRY). The grape pomace partially replaced triticale silage in the diet. Ruminal fluid (d 19 at 3 h post-feeding), blood (d 21 at 3 h post-feeding), and spot urine samples (d 19 at 0900, 1500, and 2100; d 20 at 0300, 1200, and 1800 h; d 21 at 0000 and 0600 h) were collected and analyzed for metabolites. Ruminal pH was also measured at 3 and 4 h post-feeding on d 19. All data were analyzed using the MIXED procedure of SAS. There was no diet effect (*P* ≥ 0.37) on dry matter intake (DMI) and ruminal pH at 3 h post-feeding. However, ruminal pH at 4 h post-feeding was lower for heifers fed the DRY compared to the CON and ENS diets (6.19, 6.07, and 6.30 for the CON, ENS, and DRY diets, respectively). There was no diet effect (*P* = 0.45) on ruminal NH_3-N concentration. Similarly, plasma urea-N concentration (*P* = 0.97) and urine output (*P* = 0.30) did not differ across diets. However, urine urea-N output was lower (*P* < 0.01) in heifers fed diets containing grape pomace than the CON diet (84.9, 62.1, and 67.1 g/d for the CON, ENS, and DRY diets, respectively L/d). In summary, although feeding either ensiled or sun-dried grape pomace had no effect on DMI, and rumen ammonia-N and plasma urea-N concentrations, it resulted in a decrease in urine urea-N excretion, which could be beneficial from an environmental standpoint.

Keywords: grape pomace, preservation method, nitrogen utilization

Impact of a commercial direct-fed microbial on cow performance during the periparturient transition

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The transition period is a metabolically demanding time for dairy animals because of the increased nutrient requirements for milk yield. The objective of this study was to investigate the effect of feeding a commercial direct-fed microbial in multiparous and primiparous dairy cows on productive measures during the transition period. Primiparous (n=33) and multiparous (n=35) cows were fed a close-up TMR before calving and a lactation TMR postpartum. Three weeks before expected calving, all animals were blocked to balance parity and body weight, then randomly assigned to either control group (CTRL; n=34) or a direct-fed microbial (DFM; n=34). The DFM animals received a top-dressed DFM (ProTernative) fed daily at 12.5 g per head. Feed intake was measured by weighing the amount of feed given in one day and subtracting what was left from the total both pre- and post-calving. All animals were weighed weekly for the duration of the study. Blood samples were collected weekly. Theses samples were analyzed for glucose concentration, and non-esterified fatty acid concentration (NEFA). Colostrum samples were collected at calving and will be analyzed for IgG, IgA, and IgM content. Somatic cell count was also measured in the colostrum samples. Milk samples were collected once per week postpartum, and all of the samples were analyzed for protein percentage, fat percentage, lactose percentage, urea nitrogen (MUN), and somatic cell count (SCC). All results were analyzed using PROC MIXED in SAS with significance defined as $P \leq 0.05$. All covariate models were selected based on the lowest AIC value. Results showed that the interaction of treatment, parity, and time affected DMI where the DFM multiparous cows consumed more feed week 2 postpartum ($P < 0.001$) while DFM primiparous cows consumed more feed weeks 2 and 3 postpartum. Whereas DFM animals were heavier over the experimental period ($P = 0.06$), there was not a significant difference in BW by treatment, parity, or time interaction ($P = 0.11$). The supplementation of the DFM had a significant effect on milk yield as the DFM animals produced more milk overall ($P = 0.02$). There was a significant interaction of treatment, parity, and time on milk protein percentage as the multiparous DFM animals had a greater percentage of protein at week 2 ($P < 0.01$). There was also a significant interaction of treatment, parity, and time on MUN as the DFM primiparous cows had a greater MUN at week 3 ($P = 0.05$). There was not however a significant difference in milk fat percentage ($P = 0.16$), milk lactose ($P = 0.30$), somatic cell count ($P = 0.44$), plasma glucose ($P = 0.16$), or serum NEFA ($P = 0.27$) by main effects or their interactions. Supplementation of a direct-fed microbial improved DMI, milk production, milk protein content, and increased MUN. Gross feed efficiency (energy-corrected milk/DMI) in week 1 postpartum tended to improve by feeding DFM ($P = 0.06$). Further research is needed to better understand the mechanisms involved in enhancing milk yield by DFM.

Key Words: direct-fed microbial, periparturient, dry matter intake

Relationship between nutrient metabolism during the periparturient period and health measures in a Pacific Northwest dairy herd

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During the periparturient period, dairy cows mobilize stored nutrients to support fetal development and milk production. In addition, this period is characterized by increasing risk of metabolic disorders and infectious diseases. Nutrients such as lipid-soluble vitamins affect immune responses. These vitamins may modulate immune responses toward a more anti-inflammatory status and provide protection against invading pathogens. The objective of the present study was to determine the relationship between dairy cows' serum lipid-soluble vitamins and health status during the periparturient period. Blood samples were obtained from a total of 645 periparturient cows on d-21, d-7, d1, d7 and d14 relative to calving. Sera were collected and analyzed for lipid-soluble vitamins (α-tocopherol, β-carotene, and retinol) via HPLC. Health records of the cows were collected and categorized based on the occurrence of periparturient diseases such as lameness, mastitis, and milk fever. The data were analyzed using the Proc Mixed in SAS with significance declared at $P \leq 0.05$. Results showed that there was a significant interaction between the time relative to parturition and seasons on serum α-tocopherol, β-carotene, and retinol concentration regardless of health status ($P < 0.001$). In addition, there was a significant interaction between the time relative to parturition and seasons on serum α-tocopherol, β-carotene, and retinol for cows with lameness ($P < 0.001$). Furthermore, serum α-tocopherol was affected by time, seasons, and mastitis interaction ($P = 0.001$). Cows with mastitis had significantly greater serum retinol concentration compared with that of healthy cows during postpartum ($P = 0.03$). Beta-carotene showed interaction between the time relative to parturition and seasons in cows with mastitis ($P < 0.001$) but not in cows without mastitis. In summary, the metabolic disorders may affect the lipid soluble vitamins status of periparturient cows and may be associated with cows' other health issues. Further research is taking place to determine theses relationship in calves born from the dams with diseases and disorders.

Key words: lipid-soluble vitamin, health status, dairy cow

Effects of Serum Protein Concentrations on Selected Health Measures within the First 90 Days of Life in Holstein Dairy Calf

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Immune response of neonate calf is functional, but naïve and immature; colostrum is a solution to this problem. We hypothesized that increase incidence in morbidity and mortality would be associated with low serum total protein concentration. An objective of this study was to determine the effect of passive transfer status on morbidity and mortality in neonatal Holstein dairy calves (n=1,558). Calves were purchased from several dairy farms in the western United States and placed in a calf ranch as one day old. Calves were assigned an individual electronic identification and entered into Feedlot Health Management Services proprietary software system, iFHMS (Feedlot Health Management Services, Preston, ID). Cause-specific morbidity and mortality was recorded on an individual calf basis daily from entry to exiting or death at the calf ranch. A 5 mL tube of whole blood was collected from each animal at 48 ± 6h post-arrival. Health events were recorded on an individual calf basis. Whole blood was centrifuged at 2000g for 10 minutes and serum was stored at -22°C until analyzed. Serum total protein (TP) was measured using a digital refractometer as described by (Weaver et al., 2000). Calves were categorized based on proposed USDA serum TP guidelines into poor ($TP < 5.1$ g/dL), fair ($5.1 < TP \leq 5.7$ g/dL), good ($5.8 \leq TP \leq 6.1$ g/dL) and excellent ($TP > 6.1$ g/dL). Data were analyzed using logistic regression models with significance declared at $P \leq 0.05$ and trend at $P < 0.10$. Results showed that there was a significant difference between poor and excellent in the total respiratory disease treatments as well as the total gastrointestinal disease treatments ($P < 0.001$ for both). In addition, there were differences in the ear disease treatments in relation to serum total protein status (poor vs excellent and fair vs excellent; $P < 0.01$). Higher morbidity was demonstrated in calves with lower serum TP values measured within the first few weeks of life, suggesting that other factors affecting immunity and overall health, such as lipid soluble vitamins, may be involved, which warrants further investigation.

Key words: passive transfer, serum total protein, calf health, lipid soluble vitamins