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Update on Palmitic and Stearic Acid Utilization in Dairy Cows.

J. R. Loften, Ph.D. Director of Technical Services, Milk Specialties Global.

Take Home Messages

- Stearic acid is the predominant fatty acid flowing from the rumen to the duodenum of lactating cows for absorption and utilization
- Ca salts of long chain fatty acids offer little or no protection from biohydrogenation in the rumen—they are not rumen inert
- Palmitic acid accumulates in liver triglycerides post-partum while stearic acid declines due to need for stearic acid in many body tissues
- Stearic acid contributes to the partitioning of energy during periods of upregulated gluconeogenesis, increased hepatic FA supply or both
- Feeding lactating cows highly enriched palmitic acid supplements increases circulating and tissue ceramides that cause insulin antagonism leading to body weight loss, lower BCS scores, and increased circulating NEFA in peri-parturient and mid-lactation cows

Introduction

Energy intake remains as the major nutritional challenge for increasing the lactation productivity of dairy cows. Nutritional consultants and their dairy producer clients have used high energy ingredients, such as fat, in their lactating cow diets to meet these ever-increasing energy requirements. Many different sources of fat exist, e.g., tallows, oil seeds, byproducts from ethanol production, and dry ruminally inert fats (RIF) to name a few. In the past 20 years, RIF have increased in use due to their versatility in feed mills and on dairy farms. Due to the success and failure of many different RIF products that have been tested in research trials as well as on dairy farms, interest has been placed on the actual composition of these products - the fatty acids (FA) that are contained within them. These FA acids are primarily stearic acid (C18:0), palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2). In 2014, leading lipid researchers published an invited review in the Journal of Dairy Science regarding the metabolism of C16:0 and C18:0 in lactating dairy cows (Loften et al., 2014). This paper will first address the main findings of that review and then segue into new research findings published since then to aid us in our understanding of how these two FA are metabolized and utilized by the lactating cow.

Rumen Metabolism

Palmitic and C18:0 are considered rumen inert fatty acids with little or no effect on rumen microorganisms due to their complete saturation. The amount of C16:0 leaving the rumen is similar to the amount fed (Wu et al., 1991), while C18:0 leaving the rumen is several times higher

greater than the amount fed. Biohydrogenation of long chain unsaturated FA, oleic (18:1), linoleic (C18:2) and linolenic (C18:3) make significant increases to the pool of C18:0 leaving the rumen. An excellent example of this process was shown by Loor et al. (2004). In their experiment, they compared low and high concentrate diets with or without 3% added linseed oil and observed ruminal flows of FA and their corresponding extent of biohydrogenation. The extent of individual FA biohydrogenation is shown in Table 1.

Table 1. The effects of low and high concentrate diets with or without 3% linseed oil on the extent of ruminal biohydrogenation of unsaturated fatty acids[1].

		Diets			
Fatty acid	Units	LC[2]	LC+3% oil[3]	HC[2]	HC+3% oil[3]
cis-9 C18:1	% BH	58	85	60	74
C18:2	% BH	78	89	75	82
C18:3	% BH	90	97	84	93
Total FA	% of DM	1.6	4.7	1.9	5.1

[1]Adapted from Loor et al., 2004.
[2]LC=35% concentrate-65% forage diet; HC=65% concentrate-35% forage diet.
[3]Linseed oil added to the diet

As more supplemental FA are included in the diet, the amount of biohydrogenation of these FA increased more than the effect of forage:concentrate (F:C). This would indicate that FA contained in forages and grains are less susceptible to rumen biohydrogenation by microorganisms.

Many attempts have been made to protect mono (MUFA) and polyunsaturated fatty acids (PUFA). Reacting free fatty acids with calcium oxide results in the formation of a calcium salt that is believed to resist biohydrogenation by rumen microorganisms. This process results in a dry fat product that allows fat to be added to the ration in feed mills and mixer wagons alike, thus greatly improving the versatility of use. The resulting salts are also much higher in melting point allowing their use in hot environments. However, this claim of rumen inertness is grossly overstated. The effect of rumen microorganisms on Ca salts of long chain fatty acids is similar to adding the fatty acids in the form of oil triglycerides or naturally occurring triglycerides in commonly fed byproducts. Table 2 illustrates this effect.

Table 2. Biohydrogenation of PUFA in calcium salts of unsaturated fatty acids.[1]

Study	Ca Salt	cis-9 C18:1	C18:2	C18:3
Scollan et al., 2001	PFAD[2]	75.7	86.6	88.0
Lundy et al., 2004	Soybean Oil	77.9	92.2	---
Harvatine and Allen 2006	Megalac R	71.6	86.6	84.9
Average		75.1	88.5	86.9

[1]Adated from Scollan et al.,2001, Lundy et al., 2004, and Harvatine and Allen 2006.
[2]Palm fatty acid distillate.

As illustrated in Table 1 and 2, the biohydrogenation of the MUFA and PUFA, whether in calcium salt or triglyceride form, are relatively the same indicating the calcium salts of these FA offer little protection against biohydrogenation. Stearic acid is unique to the lactating cow since it is the only saturated FA that increases in quantity in the small intestine compared to the amount of intake from the diet. A high percentage of the FA entering the small intestine of ruminants are saturated and unattached to a TG. Various isomers of C18 FA formed from incomplete biohydrogenation of long chain PUFA in the rumen also exit the rumen as free FA. These isomers of C18 are important lipid fractions that are very bioactive and interact with C16:0 and C18:0 metabolism in adipose tissue and milk fat synthesis (Linn and Loften, 2015). Only free FA are absorbed from the intestine. Once absorbed across the intestine, FA circulate as free FA bound to serum albumin or incorporated into very low-density lipoproteins (VLDL), high-density lipoproteins (HDL) or chylomicrons. Lipoprotein lipase(LPL) releases FA from these lipid moieties in tissues and cells. Adipose, mammary gland, heart and skeletal muscle have high activities of LPL (Drackley, 2000). The lactating cow has evolved over millennia to biohydrogenate MUFA and PUFA to C18:0 in the rumen and subsequently absorb large quantities in the duodenum for use in many tissues. In fact, C18:0 appears to be the currency of choice by the lactating cow for energy exchange other than glucose.

Intermediary Metabolism of Palmitic and Stearic Acids

Palmitic acid and C18:0 are chemically similar, both are saturated and only differ by 2 carbon units. Their individual metabolism in tissues of dairy cows is quite different. Douglas et al. (2007) found concentrations of C16:0, C18:0, and C18:1 differed among blood and body tissues and changed with progression of the lactation and during negative energy balance, (Table 3). In adipose tissue prior to calving, FA concentrations for C16:0, C18:0, and C18:1 were 27.0, 10.7, and 49.4 g/100g respectively. Post-partum FA concentrations in adipose tissue were similar. Weight percentages of C16:0, C18:0, and C18:1 in plasma were similar during the dry period, but C16:0 and C18:1 increased following parturition and during negative energy balance, while C18:0 decreased (Table 3). This indicates that C16:0 collects in liver tissue after parturition more so than C18:0. In fact, C18:0 declines markedly after parturition indicating its use by other tissues for oxidation for energy or incorporation into milk fat triglycerides (TG).

Rukkwamsuk et al. (2000) investigated the composition of FA in the adipose tissue, serum, and liver of prepartum cows restricted in energy intake or fed excess energy during the dry period. They found that concentrations of C16:0, C18:1, and C18:2 increased significantly in liver tissue during the first week postpartum of cows fed for high-energy intake. Liver C16:0 concentrations decreased in week 3 postpartum for energy-restricted cows, but liver C16:0 concentrations remained significantly higher in cows fed the high-energy diet prepartum. Litherland et al. (2011) found feeding of moderately excessive-energy diets (150% of requirement) early in the dry period affected palmitate metabolism in the liver at parturition. Cows fed the moderate-energy diet early in the dry period had a decreased capacity for palmitate oxidation in the liver at parturition favoring deposition of C16:0 in TG.

Table 3. Fatty acid composition of tissues in pre- and postpartum dairy cows.

	Day relative to parturition			
Fatty acids - g/100g	-45	1	21	65
Adipose				
C16:0	27.0	27.5		
C18:0	10.7	10.8		
C18:1 cis 9	49.4	48.1		
Liver Triacylglycerols				
C16:0	26.8	42.3a	39.0a	26.0b
C18:0	25.5	10.6b	12.2b	24.7a
C18:1 cis 9	23.9	26.6a	26.6a	17.2b
Plasma				
C16:0	16.7	18.2a	14.5b	12.2c
C18:0	16.5	15.6a	13.9b	13.7b
C18:1 cis 9	18.0	19.6a	20.1a	14.5b

[1]Adapted from Douglas 2007

Stearic acid concentrations in liver are relatively unaffected by energy balance during the dry period (Rukkwamsuk et al., 2000). Mashek and Grummer (2003a) observed no net uptake of C18:0 in the caprine liver when 0.3 mM concentrations of C16:0 and C18:0 were perfused into the caudate lobe. However, C16:0 uptake was significantly increased compared with C18:0. Mashek and Grummer (2003b) observed a 2-fold increase in C16:0 metabolism when C18:0 was added to bovine cell hepatic cultures compared with C16:0 alone. This may aid in the removal of excess C16:0 in hepatocytes. Sato and Inoue (2006) observed similar increases of C16:0 in liver, subcutaneous adipose, and perirenal adipose tissues of cows with fatty liver, with C18:0 decreasing in liver and adipose tissue following parturition. These data indicate that C18:0 does not accumulate in tissues of cows in negative energy balance and cows metabolize C18:0 for energy (e.g., β oxidation) in the liver and muscle or secrete large proportions of C18:0 through milk as both C18:0 and C18:1.

White et al. (2011) suggested that the circulating FA that are characteristically increased in transition cows may contribute to increased expression of pyruvate carboxylase mRNA to stimulate gluconeogenesis and maintain oxaloacetate for the tricarboxylic acid cycle. Stearic acid was shown to regulate pyruvate carboxylase promoters (P1, P2, and P3) in different tissues, with C18:0 suppressing promoter P1 and enhancing promoter P3 activity simultaneously. The ability of C16:0 to affect pyruvate carboxylase promoter activity was not tested. These results are illustrated in Table 4. These data suggest that C18:0 contributes to the partitioning of energy during periods of upregulated gluconeogenesis, increased hepatic FA supply, or both. Pyruvate carboxylase activity is critical to the rate of gluconeogenesis from lactate and carbon flux in the tricarboxylic acid cycle. In dairy cows, PC mRNA expression is significantly increased at calving and during feed restriction, whereas other gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase are unchanged (Greenfield et al., 2000).

Table 4. Activity of bovine pyruvate carboxylase promoter 1 (P1), promoter 2 (2), and promoter 3 (P3) after exposure to stearic, oleic, and linoleic acids.

	Treatments				
Item	Control	Stearic acid	Oleic acid	Linoleic acid	P value
P1	6.19[a]	1.58[c]	5.58[b]	6.31[ab]	<0.05
P2	1.09[ab]	2.56[a]	0.77[b]	0.94[ab]	<0.05
P3	0.40[a]	2.55[b]	0.25[a]	0.35[a]	<0.05

[1]Promoter expression, in arbitrary units, expressed as firefly:*Renilla* luciferase relative to pGL3-basic expression.

In adipose tissue, C18:1 was about twice the concentration (g/100 g of FA) of C16:0, and C16:0 was about 3 times the concentration of C18:0. The high concentration of C18:1 in adipose tissue arises from activity of delta-9 desaturase, encoded by the stearoyl CoA desaturase gene, which converts C18:0 to C18:1 in adipose cells (Smith et al., 2006). Age, diet (forage-to-grain ratio), and FA composition of supplemented fat have been shown to affect desaturation of C18:0 to C18:1 in beef cattle; however, cis 9-C18:1 remains the predominant FA in ruminant adipose tissue (Choi et al. 2013) even though cis 9-C18:1 flow from the rumen into the duodenum is significantly less than C18:0. Activity of delta-9 desaturase is likely an adaptive mechanism in ruminants that allows for utilization of the predominant SFA absorbed from the intestine. Acetate, not glucose, is the principal precursor for lipogenesis in ruminants. Acetyl-CoA carboxylase catalyzes the rate-determining step in fatty acid biosynthesis.

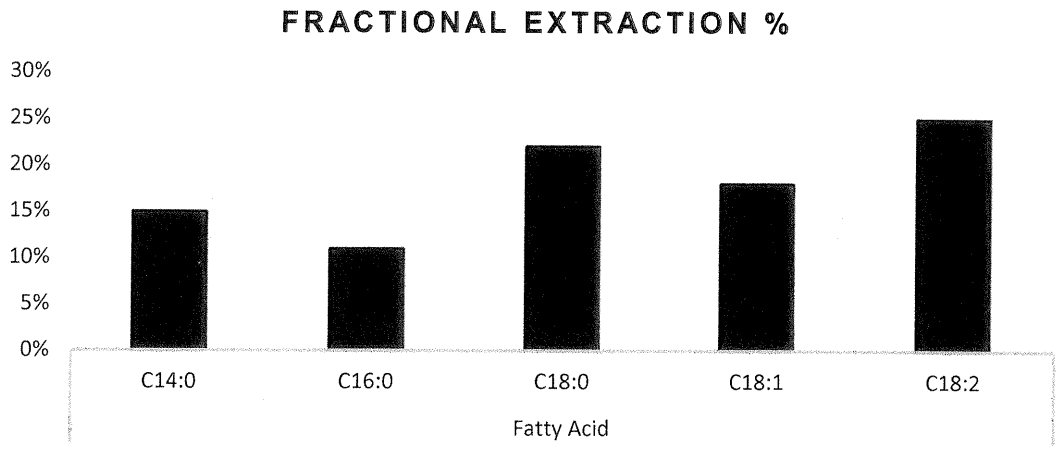
There is little or no ruminant research that has estimated the amount of dietary fatty acid intake that is deposited in adipose tissue. Conversely, there is little ruminant research that can estimate the amount of fatty acids deposited in adipose tissue that has its origin in *de novo* synthesis. Recent research has discovered other relationships between saturated fatty acids and adipose lipogenesis and lipolysis. Summers et al. (2000) reported that 10-25% of fatty acids fed to humans in a test diet were found in adipose tissue determined by arterio-venous differences. The results are illustrated in Figure 1.

There is further research necessary in this area to determine the actual transfer of dietary fatty acids to adipose tissue to more clearly understand the dynamics of transfer efficiency of fatty acids to different tissues. In this example, C16:0 and C18:0 have less than expected transfer from diet to adipose tissue, only 10-20%. If similar ratios of dietary fatty acids are transferred from diet to adipose tissue in ruminants, it would indicate that the accretion of fatty acids in adipose tissue is dependent on *de novo* synthesis from acetate and glucose.

Choi et al. (2013) measured the change in fatty acid composition of subcutaneous adipose tissue of finishing feedlot steers when a nonfat control diet was compared to either 3% added palm oil or 3% added soybean oil. They reported palm oil did not increase palmitic acid or decrease oleic acid in subcutaneous adipose tissue. Soybean oil increased the proportion of α-linolenic acid in adipose tissue. The

C16:0, C18:0 and cis-9 C18:1 composition of adipose tissue appears to be closely regulated and is not greatly affected by dietary fatty acid intake. The results are illustrated in Table 5.

Figure 1. Fractional extraction of dietary fatty acids deposited in adipose tissue in humans after a meal determined by arterio-venous differences, %. Adapted from Summers et al. (2000).



As seen in Table 5, feeding either palm oil or soybean oil to finishing steers did not greatly affect the composition of subcutaneous adipose tissue. The close regulation of adipose tissue composition is due to the maintenance of membrane fluidity of adipocytes. Burns, et al. (2012) treated bovine adipocytes cultures with 150uM solutions of C16:0, C16:1, and cis-vaccenic acid. They reported reduced total fatty acids in palmitic acid-treated cells compared with MUFA-treated cells may be due to an apoptotic effect of palmitic acid supplementation. They suspected that palmitic acid supplementation in bovine adipocyte cultures induced apoptosis, resulting in reduced total lipid accumulation and treatment fatty acid inclusion compared with MUFA-supplemented cells.

Table 5. Fatty acid composition of subcutaneous adipose tissue and muscle of feedlot steers fed a basal finishing diet or diets supplemented with 3% palm oil or 3% soybean oil.[1]

	Treatments			
Fatty acid g/100g	Control	3% Palm oil	3% soy oil	P value
C16:0	27.9	27.6	26.7	0.09
C16:1	5.0a	3.7b	4.3ab	0.05
C18:0	10.4b	12.6a	12.6a	0.02
cis-9 C18:1	42.9	42.7	42.9	0.22
C18:2	1.8	1.9	2.0	0.18
C18:3	0.08	0.07	0.1	0.22

[1]Adapted from Choi et al. 2013

Recent Research Regarding Palmitic and Stearic Metabolism and Utilization

The effects of C16:0 on lipogenesis and lipolysis in adipose tissue has drawn more interest due to field observations of dairy herds losing substantial body condition feeding as low as 0.5 lb./d for 3-6 months. A research trial reported at the 2017 ADSA annual meetings, de Souza et al. 2017, reported a 30kg loss of body weight in the first 4 weeks post-partum compared to a nonfat control. This excessive body weight loss was accompanied by a significant increase in FCM, MF%, MF yield and ECM. However, the increase in milk and component production at the cost of body weight in the first month post-partum is an expensive high interest loan which has to be paid back. Butler and Smith (1989) reported that the magnitude of loss in body weight in the first 3 weeks post-partum is positively correlated to the days to first service. Thus, the partitioning of energy from adipose depots to milk and component production early in lactation may have a marked negative effect on reproduction. It's the "unintended consequence" of high intakes of C16:0 shortly after calving.

Recent research regarding the role of ceramides and sphingolipids in lactating cows has shed new light on the effects of feeding and metabolism of C16:0 in lactating cows. Nearly all stress stimuli (e.g., inflammatory cytokines, glucocorticoids, chemotherapeutics, etc.) induce sphingolipid synthesis, leading to the accumulation of ceramides and ceramide metabolites. While the role of these lipids in the regulation of cell growth and death has been studied extensively, recent studies suggest that a primary consequence of ceramide accumulation is an alteration in metabolism. In both cell-autonomous systems and complex organisms, ceramides modify intracellular signaling pathways to slow anabolism, ensuring that catabolism ensues (Bikman and Summers, 2011). Mathews et al., (2016) observed a decrease in glucose-stimulated NEFA disappearance in mid-lactation cows fed C16:0 by week 7 suggesting the possibility of localized adipose tissue insulin resistance with prolonged C16:0 feeding. They reported C16:0 feeding rapidly increased circulating ceramide which is positively correlated to circulating NEFA. Additionally, C16:0 feeding decelerated a gradual reduction in circulating ceramide observed in control cows as lactation advanced. Rico et al. (2017) confirmed that C16:0 more effectively increases circulating ceramides than stearic acid. Rico et al. (2016) reported ceramide to be inversely associated with insulin sensitivity in mid-lactation palmitic acid-fed cattle. Thus, ceramide accrual likely contributes to insulin antagonism in ruminants. This aids in the explanation of the effects of highly enriched C16:0 fed dairy cows on body weight and body condition loss in peri-parturient and mid lactation cows by influencing insulin antagonism. McFadden (2017) reported that early lactation insulin resistance accelerates adipose tissue lipolysis. This leads to steatosis due to reduced mitochondrial beta oxidation, increased TG esterification, and reduced capacity to export TG in very low-density lipoproteins (VLDL). This effect leads to TG accumulation in hepatic tissue possibly leading to fatty liver issues. In addition, the inhibition of insulin signaling by ceramides increased by consumption of highly enriched C16:0 supplements increase lipolysis. This increased lipolysis aids in explaining the body weight and body condition score loss through mid-lactation.

The newest trend in bypass fat supplementation is to combine calcium salts of palm fatty acid distillate and highly enriched palmitic acid supplements to add C18 back into the diet to overcome these "unintended consequences" (de Souza et al. 2017). We know from rumen biohydrogenation of Ca salts discussed earlier that the net duodenal flow of oleic acid is approximately 30% of what was consumed and C18:0 flow is increased by 70g for every 100 g of intake. So, what was the response due to?

These new findings regarding ceramides, insulin antagonism, increased circulating NEFA, and body weight and body condition score loss have elucidated the "unintended consequences" of feeding highly enriched C16:0 supplements. It is a high cost to the early lactation cow that has to be addressed at some point.

Conclusions

As one can see, the metabolism and utilization of C16:0 and C18:0 is complicated and the possibility of developing the "ideal" long chain fatty acid supplement is daunting. Palmitic and C18:0 have similar structures but very different metabolic functions in the cow. The recent research findings regarding ceramides and their profound effects on insulin sensitivity and lipolysis indicate our need for further research and understanding of the long-term effects on lactation productivity. The release of NEFA from adipose tissue from parturition to mid-lactation now creates a question of how much increase in milk fat content is due to circulating NEFA and how much from feeding C16:0 supplements. As stated earlier, we may be borrowing from adipose tissue which is a high interest loan that you will have to pay back.

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Effects of Fatty Acids on Digestibility and Feed Intake in Lactating Dairy Cows

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Take Home Message

- It is important to consider rumen metabolism of FA both because of biohydrogenation and hydrolysis to free FA.
- Measuring FA digestibility is complicated by a number of technical issues, but overall there appears to be little difference in digestibility between FA.
- Fatty acid digestibility is variable and may be impacted by level of fat and other associative effects, although these are not well characterized.
- Highly enriched saturated fat supplements with very low unsaturated FA may have a strong physical structure that reduces digestibility.
- Calcium salts of FA decrease intake and enriched palmitic acid supplements have also decreased intake in some, but not all, experiments.

INTRODUCTION

Lipids are a broad group of compounds that are soluble in organic solvents and includes waxes, sterols, and compounds that include fatty acids (**FA**) including triglycerides, phospholipids, and glycolipids. Dietary FA are the nutritionally important component of lipids and serve a number of functions in animal nutrition. Fatty acids are a concentrated source of energy, but also serve as integral structural components of cellular membranes and regulatory molecules.

Palmquist and Jenkins (1980) and Palmquist and Jenkins (2017) reviewed the history of fat research in dairy cows including a 1907 review of the effect of fat on milk and milk fat yield (Kellner, 1907). It is interesting that over 100 years later we still are asking some of the same questions, but in the context of a cow with much higher metabolic demands. Interest in fat supplementation has traditionally centered around increasing dietary energy density without increasing dietary fermentability to support energy requirements of 'high producing cows.' More recently, interest in fat supplementation has broadened to increasing milk or milk fat yield, increasing reproductive efficiency (Staples et al., 1998), modifying FA profile of milk (Glasser et al., 2008a; Shingfield et al., 2013), and increasing FA digestibility. Most recently, availability of enriched palmitic acid supplements has provided additional options and spurred a new era of FA research.

Sources of Dietary Fat

Nearly all feed ingredients contain FA, but vary in type and FA profile and have different effects in the rumen. Forages and cereal grains have a low concentration of fat, but their high feeding rates make them a major dietary source of FA. Oilseeds, high fat byproduct feeds, and liquid fats are

economical sources of FA, but care must be taken to not disrupt rumen fermentation. Fatty acids in forages, cereal grains, oilseeds, and most byproducts are in triglyceride form and must undergo lipolysis before absorption. Lipolysis normally occurs at a very fast rate if available in the rumen. This does not appear to be a limiting factor beyond fully hydrogenated oils that do not disperse in the rumen. Lastly, dry fats provide the opportunity to customize absorbed FA profile, but are expensive and differ greatly in FA profile, risks for rumen disruption, and benefits.

Rumen Metabolism of Fatty Acids

Most dietary fat is esterified in triglycerides and, to a lesser extent, glycolipids and phospholipids. Microbial hydrolysis capacity is high resulting in rapid release of free FA in the rumen. Thus, there is little duodenal flow of esterified feed lipids. The exception to hydrolysis appears to be fat supplements that are fully hydrogenated and have a very low iodine value. The high melting temperature and physical form of these products appear to limit their dispersion and interaction with hydrolysis enzymes.

The rumen is a reducing environment so fermentation processes in the rumen cannot break down FA. Normally, duodenal flow of total FA is similar to intake, but rumen microbes can synthesize FA de novo. This is apparent in the rumen outflow of odd and branched chain FA not found in feeds. Ruminal synthesis of FA is increased when feeding low fat diets as the microbes require FA for synthesis of their cellular membranes, although the net increase is rather small. Loss of FA in the rumen has been reported in diets feeding high levels of unsaturated FA, but this is likely a technical error in the formation of modified FA not recovered in analysis (ex. specific trans and hydroxy FA).

The majority of FA in forage and grain feedstuffs are unsaturated and the rumen microbes will biohydrogenate these unsaturated FA forming *trans* FA intermediates and saturated FA. Rumen microbes biohydrogenate unsaturated FA because they are toxic and because they prefer saturated and *trans* FA for their cellular membranes. Biohydrogenation also severely limits absorption of unsaturated FA by the cow and makes stearic acid the most abundant FA in duodenal flow. Glasser et al. (2008b) conducted a meta-analysis of duodenal FA flow data and reported that only 24% of *cis*-9 C18:1, 5% of C18:2 n-6, and 4% of C18:3 n3 reached the duodenum. Over 50% of the *cis*-9 C18:1 and C18:2 n-6 reached the duodenum as C18:0. We recently developed a new method to observe biohydrogenation rates in the rumen by bolus dosing ~150 g of an unsaturated oil into the rumen and sampling over time (Baldin et al., 2018). The method reported very high rates of biohydrogenation (>40%/h) and highlights that rumen available unsaturated FA are rapidly isomerized by the first step of biohydrogenation. An important conclusion from this very high rate is that biohydrogenation is likely limited by the rate of release of the triglyceride from plant material. We currently do not have data on the rate of rumen availability of unsaturated FA, but expect that free oils are more rapidly available than FA in feeds, feeds will differ, for example hard seed coats like cottonseed will decrease release rate, and rate of availability will increase with increased processing. Rumen biohydrogenation of unsaturated FA has some key implications on understanding digestibility, as duodenal FA flow is drastically different from the diet. The difficulty and errors associated with observation of rumen outflow have provided significant roadblocks to this research.

Intestinal Digestibility of Fatty Acids

All FA are hydrophobic and this creates issues with mixing in digesta, interaction with digestive enzymes, and absorption. To be absorbed, fats must be emulsified into small droplets, called micelles, that aid interaction with digestive enzymes and the intestinal brush border. Nutrients are absorbed as simple molecules and dietary fat enters the body through the intestinal brush borders as free FA or monoglycerides. Since most triglycerides are hydrolyzed in the rumen there are few FA absorbed as monoglycerides. As hydrophobic molecules, lipids theoretically could move through the plasma membrane through what is referred to as a "flip-flop" mechanism where the FA would enter one side of the lipid bilayer and then flip to the other. However, there is high expression of Fatty Acid Transport Proteins (FATP) that are expected to provide the bulk of FA transport into the enterocyte. Once in the enterocyte, FA are esterified into triglycerides and packaged with phospholipids and proteins in a chylomicron particle. This packaging allows the hydrophobic lipid to be carried in the blood.

Intestinal absorption of FA is quite different in the ruminant compared to the nonruminant, as duodenal flow in the ruminant is predominantly free FA and is high in saturated FA. Non-ruminants depend on monoglycerides and unsaturated FA for formation of micelles, while in the ruminant lysolecithin is a very potent emulsifier that aids formation of micelles (Doreau and Chilliard, 1997). Lysolecithin is synthesized by a phospholipase that cleaves one FA from a phospholipid. Phospholipids are plentiful in the ruminant duodenum from microbial cell walls and pancreatic secretions. The ruminant has a lower capacity for hydrolysis of triglycerides as differences in pH and lipase enzyme capacity limit the activity of the enzyme and lipolysis may not occur until part way through the small intestine. In the ruminant, there is a large decrease in total tract digestibility when highly saturated TG are fed as they are more resistant to ruminal and intestinal lipolysis (Elliott et al., 1994; Elliott et al., 1999).

A key concept that is not well discussed or understood is the partitioning of lipids in duodenal flow, but I expect that FA are not fully homogenized within digesta. The free FA resulting from triglyceride hydrolysis in the rumen are hydrophobic and may form small fat droplets or emulsions, adhere to feed particles, or be incorporated into microbial cells. The level of dispersion of FA fed in calcium salts and prills has not been characterized to my knowledge and may impact emulsification in the duodenum. Recent work with highly enriched palmitic and stearic acid supplements that contained very low levels of unsaturated FA (<2%) reported large decreases in both palmitic and stearic acid digestibility (Piantoni et al., 2013;2015; Boerman et al., 2017). We recently characterized the physical properties of similar supplements using Differential Scanning Calorimetry (DSC), which measures energy required to heat a sample and provides high resolution melting curves and indications of formation of FA secondary structures including crystals (Shepardson et al., 2017). By this procedure, highly enriched palmitic and stearic acid supplements with very low levels of unsaturated FA had increased melting temperature, increased enthalpy of melting, and indication of formation of FA crystals. We expect this increased stability inhibits emulsification in the rumen and duodenum, but additional work is required.

There are a number of options for investigation of FA digestibility in the cow, but each has its own biases and limitations. Duodenal and ileal cannulated cows allow direct observation of absorption of FA in the small intestine, but are plagued by surgical complications leading to low intake

and digesta flow marker biases. Duodenal cannulated cows allow direct observation across the small and large intestine, but is biased by biohydrogenation of unsaturated FA in the hindgut limiting its use for comparison of individual FA. Abomasal infusion of FA allows comparison of individual FA treatments without the confounding issues of rumen biohydrogenation, but much of the experimental data using this approach used higher doses that may have overran digestive capacity. Lastly, total tract digestibility is confounded by ruminal and hindgut biohydrogenation, but is the simplest approach and has provided the most experimental data. Total tract digestibility is the best suited to characterizing saturated fat supplements that have minimal rumen biohydrogenation. Total fecal collection is the gold standard for digestion studies, but most experiments have relied on flow markers that inherently add error.

Total tract FA digestibility is roughly between 70 to 80% in dairy cows. Differences in digestibility of individual FA are controversial and are difficult to investigate because of rumen and hindgut biohydrogenation discussed above. Multiple well-conducted meta-analyses have observed little difference in digestibility between FA, although FA digestibility generally decreases with increasing fat intake (Glasser et al., 2008b; Schmidely et al., 2008; Boerman et al., 2015). The decrease in digestibility with increasing intake makes assigning digestibility values to supplements difficult. When feeding a fat supplement, we might expect that both digestibility of the basal diet and the supplement decrease due to the higher fat feeding.

There is significant variation in total tract digestibility reported in the literature that reflects both variation between diets and the technical challenges of digestion studies. Factors that explain these differences are not totally clear. For example, basal diet FA level and FA flow of basal diets may have an associative effect on digestibility of fat supplements (Rico et al., 2017; de Souza et al., 2018). However, the extensive biohydrogenation of unsaturated FA, including those in calcium salts, makes prediction of the effect of the associative effect of unsaturated FA difficult to interpret or predict. de Souza et al. (2017) recently reported very exciting work that demonstrated increased FA digestibility with abomasal infusion of a chemical emulsifier (Tween-80). This demonstrates the potential to improve FA absorption. Development of rumen protected emulsifiers may be one such mechanism.

Effect on Intake

A main goal of fat supplementation is to increase energy intake and depression of dry matter intake can limit the benefits of fat supplements. Intake is highly regulated by animal nutrient requirements and metabolic state, and also by the type and temporal pattern of fuels absorbed (Allen, 2000). Fat source, form, and FA profile are significant predictors of intake response. In a meta-analysis, Allen (2000) reported a linear decrease in intake with calcium salts of palm distillate, while saturated FA had no effect on intake. Benson et al. (2001) summarized 11 infusion studies and observed a negative relationship between infused C18:1 and C18:2 FA concentration and intake, with C18:2 creating greater intake depression. Abomasal infusions of unsaturated FA with a lower C16:C18 FA ratio decreased DMI and energy intake (Drackley et al., 1992; Christensen et al., 1994). Finally, four-day continuous intravenous infusion of both palmitic and oleic acid significantly decreased intake, while stearic acid only numerically decreased intake (Vandermeerschen-Doize and Paquay, 1984). Recent work with

enriched palmitic acid supplements have observed decreased intake compared to no fat controls (Lock et al., 2013; Rico et al., 2014), although others have not and the overall decrease in DMI was not significant and energy intake was increased in a recent meta-analysis (deSouza et al., 2016).

CONCLUSIONS

Although FA are a small part of the diet they have the potential to have a big impact on metabolism, efficiency, and profitability and provide opportunities to strategically manage. Overall, the difference in FA digestibility between FA is small, but increasing FA intake and feeding highly enriched supplements reduces digestibility. Fatty acid digestibility is dynamic, although all regulatory points and interventions to alleviate bottlenecks are not clearly understood. Renewed interest in FA supplements continues to develop our understanding of optimizing FA digestion and selection of supplements to reach herd goals.

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The Search for the Optimal Rumen Inert Fat Supplement: Milk Yield, Composition, and Potential Limitations

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Take Home Message

Three rumen-inert fats dominate the market today: calcium salts of palm fatty acids, highly enriched palmitic acid supplements, and supplements containing a blend of saturated (palmitic and stearic) fatty acids. These supplements differ in the production responses that they typically produce. The largest differences are in dry matter intake, milk yield, and milk fat concentration and yield. Moreover, these fat sources have differing effects on energy balance in early lactation. When selecting a rumen-inert supplemental fat, thought must be given to the desired production outcome, economic return on investment, and stage of lactation to maximize benefit and return.

Introduction

Rumen inert fat supplements have long been used in lactating cow rations as an energetically dense feedstuff with the purpose of increasing the energy intake of the cow to support improved production and performance. Three types of rumen inert fat sources account for a majority of the rumen inert fat supplemented to lactating cows today: calcium salts of palm fatty acid distillate (Ca Salts), prilled free fatty acid products that contain primarily palmitic acid (highly enriched palmitic acid; HEPA), and prilled free fatty acid products composed primarily of a combination of saturated palmitic and stearic acids (saturated fatty acids; SFA). Consumer perceptions of these products largely influence the purchasing decision, but these perceptions aren't always accurate. These fat sources differ greatly in cost, dietary composition, and handling characteristics. Moreover, these fat sources differentially affect dry matter intake, milk yield, and milk component concentration and yield. These three supplemental fat sources also possess unique advantages and disadvantages that make them more or less adept for certain feeding situations. Ultimately, when choosing a supplemental fat, the decision should be based on (1) the specific needs and production goals of the target animals, and (2) the expected performance characteristics of the supplemental fat. When a single fat supplement can't meet the requirements, it may be best to combine fatty acid supplements to achieve desired performance outcomes.

CHARACTERISTICS OF THE OPTIMAL RUMEN INERT FAT SUPPLEMENT

The dairy industry has been searching for the 'optimal' rumen inert fat supplement for several decades. An entire body of peer-reviewed literature is dedicated to characterizing the effects of rumen inert fat supplements on dry matter and energy intake, milk and component production responses, and changes in body weight and body condition in dairy cows during various stages of lactation.

If one were to poll members of the dairy industry and inquire about how they would want the optimal supplemental fat to perform, the answers

would likely be variable, but some consistencies would emerge. Most would agree that the optimal rumen inert fat supplement would improve milk yield as well as milk component concentration and yield. Opinions may differ regarding desired effects on dry matter intake. On one hand, the concept of feed efficiency dictates that if cows can maintain a given level of production while consuming less feed, the cows become more efficient and therefore more profitable. On the other hand, increased energy intake is essential to improving production, and it can be challenging to increase energy intake without maintaining or improving dry matter intake. Additionally, the causative link between energy balance, body weight (BW), and body condition score (BCS) in early lactation and subsequent reproductive efficiency cannot be overstated (Butler and Smith, 1989; Carvalho et al., 2014). Perhaps the most important characteristic of the optimal rumen inert fat supplement is the ability to consistently provide a positive economic return on the investment required to feed the supplement. With milk and component prices and heifer replacement costs in constant flux, the characteristics of the optimal rumen inert fat supplement are prone to change and must be re-evaluated frequently.

Performance Responses

With the intent of characterizing the intake and production responses of Ca Salts, HEPA, and SFA supplements, three separate meta-analyses were conducted. Each meta-analysis included studies where lactating cows were either supplemented with the target rumen inert fat source or fed a no-fat control diet. Performance responses reported included dry matter intake (DMI) and NE_L intake, milk yield, milk composition, and milk component yield. Within each analysis, a random-effects model with the random effect of study was fitted as recommended by St-Pierre (2001), and studies were weighted by the inverse of their variance as suggested by Borenstein et al. (2009). Weighted means, mean differences, and standard errors of the differences between means were reported. The average amount of supplemental fat fed was 662 ± 275.5 g/d, 560 ± 92.5 g/d, and 632 ± 222.4 g/d for Ca Salts, HEPA, and SFA supplements, respectively. Results of the Ca Salt meta-analysis (Harris et al., 2017), the HEPA meta-analysis (Sellers et al., 2017a), and the SFA meta-analysis (Sellers et al., 2017b) are shown in Table 1, Table 2, and Table 3, respectively.

Dry Matter and Net Energy Intake

Effects on DMI and NE_L intake were variable across fat sources. DMI was reduced by an average of 0.93 kg/d when Ca Salts were supplemented versus a no-fat control diet ($P < 0.001$), but there was a tendency for Ca Salts to increase NE_L intake (0.87 Mcal/d; $P = 0.115$) likely due to increased energy density of the ration with supplemental fat. Net energy intake was only reported in a small fraction of Ca Salt studies, however. The decrease in DMI when Ca Salts are fed is well characterized. In a review of dietary factors affecting feed intake in lactating dairy cows, Allen (2000) reported that DMI decreased by 2.52% for each 1% inclusion of Ca Salts in the diet. This statement was also reported in the most recent edition of the Dairy NRC (National Research Council, 2001). Onetti and Grummer (2004), Rabiee et al. (2012), and Boerman and Lock (2014) reported similar reductions in DMI in separate meta-analyses (-0.97, -0.64, and -0.58 kg/d, respectively).

Table 1. Effect of supplemental calcium salts of palm fatty acid distillate on dry matter and net energy intake, milk yield, and milk component concentration and yield in lactating dairy cows.

Item	n[1]	Control[2]	Ca Salt[2]	Difference	SED[3]	*P*
DMI, kg/d	66	20.97	20.04	-0.93	0.181	<0.001
NE_L Intake, Mcal/d	13	35.98	36.85	0.87	0.550	0.115
Milk Yield, kg/d	68	31.82	32.61	0.79	0.260	0.002
Milk Fat, %	74	3.42	3.48	0.06	0.023	0.012
Milk Protein, %	67	3.12	3.03	-0.09	0.011	<0.001
Milk Fat, kg/d	59	1.08	1.13	0.05	0.012	<0.001
Milk Protein, kg/d	54	1.01	1.00	-0.01	0.011	0.452

[1]Number of Control vs. Calcium Salt of Palm Fatty Acid Distillate comparisons included
[2]Values reported as weighted means
[3]Standard error of the difference between means

Table 2. Effect of supplemental highly enriched palmitic fatty acids on dry matter intake, milk yield, and milk component concentration and yield in lactating dairy cows.

Item	n[1]	Control[2]	HEPA[2]	Difference	SED[3]	*P*
DMI, kg/d	14	26.98	26.44	-0.54	0.153	0.004
Milk Yield, kg/d	14	38.90	39.05	0.15	0.265	0.568
Milk Fat, %	14	3.74	3.98	0.24	0.026	<0.001
Milk Protein, %	14	3.19	3.15	-0.04	0.013	0.005
Milk Fat, kg/d	14	1.39	1.48	0.09	0.009	<0.001
Milk Protein, kg/d	14	1.24	1.23	-0.01	0.011	0.290

[1]Number of Control vs. Highly Enriched Palmitic Acid Supplement comparisons included
[2]Values reported as weighted means
[3]Standard error of the difference between means

Table 3. Effects of supplemental palmitic and stearic saturated fatty acids on dry matter and net energy intake, milk yield, and milk component concentration and yield in lactating dairy cows.

Item	n[1]	Control[2]	SFA[2]	Difference	SED[3]	*P*
DMI, kg/d	40	22.07	22.01	-0.06	0.181	0.748
NE_L Intake, Mcal/d	13	35.46	37.59	2.13	0.617	0.005
Milk Yield, kg/d	39	32.55	33.78	1.23	0.260	<0.001
Milk Fat, %	39	3.45	3.53	0.08	0.028	0.009
Milk Protein, %	39	3.14	3.12	-0.02	0.017	0.336
Milk Fat, kg/d	38	1.11	1.17	0.06	0.012	<0.001
Milk Protein, kg/d	38	1.01	1.04	0.03	0.007	<0.001

[1]Number of Control vs. Saturated Fatty Acid Supplement comparisons included
[2]Values reported as weighted means
[3]Standard error of the difference between means

When HEPA was supplemented, DMI was reduced by 0.54 kg/d ($P < 0.004$). Effects of HEPA on DMI are inconclusive. Hu et al. (2017) reported no significant decrease in DMI (-0.28 kg/d; $P = 0.556$) when supplements rich in *either* palmitic or stearic acid were fed versus no-fat control diets, and Boerman and Lock (2014) did not differentiate between palmitic acid prills and palmitic/stearic acid prills. No HEPA studies included in the meta-analysis reported effects on NE_L intake, although some very recent studies have demonstrated increased NE_L intake with

HEPA and palmitic acid-enriched triglyceride supplementation in mid to late lactation (de Souza and Lock, 2018a; b).

Supplementation with SFA did not suppress DMI to any appreciable degree (-0.06 kg/d; $P = 0.748$), and increased NEL intake by 2.13 Mcal/d ($P < 0.005$). Rabiee et al. (2012), Boerman and Lock (2014), and Hu et al. (2017) also reported no change in DMI when SFA were supplemented ($P = 0.72$, $P = 0.71$, and $P = 0.45$, respectively).

The relationship between fatty acid composition and effects on dry matter intake appears complicated. Allen (2000) suggests that neither fatty acid chain length nor degree of saturation affect DMI. Instead, the amount of unsaturated fatty acids that reach the duodenum appears to determine degree of DMI suppression (Drackley et al., 1992). The amount of unsaturated fatty acids that reach the duodenum is dependent upon degree of unsaturation of the fat supplement as well as rate of rumen biohydrogenation. The consistent DMI reduction observed with Ca Salts versus HEPA and SFA is therefore likely attributable to a comparatively high degree of unsaturation combined with some amount of rumen protection that delivers a larger amount of unsaturated fatty acids to the duodenum.

Milk Yield

Feeding Ca Salts increased milk yield by 0.79 kg/d ($P < 0.002$), while feeding HEPA did not improve milk yield (0.15 kg/d, $P = 0.568$). The greatest improvement in milk yield was observed when feeding SFA, an increase of 2.13 kg/d ($P < 0.001$). Onetti and Grummer (2004) reported a larger increase in milk yield with Ca Salt supplementation (1.29 kg/d; $P < 0.02$). Rabiee et al. (2012) reported a tendency for a larger increase in milk yield with Ca Salt supplementation (1.55 kg/d; $P = 0.075$), but failed to detect an increase in milk yield with SFA supplementation (0.997 kg/d; $P = 0.312$), likely due to the very small number of SFA studies included in the analysis (n=4 studies). Boerman and Lock (2014) reported similar increases in milk yield for Ca Salts and prilled fatty acids (1.20 and 1.19 kg/d, respectively). Most recently, Hu et al. (2017) reported increases in milk yield for both palmitic/stearic supplements and supplements containing *either* palmitic or stearic acids (1.80 and 1.21 kg/d, respectively; $P < 0.035$).

A correlation between either DMI or NE_L intake and milk production would be expected, as milk production is an energetically demanding process, and within a production system, cows that consume more feed of energy generally produce greater volumes of milk. In the current meta-analyses, however, Ca Salt supplementation resulted in the greatest decrease in DMI, a tendency for improved NE_L intake, and a substantial improvement in milk yield, while HEPA supplementation caused only a moderate reduction in DMI and did not result in any improvement in milk yield. Supplementation with SFA, contrastingly, did not decrease DMI, improved NE_L intake, and resulted in the greatest increase in milk yield. The relationship between DMI and milk yield is complicated by factors such as stage of lactation, energy balance, and genetic potential, among others.

Milk Fat Concentration and Yield

Supplementation with Ca Salts increased milk fat concentration (0.06%; $P < 0.012$) and yield (0.05 kg/d; $P < 0.001$). Onetti and Grummer (2004) and Rabiee et al. (2012) reported similar increases in milk fat concentration and yield, while Boerman and Lock (2014) reported no increase in milk fat concentration ($P = 0.25$) but a similar increase in milk fat yield with Ca Salt supplementation. A similar increase in milk fat concentration (0.08%; $P < 0.009$) and yield (0.06 kg/d; $P < 0.001$) was observed when SFA was supplemented. Rabiee et al. (2012), Boerman and Lock (2014), and Hu et al.(2017) report similar, consistent increases in both milk fat concentration and yield with prilled fat supplementation. The largest increases in milk fat concentration and yield were observed with HEPA supplementation (0.24% and 0.09 kg/d; $P < 0.001$). Hu et al. (2017) reported a much smaller increase in milk fat concentration (0.097%; $P = 0.078$) and yield (0.066 kg/d; $P < 0.006$) for supplements containing *either* palmitic or stearic acid, and reported very similar increases in milk fat concentration and yield for combined palmitic/stearic supplements and supplements contains *either* palmitic or stearic acid.

The interrelationships between dietary fatty acids and yields of milk fatty acids are complex. Dorea and Armentano (2017) recently summarized the effects of various fatty acids and fatty acid combinations on yields of *de novo* synthesized, mixed origin, and preformed fatty acids. They noted that supplementation with a combination of palmitic and stearic acids (SFA) or with a combination of palmitic and oleic acids (Ca Salts) did not change milk fat yield ($P = 0.25$ and $P = 0.76$, respectively), but supplementation with palmitic acid (HEPA) increased milk fat yield ($P < 0.03$). Specifically, palmitic acid supplementation had no effect on yield of *de novo* synthesized fatty acids ($P = 0.55$), greatly increased yield of mixed origin 16-carbon fatty acids ($P < 0.001$), and had no effect on 18-carbon fatty acid yield ($P = 0.66$). Supplementation with palmitic and stearic acid also had no effect on *de novo* synthesized fatty acid yield ($P = 0.73$), and there was a tendency for increased 16- and 18-carbon fatty acid yields ($P = 0.10$ and $P = 0.12$, respectively). In contrast, palmitic and oleic acid supplementation tended to decrease yield of *de novo* fatty acids ($P = 0.06$), had no effect on 16-carbon fatty acid yield ($P = 0.25$), and tended to increase 18-carbon fatty acid yield ($P = 0.11$). The reduction in *de novo* fatty acid yield with combined palmitic and oleic acid supplementation may be due to the interaction of oleic acid with linoleic acid in the basal diet leading to diet-induced milk fat depression (He et al., 2012; Dorea and Armentano, 2017).

Milk Protein Concentration and Yield

Supplementation with Ca Salts decreased milk protein concentration by 0.09% ($P < 0.001$), but did not decrease milk protein yield ($P = 0.452$). Onetti and Grummer (2004), Rabiee et al. (2012), and Boerman and Lock (2014) report a similar decrease in milk protein concentration but no reduction in milk protein yield with Ca Salt supplementation. Similarly, feeding HEPA decreased milk protein concentration by 0.04% ($P < 0.005$), but also did not decrease milk protein yield ($P = 0.29$).

Supplementation with SFA, in contrast, did not decrease milk protein concentration ($P = 0.336$) and increased milk protein yield (0.03 kg/d; $P < 0.001$). Rabiee et al. (2012) reported no change in milk protein concentration or yield with prilled fats, while Boerman and Lock (2014) reported decreased milk protein concentration (0.05%, $P < 0.04$) but increased milk protein yield with prilled fat feeding. Hu et al. (2017) reported very similar results for supplementation with combined palmitic/stearic acids, or with *either* palmitic or stearic acid alone, in that milk protein concentration was decreased to a very small degree (-0.02%), but milk protein yield was increased (0.035 kg/d).

Wu and Huber (1994), in reviewing the relationship between dietary fat supplementation and milk protein concentration, concluded that decreased milk protein concentration when feeding supplemental fat is largely attributable an increase in milk yield in conjunction with insufficient amino acid supply to the mammary gland. Specifically, fat supplementation tends to increase energy available for milk yield, but does not increase amino acids available to the mammary gland, and may actually decrease the amount of amino acids available to the mammary gland due to negative effects of supplemental fat on rumen microbial protein synthesis. Feeding saturated or rumen inert fatty acids should limit any decrease in rumen microbial protein synthesis. The authors (Wu and Huber, 1994) suggested that increasing absorption of limiting amino acids, either through supplementation or by increasing rumen microbial protein synthesis, would help sustain milk fat protein concentration when fat was supplemented.

POTENTIAL LIMITATIONS

Interactions with Energy Balance in Early Lactation

Several authors have demonstrated the relationship between energy balance, body weight, or body condition change in early lactation and subsequent reproductive performance (Butler and Smith, 1989; Jorritsma et al., 2003; Roche et al., 2007; Carvalho et al., 2014). Likewise, cows with increased plasma non-esterified fatty acid (NEFA) concentrations have a lower probability of conception by 150 days in milk (Westwood et al., 2002), as elevated plasma NEFA in early lactation are indicative of mobilization of body energy reserves (Adewuyi et al., 2005). Recently, some focus has been placed on the relationships between rumen inert fat supplementation and changes in energy balance, body weight, and body condition score in early lactation. While the majority of recent supplemental fat experiments are latin square or crossover designs completed during mid-lactation, several studies have determined effects of rumen inert supplemental fat in early lactation.

Supplementing cows in early lactation with Ca Salts appears to have mixed results on BW and BCS change. In multiple experiments, Atwal et al. (1990) reported both increased and decreased BW change when cows were supplemented with 5% Ca Salts in the first 8 weeks of lactation. Simas et al. (1995) and Schneider et al. (1988) reported decreased body weight change. Erickson et al. (1992) reported no effect of Ca Salts on BW, BW change, or NCS when fed starting at 15 days in milk. Kim et al. (1993) and Sklan et al. (1994) reported increased BW change when Ca

Salts were fed. Rodney et al. (2015), in a meta-analysis aimed at determining effects of supplemental fat on reproduction, reported no change in relative risk of pregnancy to service (P = 0.164) or calving to pregnancy interval (P = 0.455) when cows were supplemented with calcium salts of fatty acids in early lactation. Several early lactation Ca Salt studies (Erickson et al., 1992; Sklan et al., 1994; Garcia-Bojalil et al., 1998; Moallem et al., 2007) report increased plasma NEFA values.

There exists very little data regarding effects of HEPA supplementation in early lactation. The HEPA studies included in the current meta-analysis were all conducted on mid-lactation cows 109 – 149 days in milk. Very little effect is seen on body weight and body condition change in these trials, although several report increased plasma NEFA, indicative of body energy store mobilization (Piantoni et al., 2013; Boerman et al., 2015; de Souza et al., 2016). A single experiment recently characterized changes in BW, BCS, and plasma NEFA concentration when HEPA was supplemented during the first 24 DIM (de Souza and Lock, 2017). Cows supplemented with 1.5% HEPA from calving to 24 DIM had decreased BW (668 vs. 709 kg; P = 0.05) and BCS (3.25 vs. 3.34; P = 0.04), decreased plasma insulin concentration (0.21 vs. 0.24 μg/L; P = 0.05), and increased plasma NEFA concentration (0.65 vs. 0.59 mEq/L; P = 0.03) versus non-supplemented control cows, suggesting increased mobilization of body energy stores during the fresh period. Increased body energy mobilization in early lactation with HEPA supplementation may be due to increased hepatic synthesis and accrual of ceramide, which is associated with elevated plasma NEFA concentration (Rico et al., 2016).

In contrast to Ca Salts and HEPA, supplementing SFA in early lactation did not decrease BW , and also did not increase plasma NEFA concentrations (Skaar et al., 1989; Elliott et al., 1995; Moallem et al., 2007; Piantoni et al., 2015), suggesting that SFA may not increase body energy mobilization to the same degree as Ca Salts and HEPA. This may be related to the stearic acid content of SFA. When Rico et al. (2014) supplemented mid-lactation cows with HEPA or highly enriched stearic acid supplements, there was a tendency for cows fed stearic acid to have increased BW and BCS (P = 0.12 and P = 0.11, respectively). Cows fed stearic acid also had lower plasma NEFA concentrations (P = 0.008).

These rumen inert fat sources differ in their effects on energy balance in early lactation, as evidenced by differing effects on BW, BCS, and plasma NEFA concentrations. The importance of reproductive efficiency may support stage-of-lactation dependent supplemental fat feeding in order to limit the decrease in BW and BCS in early lactation.

Conclusion

The search for the optimal rumen-inert supplemental fat source continues. Production responses to Ca Salt, HEPA, and SFA supplements differ drastically, particularly in terms of DMI, milk yield, and milk fat response. The situation is further complicated by effects of rumen-inert supplemental fats on energy balance in early lactation, which can cause detriment to reproductive success. When choosing a rumen-inert fat source, several factors must be considered, including (1) desired change in performance and (2) stage of lactation of the target group of

cows in order to maximize benefit of supplemental fat feeding and provide the greatest amount of return. Priorities should be re-evaluated often, as prices of supplemental fat, milk and milk components, and replacement animals change quite regularly.

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Nutritional Management of Transition Cows

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Take Home Message

- The metabolic, inflammatory, and infectious disease processes that occur during the transition period following calving, are interrelated and can be predicted by alterations in biochemical markers, DMI, and feeding behavior weeks before calving.

- Negative energy balance is a normal homeorhetic process of transition dairy cows, but excessive body weight loss and tissue catabolism is associated with increased risk of disease and death during the peripartum period.

Introduction

The transition period in dairy cows, three weeks before calving to three weeks post calving, is the costliest and most labor intensive for dairy producers. During the transition period, cows have their lowest positive cash flows because of no or low saleable milk production and significant time is spent screening for and treating periparturient diseases. Approximately 90% of all metabolic diseases seen during lactation occur during the transition period (Montagner et al., 2017) with post-partum metritis and mastitis being particularly at high risk during this time (Huzzey et al., 2015). The health risk factors increase the odds of culling or death loss (Wankhade et al., 2017). While increased treatment costs and costs associated with culling or death are the most visible, the most insidious financial losses come from reduced milk yield. Poor transition into lactation can result in the loss of 5 to 10 kg of peak milk production resulting in losses of 900 to 1800 kg for the whole lactation (Wankhade et al., 2017).

Metabolic Challenges during transition

Increased energy demands by the growing calf and the onset of lactation, coupled with reduced dry matter intake (DMI) as parturition approaches causes cows to undergo negative energy balance (NEB) and protein catabolism. Low DMI decreases circulating levels of insulin and increases the release of growth hormone(GH)that increases insulin resistance resulting in decreased available glucose for tissue utilization. This results in lipolysis of fat from adipose tissue and fat mobilization, mainly in the form of nonesterified fatty acids (NEFA). Low glucose availability in hepatocytes causes an incomplete oxidation of NEFA to form ketone bodies, primarily beta-hydroxybutyrate (BHBA). The hormonal and nutrient interactions related to metabolic challenges during the transition period are illustrated in Figure 1. Elevated NEFA and BHBA have strong associations with suppression of DMI, immunosuppression, increased peripartum metabolic and inflammatory disease, and subsequent decreased milk production (Wankhade et al. 2017).

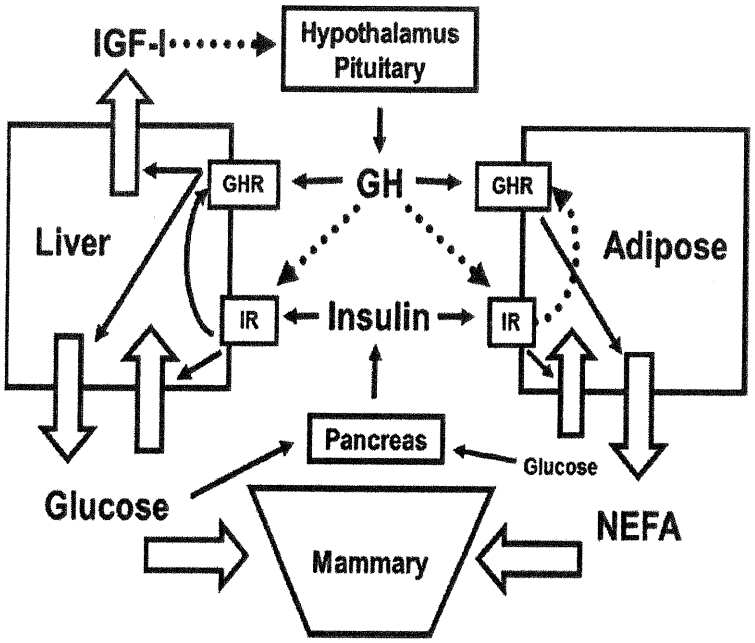

Figure 1. Interaction between growth hormone (GH) and insulin in postpartum dairy cows. Solid lines indicate stimulatory actions, while broken lines infer negative feedback or inhibitory actions. IR: Insulin receptor, GHR: GH receptor (Wankhade et al., 2017).

Mechanism for Inflammatory Status

The classic pathway to increased inflammatory status is pathogen exposure. For example, exposure to a gram-negative organism via the udder or digestive tract excites the inflammatory cascade. A second, very important mechanism is oxidative stress. Cows losing body weight in late gestation or early lactation can release inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) which then causes release of interleukin (IL)-1 and IL-6 from immune cells. In turn, these cytokines cause the liver to produce positive acute phase proteins (+APP), like haptoglobin and ceruloplasmin (Bionaz et al., 2007). These same cytokines decrease the production of negative acute phase proteins (-APP), which may be important for normal liver function. The subsequent hyperexcited immune status can paradoxically make cows susceptible to periparturient disease because the chronic inflammation can cause innate immune cells, neutrophils, to undergo apoptosis (Dervishi et al., 2016b).

Associations between biomarker levels and disease

In a study by Dervishi et al. (2016a), blood samples were taken from 100 cows weekly starting 8 weeks before parturition to 8 weeks post calving. Of the 100 cows, 9 developed a retained placenta (RP). Three of these cows were also affected concurrently with another disease and removed from the study. Six cows remained and were compared to the cows classified as control (CON) that did not develop an RP. Health parameters and biochemical markers in plasma of RP and CON cows -8 and -4 weeks before parturition are in Table 1.

Table 1. Dry matter intake and serum variables at -8 and -4 weeks prepartum for cows developing retained placenta (RP) post-calving.[1]

	-8 weeks parturition			-4 weeks parturition		
Item	CON[2]	RP[3]	P value	CON	RP	P value
DMI, kg/day	16.3	12.5	0.02	15.9	14.3	0.08
Body Temperature, °C	38.2	37.9	0.42	38.5	38.4	0.81
Body Condition Score	2.75	3.13	0.18	3.04	3.21	0.41
Serum Parameters						
Lactate, µmol/L	2455.0	4855.0	0.05	2162.0	5507.0	0.03
NEFA, mmol/L	140.8	193.3	0.41	194.5	182.0	0.87
BHBA, µmol/L	352.0	340.0	0.87	312.0	366.0	0.30
IL-1, pg/mL	317.0	347.0	0.04	321.0	337.0	0.02
IL-6, pg/mL	19.2	100.0	0.02	48.2	69.4	0.34
TNF-α, ng/mL	0.34	1.26	0.03	0.27	1.31	<0.01
Haptoglobin, mg/mL	0.19	0.34	0.58	0.15	0.07	<0.01
SAA, ug/mL	8447.0	24584.0	0.04	3461.0	19378.0	0.03

[1]Dervishi et al. (2016a)
[2]Cows not diagnosed with RP post-calving.
[3]Cows (n=6) diagnosed with an RP within the first 2 weeks post-calving.

The authors concluded cows developing an RP had significantly higher levels of lactate at -8 weeks and -4 weeks pre-partum. Cows developing RP increased in concentration of lactate in plasma from -8 to -4 weeks, whereas CON cows decreased. Comparisons of serum cytokines yielded significant elevations of IL-1 and TNF-α at both -8 and -4 weeks prior to parturition.

In this study (Dervishi et al., 2016a), the +APP serum amyloid A (SAA) was more closely associated with development of RP than serum haptoglobin (Hp). Cows with RP had significantly higher SAA throughout the study (P>0.05). Paradoxically, in the week prior to diagnosis, serum Hp was lower for cows that developed RP. No differences were found in serum NEFA or BHBA between CON and RP cows at -8 or -4 weeks. Cows that developed RP had a lower DMI at -8 weeks (P<0.02) and tended (P>0.8) to be lower at -4 weeks prior to parturition. The interrelationship between DMI, placenta, liver and mammary gland on metabolism and inflammation have been illustrated by Loor et al. (2005) and shown in figure 2. Cows that developed RP produced significantly less milk than control cows (32.6 vs. 42.4 Kg/day; P>0.05) during week 4 post-calving. At the time of RP diagnosis, no differences in milk fat percent or milk protein percent between CON and RP cows was found, however, somatic cell count (SCC) was higher in milk of RP cows (108,670 cells/ml) than CON cows (28,330 cells/ml).

The results of principal component analysis on the data set comparing healthy cows and cows that developed RP showed that the first 2 principle components, TNF-α and IL-6 covered 69.1% of the observed variance (Dervishi et al., 2016a).

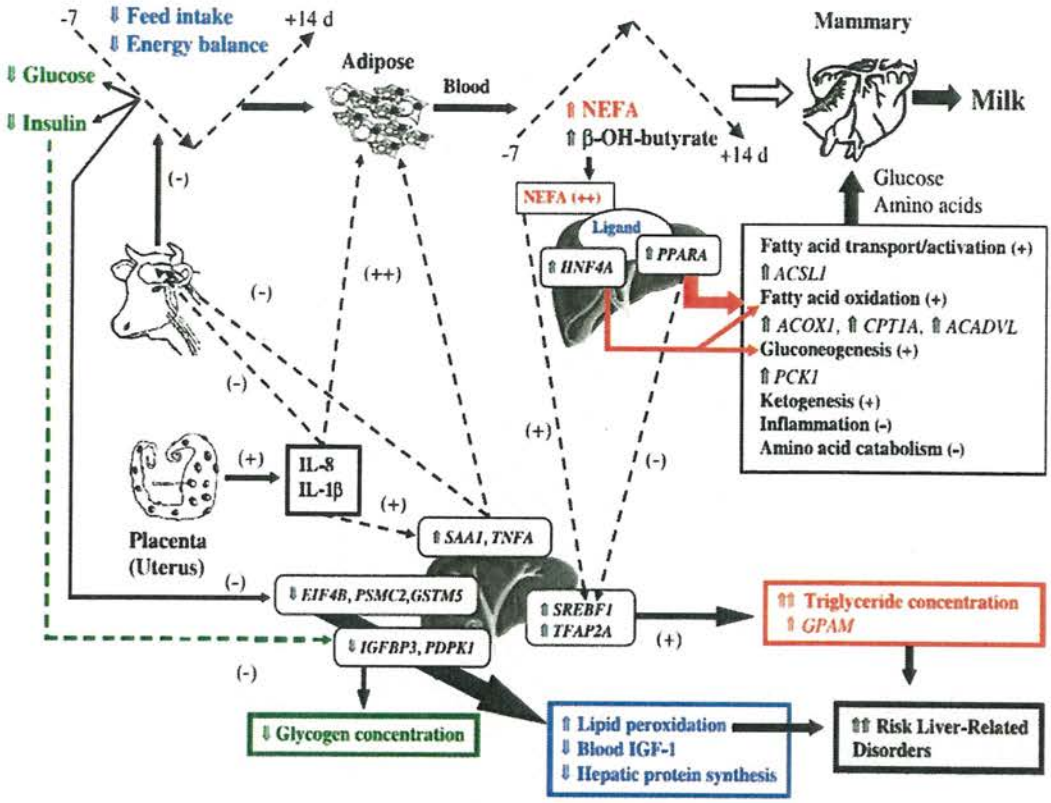

Figure 2. Interrelationships between dry matter intake and physiological events in liver, placenta and inflammation during the periparturient period. Loor et al. (2005).

Associations with body condition loss

Montagner et al. (2017) published a study on thirty-five mature (≥3rd lactation) Holstein cows housed together and fed the same ration from 21 days pre-partum to 30 days post-partum. Blood samples we taken for biochemical analysis at -21, -14, -7, -3, 0, 3, 6, 9, 23, and 30 days relative to calving. Body weights and body condition score (BCS) were measured weekly throughout the study period. In this study, all cows with a negative health event were excluded (15 cows) and the remaining 20 comprised the data set. Cows were retrospectively split into two cohorts, cows that either gained or lost BCS -3 to -1 week prior to parturition.

Cows in the group that lost BCS (LBC) had higher serum Hp prepartum ($P>0.004$) and post-partum ($P>0.004$). There was no difference in -AAP or paraoxonase (PON) pre-calving, but levels were significantly reduced post calving in the LBC group. Another -APP and albumen (ALB) concentration were lower both pre-partum ($P>0.03$) and post-partum for the LBC group. Cows in the LBC group were at a higher inflammatory state at the cellular level as indicated by a higher monocyte count pre- (1121 vs. 708, $P<0.05$) and post-partum (663 vs. 331, $P<.03$) compared to CON. The LBC also had a greater number of animals with a neutrophil: monocyte ratio >1, 41.6% vs. 13.7% for CON ($P<0.03$).

Cows in this study by Montagner et al., (2017) that gained BCS (GBS) produced significantly more milk (3.0 kg/day, $P<0.03$) from 16 to 41

days in milk. This is important because by eliminating cows with negative health events, this study could measure the negative association between systemic inflammation and production outcomes. No differences between these groups were found in relation to energy metabolism. Serum levels of glucose, insulin, and NEFA were not different. The authors (Montagner et al., 2017) theorized LBC cows demonstrated a more intense inflammatory status resulting in decreased -APP, PON and ALB. These decreases were attributed to impaired liver function directly as caused by proinflammatory conditions (BCS loss) during the transition period.

Associations with Reduced Feeding times and DMI

Huzzey et al. (2007) and Goldhawk et al. (2009) separately published papers on data from the same group of study subjects, 101 cows of mixed parity housed in group pens of 20 cows with free stalls. These cows entered the study 25 days prior to their expected calving date and monitored for 21 days post-parturition. Cows pre- and post-calving were monitored for DMI, feeding (eating) time and drinking behavior. They were further monitored for signs of puerperal metritis (Huzzey et al., 2007) and sub-clinical ketosis (SCK) (Goldhawk et al., 2009).

Huzzey et al. (2007) divided cows into groups retrospectively, based on uterine health status post-partum. Three cohorts were examined, healthy, mild metritic, and severely metritic. Severely metritic cows consumed less DM than healthy cows beginning two weeks prior to parturition through the end of the study period, 3 weeks postpartum ($P<0.05$). Cows that developed mild metritis, ate less DM beginning the week prior to parturition through 3 weeks following parturition ($P<0.05$) (Figure 3). In the last week of gestation, healthy cows, mildly metritic cows, and metritic cows decreased their DMI 0.15, 0.21, 0.33 kg/day respectively. Thus, cows that were eating less going into the last week of gestation had a more precipitous drop in DMI. Cows that subsequently developed severe metritis, ate less feed than healthy cows beginning 2 weeks prior to parturition ($P<0.05$). The authors (Huzzey et al., 2007) concluded each 10-min reduction in feeding time increased the odds for severe metritis by 1.72. Each kg of decreased DMI increased the odds for metritis nearly 3 times. Also of note, healthy cows had greater daily milk production than the mildly and severely metritic group with the mildly metritic cows averaging 5.7 kg/day less milk and the severely metritic group 8.3 kg/day less milk ($P < 0.001$) than healthy cows during the first 3 weeks of lactation. The magnitude of the milk production difference increased as days from parturition increased throughout the first three weeks of lactation ($P<0.05$).

Average daily DMI pre-partum was also the best predictor for post-calving SCK (Goldhawk et al., 2009). During the week before calving and the 2 weeks post-calving, cows with SCK had lower DMI ($P<0.05$). Every 10 min decrease in daily eating time increased the risk of SCK 1.9 times. During the same time, a 1 kg decrease in DMI increased the risk of SCK by 2.2 times. Milk production did not differ between healthy and SCK cows during the first 21 days postpartum.

The effect of feeding rumen protected methionine and/or choline:

To mitigate the effects of the NEB and protein catabolism during the transition period, a variety of housing and feeding management

strategies have been studied. Many of these have attempted to either increase DMI or caloric density of the diet. Sun et al. (2016) compared feeding rumen protected choline (RPC), rumen protected methionine (RPM), or a combination of both during the transition period to mitigate the negative sequala associated with energy balance and systemic inflammation. In their study, 48 cows were randomly assigned to four treatment groups: control, 15 g/d RPC, 15 g/d RPM, or 15 g/d RPC and 15 g/d RPM. Cows were fed treatments starting 21 days prepartum through 21 days postpartum. The study investigated the effect of RPC and RPM on energy balance, production, antioxidant capacity and immune response.

In the prepartum period, only serum glucose was increased for the RPC treatment ($P<0.04$). On the day of calving, RPC group had higher DMI ($P<0.04$) and higher serum glucose ($P<0.001$) than the control group. The RPM also had higher serum glucose($P<0.002$) and lower BHBA ($P<0.043$) on the day of calving vs. control. Post-partum, DMI and glucose were increased and NEFA and BHBA were decreased in RPC, RPM and RPC-RPM treatment groups vs. control $P<0.05$).

Lipid metabolism was also positively affected by both RPC and RPM. Supplementation with RPC, RPM, or both significantly reduced total cholesterol, low-density lipoproteins, and increased plasma very low-density lipoproteins and apolipoprotein B 100 ($P<0.05$). Plasma level of total bilirubin and alkaline phosphatase in cows also was reduced with feeding RPC, RPM or both compared to controls. Only RPM lowered blood urea nitrogen. Both RPC and RPM reduced inflammatory cytokines IL-2, and IL-6 ($P<0.05$) while only RPC lowered TNF-α ($P<0.007$).

This study by Sun et al. (2016) shows RPM and RPC can have an important role in the health of transition cows as well as minimizing NEB and optimizing protein nutrition in early lactation. Rumen protected methionine as well as RPC help modulate lipid metabolism which is an essential component of energy metabolism in dairy cows. Exact mechanism(s) of how RPM and RPC function in minimizing NEB remains to be elucidated, but possibilities include promoting beta oxidation of fatty acids such as NEFA and C18:0 in the liver, reducing lipid deposition in the liver and/or enhancing gluconeogenesis (Sun et al. 2016). Through improved liver function and minimizing NEB, RPM and RPC reduce inflammatory cytokines, TNC-α factor and oxidative stress which are all indices of health in transition cows as shown in the studies of Dervishi et al. (2016a) and Montagner et al., (2017). While RPM and RPC appear to function similarly in the health and immunity of transition cows, they also appear to be act independently. Methionine, unlike choline, has a role in protein metabolism and utilization in dairy cows. As shown by Sun et al. (2016), transition cows fed RPM had lower blood urea nitrogen values than cows fed control or RPC suggesting RPM, and not RPC, promoted nitrogen utilization.

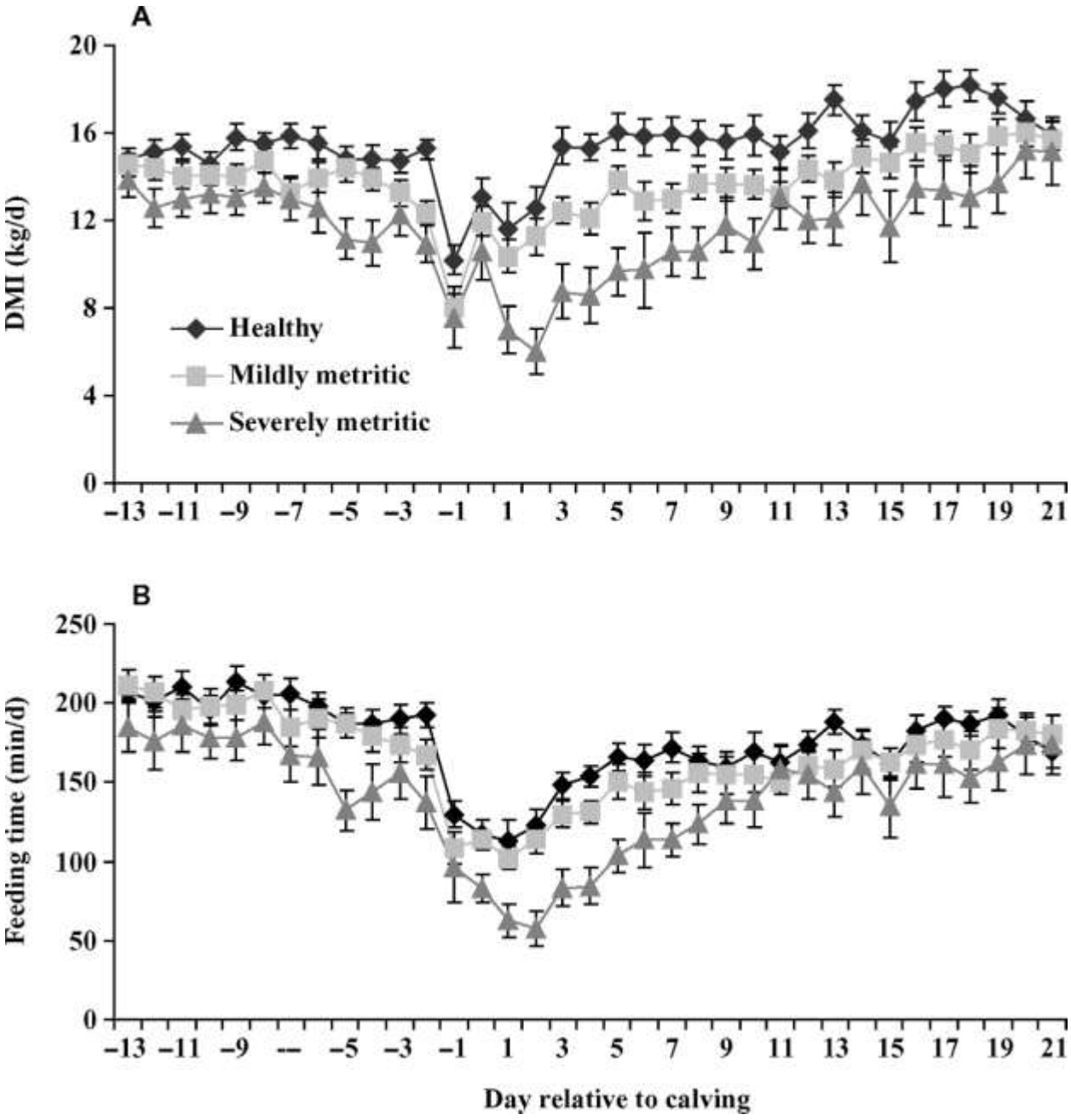

Figure 3. Arithmetic mean (□} SE) daily DMI (kg/d; A) and feeding time (min/d; B) of healthy (n = 23), mildly metritic (n = 27), and severely metritic (n = 12) Holstein dairy cows from 13 d before until 21 d after calving. Huzzey et al. 2007.

Summary

- Presence of increased levels of proinflammatory cytokines, and acute phase proteins in systemic circulation is associated with increased risk of metabolic, inflammatory, and infectious disease.
- Cows that lost body condition during the peripartum, had altered levels of acute phase proteins, and signs of reduced liver function.

- A decrease of 10 min in daily feeding time the week before calving increases the risk of subclinical ketosis 1.9 times.

- Feeding RPM or RPC during the transition period lowered levels of proinflammatory cytokines, acute phase proteins and NEFA and increased DMI both pre- and post-partum.

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Effects of Altering Energy Balance and Feed Intake on Reproductive Performance in Lactating Cows

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TAKE HOME MESSAGE

- Nutritional strategies and feeding management during precalving and post-calving periods impact health, productivity and fertility of high-producing dairy cows.
- Formulating diets to meet requirements of the cows but avoid over-consumption of energy may improve outcomes of the transition period and lead to improved fertility.
- Management to improve cow comfort and ensure good intake of the ration is pivotal for success.
- Rumen-protected methionine and lysine added to the diet of Holstein cows during the transition period and early lactation improves the survival rate of preimplantation embryos.
- Impacts of the transition program should be evaluated in a holistic way that considers disease occurrence, productivity, and fertility.

INTRODUCTION

During the transition period from late gestation through early lactation, the dairy cow undergoes tremendous metabolic adaptations (Bell, 1995). The endocrine changes during the transition period are necessary to prepare the dairy cow for parturition and lactogenesis. As peak milk yield increases, the transition period for dairy cows becomes much more challenging with most infectious diseases and metabolic disorders occurring during this time (Drackley, 1999; Grummer, 1995). Decreased dry matter intake (DMI) during late gestation influences metabolism leading to fat mobilization from adipose tissue and glycogen from liver.

Nutrient demand for milk synthesis is increased in early lactation; if no compensatory intake of nutrients is achieved to cope with the requirement, reproductive functions (i.e., synthesis and secretion of hormones, follicle ovulation, and embryo development) may be depressed. Milk production increases faster than energy intake in the first 4 to 6 weeks after calving, and thus high yielding cows will experience negative energy balance (NEB). Nutritional strategies and feeding management during pre-calving and post-calving periods impact health, productivity and fertility of high producing dairy cows. Formulating diets to meet requirements of the cows but avoid over-consumption of energy may improve outcomes of the transition period and lead to improved fertility. Management to improve cow comfort and ensure good intake of the ration is pivotal for success. Impacts of the transition program should be evaluated in a holistic way that considers disease occurrence, productivity and fertility.

Studies over the last 2 decades clearly established the link between nutrition and fertility in ruminants (Robinson et al., 2006; Wiltbank et al., 2006; Grummer et al., 2010; Santos et al., 2010; Cardoso et al., 2013; Drackley and Cardoso, 2014). Dietary changes can cause an immediate and rapid

alteration in a range of humoral factors that can alter endocrine and metabolic signaling pathways crucial for reproductive function (Boland et al., 2001; Diskin et al., 2003).

Strategies have been used to improve the reproductive performance of dairy cows through alteration of nutritional status (Santos et al., 2008; Santos et al., 2001). In other species, dietary supplementation with specific amino acids (AA), such as arginine, glutamine, leucine, glycine, and methionine, had beneficial effects on embryonic and fetal survival and growth through regulation of key signaling and metabolic pathways (Del Curto et al., 2013; Wang et al., 2012). Methionine and lysine are the most limiting AA in lactating cows (NRC, 2001), but supplementation of diets with crystalline methionine and lysine has been excluded because free methionine and lysine are quickly and almost totally degraded by the microorganisms in the rumen (NRC, 2001).

REPRODUCTION, NUTRITION, AND HEALTH

A widespread assumption is that fertility of modern dairy cows is decreasing, particularly for Holstein-Friesen genetics, at least in part because of unintended consequences of continued selection for high milk production. This assumption has been challenged recently (LeBlanc, 2010; Bello et al., 2012). There is a wide distribution of reproductive success both within and among herds. For example, within five California herds encompassing 6,396 cows, cows in the lowest quartile for milk yield in the first 90 days postpartum (32.1 kg/day) were less likely to have resumed estrous cycles by 65 days postpartum than cows in quartiles two (39.1 kg/day), three (43.6 kg/day) or four (50.0 kg/day); milk production did not affect risk for pregnancy (Santos et al., 2009). Changes in management systems and inadequacies in management may be more limiting for fertility of modern dairy cows than their genetics per se.

Dairy cows are susceptible to production disorders and diseases during the peripartal period and early lactation, including milk fever, ketosis, fatty liver, retained placenta, displaced abomasum, metritis, mastitis, and lameness (Mulligan et al., 2006; Ingvartsen and Moyes, 2013; Roche et al., 2013). There is little evidence that milk yield per se contributes to greater disease occurrence. However, peak disease incidence (shortly after parturition) corresponds with the time of greatest NEB, the peak in blood concentrations of nonesterified fatty acids (NEFA), and the greatest acceleration of milk yield (Ingvartsen et al., 2003). Peak milk yield occurs several weeks later. Disorders associated with postpartum NEB also are related to impaired reproductive performance, including fatty liver (Rukkwamsuk et al., 1999; Jorritsma et al., 2003) and ketosis (Walsh et al., 2007; McArt et al., 2012). Cows that lost >1 body condition score (BCS) unit (1-5 scale) had greater incidence of metritis, retained placenta, and metabolic disorders (displaced abomasum, milk fever, ketosis) as well as a longer interval to first breeding than cows that lost <1 BCS unit during the transition (Kim and Suh, 2003).

Indicators of NEB are highly correlated with lost milk production, increased disease and decreased fertility (Ospina et al., 2010; Chapinal et al., 2012. However, the extent to which NEB is causative for peripartal health problems rather than just a correlated phenomenon must be examined critically (Roche et al., 2013). For example, in transition cows inflammatory responses may decrease DMI, cause alterations in metabolism, and predispose cows to greater NEB or increased disease (Bertoni et al., 2008; Graugnard et al., 2012 and 2013; Ingvartsen and Moyes, 2013). Inducing a degree of calculated NEB in

mid-lactation cows similar to what periparturient cows often encounter does not result in marked increases in ketogenesis or other processes associated with peripartal disease (Moyes et al., 2009). Nevertheless, early postpartal increases in NEFA and decreases in glucose concentrations were strongly associated with pregnancy at first insemination in a timed artificial insemination (TAI) program (Garverick et al., 2013). Although concentrations of NEFA and glucose were not different between cows that ovulated or did not before TAI, probability of pregnancy decreased with greater NEFA and increased with greater glucose concentrations at day 3 postpartum (Garverick et al., 2013). In support of these findings, early occurrence of subclinical ketosis is more likely to decrease milk yield and compromise fertility. McArt et al. (2012) reported that cows with subclinical ketosis detected between 3-7 days after calving were 0.7 times as likely to conceive to first service and 4.5 times more likely to be removed from the herd within the first 30 days in milk compared with cows that developed ketosis at 8 days or later.

Cows that successfully adapt to lactation (Jorritsma et al., 2003) and can avoid metabolic (Ingvartsen et al., 2003) or physiological imbalance (Ingvartsen and Moyes, 2013) are able to support both high milk production and successful reproduction while remaining healthy. Decreased fertility in the face of increasing milk production may be attributable to greater severity of postpartal NEB resulting from inadequate transition management or increased rates of disease. Competition for nutrients between the divergent outcomes of early lactation and subsequent pregnancy will delay reproductive function. Because NEB interrupts reproduction in most species, including humans, inappropriate nutritional management may predispose cows to both metabolic disturbances and impaired reproduction. Cows must make "metabolic decisions" about where to direct scarce resources, and in early lactation nutrients will be directed to milk production rather than to the next pregnancy (Friggens, 2003).

Different nutritional strategies have been proposed to improve reproduction of the dairy cow with no detrimental effect on lactation performance. Feeding high quality forages, controlled-energy (CE) diets, or adding supplemental fat to diets are some of the most common ways to improve energy intake in cows (Cardoso et al., 2013; Drackley and Cardoso, 2014; Mann et al., 2015). Reproduction of dairy cattle may be benefited by maximizing DMI during the transition period and minimizing the incidence of periparturient problems (Cardoso et al., 2013; Drackley and Cardoso, 2014).

Some AA are limiting for optimal milk production as evidenced by an increase in milk yield, percentage of milk protein, and milk protein yield after supplementation with specific, rumen-protected amino acids. The first two limiting amino acids for milk production are considered to be methionine and lysine (NRC, 2001). There is evidence that methionine availability alters the follicular dynamics of the first dominant follicle (Acosta et al., 2017), the transcriptome of bovine preimplantation embryos *in vivo* (Penagaricano et al., 2013) and its contents (Acosta et al., 2016).

PREPARTUM DIETARY CONSIDERATIONS

Controlling energy intake during the dry period to near calculated requirements leads to better transition success (Grum et al., 1996; Dann et al., 2005 and 2006; Douglas et al., 2006; Janovick et al., 2011; Graugnard et al., 2012 and 2013; Ji et al., 2012). Research drew from earlier reports that limiting nutrient intakes to requirements of the cows was preferable to over-consumption of energy (e.g., Kunz et al., 1985). Cows fed even moderate-energy diets (1.50 – 1.60 Mcal NEL/kg DM) will easily consume 40 – 80% more

NEL than required during both far-off and close-up periods (Dann et al., 2005 and 2006; Douglas et al., 2006; Janovick and Drackley, 2010). Cows in these studies were all less than 3.5 BCS (1-5 scale) at dry-off, and were fed individually TMR based on corn silage, alfalfa silage, and alfalfa hay with some concentrate supplementation. We have no evidence that the extra energy and nutrient intake was beneficial in any way. More importantly, our data indicate that allowing cows to over-consume energy even to this degree may predispose them to health problems during the transition period if they face stressors or challenges that limit DMI (Cardoso et al., 2013).

Prolonged over-consumption of energy during the dry period can decrease post-calving DMI (Douglas et al., 2006; Dann et al., 2006; Janovick and Drackley, 2010). Over-consuming energy results in negative responses of metabolic indicators, such as higher NEFA and beta-hydroxybutyrate (BHB) in blood and more triacylglycerol (TAG) in the liver after calving (Douglas et al., 2006; Janovick et al., 2011). Alterations in cellular and gene-level responses in liver (Loor et al., 2006 and 2007) and adipose tissue (Ji et al., 2012) potentially explain many of the changes at cow level. Over-consumption of energy during the close-up period increases the enzymatic "machinery" in adipose tissue for TAG mobilization after calving, with transcriptional changes leading to decreased lipogenesis, increased lipolysis and decreased ability of insulin to inhibit lipolysis (Ji et al., 2012). Controlling energy intake during the dry period also improved neutrophil function postpartum (Graugnard et al., 2012) and so may lead to better immune function.

Allowing dry cows to consume more energy than required, even if cows do not become noticeably over-conditioned, results in responses that would be typical of overly fat cows. Because energy that cows consume in excess of their requirements must either be dissipated as heat or stored as fat, we speculated that the excess is accumulated preferentially in internal adipose tissue depots in some cows. Moderate over-consumption of energy by non-lactating cows for 57 days led to greater deposition of fat in abdominal adipose tissues (omental, mesenteric, and perirenal) than in cows fed a high-bulk diet to control energy intake to near requirements (Drackley et al., 2014). The NEFA and signaling molecules released by visceral adipose tissues travel directly to the liver, which may cause fatty liver, subclinical ketosis and secondary problems with liver function.

Data from our studies support field observations that controlled-energy dry cow programs decrease health problems (Beever, 2006). Other research groups (Rukkwamsuk et al., 1998; Holcomb et al., 2001; Holtenius et al., 2003; Vickers et al., 2013) have reached similar conclusions about controlling energy intake during the dry period, although not all studies have shown benefits (Winkleman et al., 2008). Application of these principles can be through controlled limit-feeding of moderate energy diets or ad libitum feeding of high-bulk, low-energy rations (Janovick and Drackley, 2010; Janovick et al., 2011; Ji et al., 2012) as proposed by others (Beever, 2006).

Nutritionally complete diets must be fed and that the TMR must be processed appropriately so that cows do not sort the bulkier ingredients (Janovick and Drackley, 2010). Feeding bulky forage separately from a partial TMR or improper forage processing will lead to variable intake among cows, with some consuming too much energy and some too little. Underfeeding relative to requirements, where nutrient balance also is likely limiting, leads to increased incidence of retained placenta and metritis (Mulligan et al., 2006). Merely adding a quantity of straw to a diet is not the key principle; rather, the diet must be formulated to limit the intake of energy (approximately 1.3 Mcal NEL/kg DM, to limit intake to about 15 Mcal/day for typical Holstein cows) but meet the requirements for protein, minerals and

vitamins. Reports of increased transition health problems or poor reproductive success (Whitaker et al., 1993) with "low energy" dry cow diets must be examined carefully to discern whether nutrient intakes were adequate.

FRESH COW (POSTPARTUM) DIETARY CONSIDERATIONS

Less is known about diet formulation for the immediate postpartum period to optimize transition success and subsequent reproduction. Increased research is needed in this area. Proper dietary formulation during the dry period or close-up period will maintain or enable rumen adaptation to higher grain diets after calving. Failure to do so may compromise early lactation productivity. For example, Silva-del-Rio et al. (2010) attempted to duplicate the dietary strategy of Dann et al. (2006) by feeding either a low-energy far-off diet for 5 weeks followed by a higher-energy diet for the last 3 weeks before parturition, or by feeding the higher-energy diet for the entire 8-week dry period. They found that cows fed the higher-energy diet for only 3 weeks before parturition produced less milk than cows fed the diet for 8 weeks (43.8 vs. 48.5 kg/day). However, the far-off dry period diet contained 55.1% alfalfa silage and 38.5% wheat straw but no corn silage. In comparison the higher-energy dry period diet and the early lactation diet both contained 35% corn silage. Ruminal adaptation likely was insufficient for cows fed the higher energy diet for only 3 weeks.

A major area of concern in the fresh cow period is sudden increase in dietary energy density leading to subacute ruminal acidosis (SARA), which can decrease DMI and digestibility of nutrients (Mulligan and Doherty, 2008). Adequate physical form of the diet, derived either from ingredients or mixing strategy, must be present to stimulate ruminal activity and chewing behavior (Zabeli and Metzler-Zabeli, 2012), although good methods to quantify "adequacy" remain elusive. Dietary starch content and fermentability likely interact with forage characteristics and ration physical form. Dann and Nelson (2011) compared three dietary starch contents (primarily from corn starch) in the fresh cow period for cows fed a CE-type ration in the dry period. Milk production was greatest when starch content was moderate (23.2% of DM) or low (21.0% of DM) in the fresh cow diet compared with high (25.5% of DM). If SARA decreases DMI and nutrient availability to the cow, NEFA mobilization and increased ketogenesis may follow. In addition, rapid starch fermentation in the presence of NEFA mobilization leads to bursts of propionate reaching the liver, which may decrease feeding activity and DMI according the hepatic oxidation theory (Allen et al., 2009). A moderate starch content (ca. 23-25% of DM) with starch of moderate fermentability (for example, ground dry corn rather than high-moisture corn or ground barley) along with adequate effective forage fiber may be the best strategy for fresh cows. Recent research also has demonstrated that high grain diets can lead to greater numbers of gram-negative bacteria such as *E. coli* with resulting increases in endotoxin present in the rumen, which may decrease barrier function and inflammatory responses in the cow (Zebeli and Metzler-Zebeli, 2012).

Supplemental fats have been widely investigated as a way to increase dietary energy intake and improve reproduction (Thatcher et al., 2011). A novel strategy to use polyunsaturated fatty acid (PUFA) supplements to improve reproduction has been reported (Silvestre et al., 2011). Cows fed calcium salts of safflower oil from 30 days before to 30 days after calving, followed by calcium salts of fish oil to 160 days postpartum, had greater pregnancy rates and higher milk production. The mechanism is believed to be provision of greater amounts of linoleic acid (omega-6 PUFA) until early postpartum, which improves uterine health, followed by greater amounts of omega-3 PUFA

from fish oil to decrease early embryonic loss (Thatcher et al., 2011). The effects of turbulent transitions on reproduction are established early postpartum, likely during the first 10 days to 2 weeks postpartum (Butler, 2003; McArt et al., 2012; Garverick et al., 2013). By 8 weeks postpartum, >95% of cows should be at or above energy balance (Sutter and Beever, 2000). Use of targeted prepartum and postpartum strategies may minimize health problems and lessen NEB, and thereby improve subsequent fertility.

BODY CONDITION SCORE

The role of excessive BCS in contributing to transition problems and impaired subsequent reproduction is well established and has been discussed by many authors (Drackley et al., 2005; Garnsworthy et al., 2008; Roche et al., 2013). Cows with excessive body lipid reserves mobilize more of that lipid around calving, have poorer appetites and DMI before and after calving, have impaired immune function, have increased indicators of inflammation in blood and may be more subjected to oxidative stress (Contreras and Sordillo, 2011). What constitutes "excessive" BCS relative to the cow's biological target remains controversial. Garnsworthy (2007) argued that the average optimal BCS has decreased over time with increased genetic selection for milk yield, perhaps related to correlated changes in body protein metabolism. Recommendations for optimal BCS at calving have trended downward over the last two decades, and in the author's opinion a score of about 3.0 (1-5 scale) represents a good goal at present. Adjustment of average BCS should be a longstanding project and should not be undertaken during the dry period.

Cows fed high-energy (1.58 Mcal NEL/kg DM) diets during the last 4 weeks before calving lost more BCS in the first 6 weeks postpartum than those fed controlled energy (1.32 Mcal NEL/kg DM) diets (−0.43 and −0.30, respectively) (Cardoso et al., 2013). The effect of BCS change on cow's fertility is clear. Carvalho et al. (2014) showed that cows that either gained or maintained BCS from calving to 21 days after calving had higher (38.2 and 83.5%, respectively) pregnancy per AI at 40 days than cows that lost BCS (25.1%) during that same period. Previously, Santos et al. (2009) had shown that cows that had > 1.0 BCS unit change from calving to AI at approximately 70 days postpartum had lower pregnancy per AI (28%) than cows that lost < 1.0 BCS unit change (37.3%) or did not have a BCS change (41.6%). In a grazing system, researchers from New Zealand suggested that BCS at calving should be targeted at 2.75-3.0, to optimize production, while reducing liver lipid accumulation and the negative effects of inflammation on liver function (Roche et al., 2013; Akbar et al., 2015).

CONCLUSIONS

Formulation and delivery of appropriate diets that limit total energy intake to requirements but also provide proper intakes of all other nutrients before calving can help lessen the extent of NEB after calving. Effects of such diets on indicators of metabolic health are generally positive, suggesting the potential to lessen effects of periparturient disease on fertility. Supplementation of cows with rumen-protected methionine during the final stages of follicular development and early embryo development, until day 7 after breeding, lead to lipid accumulation changes in the embryos and resulted in differences in gene expression in the embryo.

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Characteristics of Effective Rumen-Protected Methionine Sources and How to Evaluate Them

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Take Home Message

We note that RPM sources present particular considerations in evaluation because of approaches to yield consistently high ruminal escape can yield decrease intestinal availability. Appropriate shell and coating might represent larger particles with more highly saturated fatty acids that could limit the intestinal absorption from in vitro assays unless verified against in vivo approaches. Moreover, we caution against bolus-dosing of products that have lower rumen-protection because there is the potential for perturbation increasing escape compared with normal consumption of the daily dose over multiple meals. In addition, estimation of an appropriate passage rate (kp) continues to present a challenge for models that derive rates of digestions (kd). Dose-response feeding and blood sampling is useful, but throughput is limited and important dietary factors must be considered. Therefore, we recommend multiple time incubation in situ to represent protection from the rumen but a standardized 16-h incubation for screening and quality control. We find value in both enzymatic and mobile bag evaluation of intestinal digestibility, with the latter more representative of true intestinal conditions but enzymatic digestion, once calibrated, appropriate for screening and quality control. All approaches require evaluation with real handling and dietary conditions and with lactation responses.

Introduction

Amino Acid (AA) nutrition in dairy cattle is still evolving. We note the landmark efforts of two of our mentors, Jimmy Clark and Chuck Schwab, who laid the groundwork for rumen-protected methionine (RPM) sources through abomasal infusion studies, duodenal flow studies, and blood measurements. We acknowledge that readers have used RPM sources effectively and will continue to do so. We assume that methionine (Met) is THE (as in THE Ohio State university) limiting AA in many North American diets, so RPM are useful to increase Met supply to the mammary gland. Instead, we aim here to address one component of AA nutrition: assessing RPM for lactating dairy cows. Our goal is to provide a framework for the breadth of approaches each with pros and cons to estimate the metabolizability of RPM sources to continue to advance in protein nutrition of dairy cattle.

We must start by outlining a few key assumptions. First, past researchers ignored the common losses of about 10 to 20% of Met during acid hydrolysis (Mason et al., 1980), and we and others make the point that hydrolysis without protecting the sulfur AA is no longer acceptable. We note that this error is less severe if it was applied to both feed and residue after ruminal or intestinal incubation by approximating a constant recovery that can be factored out in the disappearance calculation (White et al., 2017a); however, this assumption shouldn't be needed. We also acknowledge some confusion about the definition of metabolizability; we will define it as the AA

from the diet is absorbed into blood. Although the Met precursor hydroxymethylthiobutyrate can be absorbed from ruminal or omasal tissue, we will assume Met must reach the small intestine and be absorbed there i.e., protein must be digested. Various other usages of Met besides for protein synthesis in tissues should be considered (e.g., could influence milkfat production). For example, an interested reader could follow Dr. Loor's (University of Illinois) recent work. Finally, RPM response might differ among animal, dietary, and managerial conditions beyond these known nutritional factors. These issues are beyond our scope.

Despite these caveats, great progress has been made in the past 20 years regarding usage of metabolizable AA systems. Various laboratory tests were derived and standardized to measure the rumen-undegraded protein (RUP) and its intestinal digestibility (d), which we will term dRUP. Classically, cannulas would be placed after the abomasum (but before the pancreatic duct) and at the end of the ileum (as would be done with ileal cannulated swine). Markers would be used to measure duodenal flow of non-ammonia-non-microbial N; if multiplied x 6.25 and an estimated endogenous contribution is subtracted, these flows are the basis for evaluation and potentially derivation of RUP systems but also of methods used to estimate metabolizability of AA and RPM.

Dairy nutritionists understandably are focused on the mammary gland, but it competes with tissues for AA and other nutrients that are regulated by transport and blood flow. The splanchnic tissues and liver extract AA for itself but also excess AA that are not needed for other tissues as the blood recirculates. There is some metabolism (about 1/3) by the portal-drained viscera (Berthiaume et al., 2006). In that study, a RPM source's metabolizability and increase in arterial plasma (after the liver) was confirmed, although they noted large differences among cows. This background must be considered when we discuss usage of blood Met concentrations to assess RPM efficacy.

Because of the complexity of studies measuring tissue metabolism, much of our progress in applied feeding has resulted from the usage of laboratory methods as proxies for these in vivo approaches. Currently, more people are collecting omasal digesta rather than duodenal digesta to avoid duodenal cannulation. Omasal sampling typically increases estimates of microbial N flow but decreases estimates of non-ammonia-non-microbial N (Roman-Garcia et al., 2016). Unfortunately, we do not know which –duodenal or omasal sampling– is "right". These limitations must be acknowledged because assessing metabolizability is a function of biological accuracy, which depends on how well these conditions represent an intact cow, whereas too many conclusions have been based on statistics without realizing that these conclusions were based on precision. Unlike in swine and poultry, where standardized digestibility can be assessed, dairy cattle have that wonderful organ called the rumen. Therefore, this paper will reflect our views on the accuracy of assessing the metabolizability of RPM–warts and all.

Rumen Undegradability In Situ and In Vitro

Over the years, many approaches were designed to estimate RUP based on in situ (Dacron bags placed in the rumen), in vitro (rumen fluid with live rumen microbes inoculated into a test tube that is kept anaerobic and warmed to the cow's body temperature), or various preparations of

enzymes or solvents. The underlining principle for estimating ruminal degradability of nutrients using the in situ procedure is that rumen microbes will enter the bag representatively of those microbes in the larger rumen after which they colonize and degrade the feed's nutrients inside the bag as they would in the rumen. We consider that all approaches have pros and cons, and not every method is appropriate for every feed. For example, corn gluten meal was called out a couple of decades ago for gumming up the inside of the Dacron bags, and some animal protein sources have large soluble fractions that wash out of the bags but might still be undegraded. Because we cannot fix all potential confounding factors, the ruminal in situ procedure has been standardized for various factors such as grind size, sample weight, pore sizes of bags, and animal differences (Vanzant et al., 1998; NRC, 2001).

The standard kinetics approach used in NRC (2001) and most other models is a simple first-order, one-compartment model. Typically, the feed source would be incubated at several time intervals between a zero-hour (immediately after incubation starts; A fraction) and an end-time chosen to be long enough (C fraction), with rates of disappearance to be computed for the B fraction (A – C). Unfortunately, historically, many authors did not incubate long enough for the C fraction to be computed for high bypass feeds such as heated soy or animal proteins, which then underestimated degradation rates (kd) inversely proportional to an overestimated B pool. A standard rule in kinetics is to have 5 time points per rate. Clearly, each time point used adds cost to an analysis, but reducing the number of points increases the risk of either decreased accuracy or precision.

Because the bacteria are high in N, bacterial adherence must be considered. The feed residue inside a Dacron bag increases in bacterial N relative to the decreasing original feed N with increasing duration of incubation. This artifact decreases the true rate of feed N degradation but overestimates the % N in the feed's C-fraction (Woods et al., 2003). This error is modest for most high protein feeds but becomes particularly important for feeds lower in protein but higher in NDF, which provides more substrate for bacterial adhesion (Stern et al., 1997; Paz et al., 2014). To minimize this contamination, investigators often wash bags thoroughly with cold water either by hand or by using a clothes washing machine (Alexandrov, 1998); others have used chemical, mechanical, or ultrasonic methods (Krawielitzki et al., 2006) for standardization. Unfortunately, none of these procedures work consistently.

Rather than trying to dislodge bacteria, other investigators have mathematically accounted for bacterial N contamination by measuring a microbial marker in the feed residue and relating it to the marker:N ratio of a corresponding rumen bacterial sample collected from those same cows. Unfortunately, various procedures to quantify bacterial N adherence all add time and are not fully consistent, but we think microbial rRNA genes (Paz et al., 2014) has the best potential to overcome these limitations. Although consistently emphasizing the need for bacterial N correction for residual N in fibrous concentrates and forages, such corrections have largely been ignored in feed libraries such as NRC (2001). Although we know of no studies that have evaluated the extent of bacterial N contamination in RPM products tested in situ, this potential bias is not likely to be of significant concern for RPM

strategies because of limited bacterial adherence to the lipid-based or other type of coatings that are not normal adhesion sites in the rumen.

The Dacron bag technique has estimated rumen protection of prototypes in preliminary research and for comparisons among rumen-protected AA products, including those protected by lipid coating; for example, see Wu et al. (2012). However, there are several potential limitations that should be considered. Firstly, test products are not exposed to physical disruption by chewing by the animal or storage or handling factors such as changes in environmental temperatures, decreased pH in silage-based diets, and physical mixing of rations (Wu and Papas, 1997; Ji et al., 2016). Secondly, despite being a reasonably accurate estimate of resistance to degradability in the rumen, ruminal incubation in situ ignores variable intestinal digestion (Wu and Papas, 1997). Thirdly, fine particles that invariably get washed out of the bag are not necessarily ruminally degraded or intestinally absorbed, so the technique will not be valid for very fine or soluble RPM supplements (Whitehouse et al., 2017).

In vitro methods can use filter paper and therefore be valuable for highly soluble protein sources. For in vitro methods assessing a soluble RPM, abrupt Met metabolism in bacteria can influence the pooling of extracellular Met that might otherwise be effluxed from bags incubated in situ. If incubations are short-term, this pooling can be intentional for a direct measurement of RUP (Colombini et al., 2011). For longer incubations, we should be considering not just the protein that is being degraded but the carbohydrates needed to support microbial usage of RDP or degraded RPM sources in vitro. Rumen bacteria can synthesize L-Met from normal metabolic intermediates derived from glucose (e.g., typically from starch or cellulose) and inorganic sulfur (Hackmann and Firkins, 2015). When preformed Met is transported and therefore increasing intracellular Met concentrations, rumen bacteria almost certainly down-regulate these metabolic pathways used to synthesize Met to conserve resources. Firkins et al. (2015) noted that L-Met is rapidly transported into ruminal microbes; some is degraded, but considerable L-Met is recycled intracellularly via highly conserved pathways. For example, L-Met recycling provides critical polyamines needed for cell replication. If DL-Met is dosed, the D racemer is more likely to pool in rumen fluid. Assuming a large bolus-dose, the accumulating intracellular L-Met might even be excessive and therefore racemized to D-Met for excretion extracellularly.

Bacteria typically control expression of multiple genes by a single transcriptional regulator. For example, most of the enzymes needed in a single AA's synthetic pathway are expressed simultaneously and typically related to an ability to sense that AA's concentration. Simply adding urea increased proteolysis of a low RDP diet in vitro (Griswold et al., 2003). Moreover, some AAs can cause antagonisms; although Met appears to be less prone than the aromatic and branched AAs, the metabolism of Met and the branched-chain AA also might be related (Firkins et al., 2015). All of these reasons suggest that bolus-dosing of RDP sources or RPM overestimates are not necessarily physiological and could overestimate their escape. In contrast, when well protected, perturbation is far less likely, so bolus dosing might be a useful option. Using isotopes is the most appropriate but also the most costly.

Feed testing has a trade-off of more feed samples with a single incubation time to account for normal nutrient variability (St-Pierre and Weiss, 2015) compared with fewer feeds with more time points to estimate a kd to be directly used in a nutrition model. Of course, a kd can be computed from one time point, but this "rate" increases risk for inaccuracy. If your aim is to derive a kd, a compromise is to use single bags or flasks per each time (Mertens, 1993), thereby replicating the rate, not the time. However, if your aim is not to derive a true rate, then which single incubation time? Some have argued that 16 h approximates the residence time in the rumen, but this argument ignores differences in degradability and other considerations among individual feeds. Of course, the real reason to choose a single time is that it is most highly correlated with in vivo measurements for most feeds for a standardized approach. Incubation for 16 h has been prioritized over 12 h for RUP (Boucher et al., 2009a).

Based on above assumptions, we assume 16 h is an appropriate time to estimate the undegradability of RPM sources, particularly for exploratory research or subsequent quality control of established products. We recommend longer incubation and to derive a first-order kd of the B fraction for those models that use it and to evaluate the sustainability of the protection mechanism. That said, a caveat for any model using kd is estimating a ruminal passage rate (kp) of the B fraction in a first-order effective degradability calculation [A + Bkd/(kd+kp)]. For example, the kp in the NRC were derived with minor bias (after a correction), but those kp still represent passage of primarily rare-earth markers and not the kp of the B protein fractions of feeds, which are not really known (White et al., 2017b). Also, at least for some feeds, some (perhaps ≥10%) of the A fraction passes from the rumen.

The Mobile Bag Technique for estimating Intestinal Digestibility

In this technique, a small (~ 1.5 g) sample of the feed is placed in small Dacron bags (i.e., 5 x 10 cm). The original landmark work was derived in Denmark; see Hvelplund et al. (1992). Although not necessary for all feeds, at least some feeds should be pre-incubated for estimation of RUP but also because pre-incubation in the rumen optimizes the simulation of intestinal digestion for some feeds. Thus, companion bags (besides those that are collected for RUP) are incubated in the rumen, washed, and placed in a pepsin-HCl solution to simulate abomasal digestion. Then, they are rinsed and inserted through a duodenal cannula and retrieved (typically from feces). Although some have suggested greater digestibility resulting from bacteria in the large intestine, when directly compared to those retrieved from ileal cannulas, there has been relatively minor difference (Borucki Castro et al., 2007). In another study, feed N disappearance from bags increased from duodenum to feces compared with those retrieved from an ileal cannula but also sometimes decreased if the feed was highly fibrous and therefore contained more contaminating bacteria (Jarosz et al., 1994). In general, feeds were ranked very similarly for intestinal digestibility whether retrieved from ileum or feces, so we think fecal collection is adequate so long as bags are inserted in intervals no less than 5 min apart and retrieved in periodic increments.

We have outlined considerations and potential interactions for mobile bags versus in vitro to simulate AA availability in dRUP (White et al.,

2017a). With fewer studies assessing the intestinal availability of RPM sources, we must adhere to protocols derived from studies using typical feedstuffs. Commercially available rumen-protected lysine and RPM have been evaluated using the mobile bag technique; e.g., see these sources (Berthiaume et al., 2000; Wu et al., 2012). The mobile bag technique is the only technique that ensures samples are exposed to all physiological digestive processes, but we recommend visually inspecting the bags' RPM residues to ensure the physical nature is reasonably intact to avoid inflated dRUP from washout.

In Vitro Assays for Intestinal Digestibility

The original three-step procedure (TSP) was modified (MTSP) to exclude trichloroacetic acid and includes the use of a batch rumen incubator (Gargallo et al., 2006). An in vitro assay was developed at Cornell University (Ross et al., 2013) and is also offered by many commercial feed testing laboratories. In this assay, a sample of the feed is exposed to 16 h fermentation in an Erlenmeyer flask after which time the residue is recovered by lyophilization or filtering. Then, isolated residue is exposed to pepsin followed by trypsin, chymotrypsin, amylase, lipase, and pancreatin. The MTSP (previously TSP) plus the Ross Assay all appear useful to commercially estimate dRUP because they do not require the use of cattle fitted with duodenal cannulas and can analyze large numbers of samples rapidly and precisely. We suggest that each needs quality control e.g., using standardized feeds for comparison. If using protein sources from feedstuffs, researchers should report both the ruminal undegradability and the intestinal digestibility of the sum of total AA to standardize results (White et al., 2017a).

In a comparison of the mobile bag with the TSP and MTSP, there was fairly good agreement across methods, but the mobile bag technique tended to increase estimates of dRUP, especially in forages, followed by the MTSP and then TSP (Wang et al., 2016). These results are generally consistent with previous results (Borucki Castro et al., 2007). Although many people are using bags with the same pore size (53 μm) and open space as used for RUP estimation in situ, a smaller pore size might be needed for some feeds because even 15 μm was suggested to be too large compared with the original research that relied on bags with a 9-μm pore size (Harstad and Prestløkken, 2000). Fewer studies have used in vitro techniques to estimate intestinal digestibility of rumen-protected AAs. The natural enzymatic environment is obviously retained with mobile bags. Moreover, in vitro results might be sensitive to concentration of pancreatin (Rossi et al., 2003) or perhaps other factors that might need further attention to simulate intestinal digestibility of coated RPM. For example, attention is needed to mimic the normal emulsification from bile. Until further research sorts these issues out, we think both techniques have pros and cons and both should be done to ensure a robust evaluation.

Cecectomized Chicken Assays for Intestinal Digestibility

The chick assay provides useful comparisons of intestinal digestibility of most proteins, including those that are heat damaged (Boucher et al., 2009b; a), and even rumen microbial protein (Fonseca et al., 2014). However, the ruminant intestine probably has a better ability to digest bacterial proteins. In addition, caution is needed when using

chickens to assess intestinal digestibility of fat-coated RPM sources. Ruminants use a combination of dietary and microbial detergents plus rely on taurine conjugation to bile salts to greatly increase emulsification of saturated fatty acids compared with non-ruminants (Palmquist and Jenkins, 1980).

Blood AA approaches for Dairy Cows

With a well-protected RPM, peripheral blood concentration can be used as an index of ruminal escape (preceding blood appearance) plus intestinal absorption so long as it is compared within cow and typically with certain assumptions. Methionine lends itself to such measurements compared with other AAs. For example, Met is not appreciably oxidized by intestine for fuel and is extracted by mammary tissue approximately as needed for milk protein synthesis (Manjarin et al., 2014). Although it is used as a methyl donor for many important reactions, Met also is extensively recycled and conserved. Excess Met is degraded in splanchnic tissues and liver that potentially even exceeds Met extracted by the mammary gland (Arriola Apelo et al., 2014). Even so, peripheral blood samples have been extensively used to assess Met availability, whereas other essential AA might not lend themselves so readily to assessing metabolizability (Rulquin and Kowalczyk, 2003). Differences among animal and other issues disrupting linearity (e.g., the AA should not be limiting in the diet or the cows should not be mobilizing tissue AA) need careful consideration (Whitehouse et al., 2017).

Some workers have used dose responses of RPM followed by periodic blood draws and assessed linear responses in Met concentration versus dose to assess bioavailability (Rulquin and Kowalczyk, 2003). Ideally, the other essential AA should remain unaffected by this dose response and diet energy and metabolizable protein (MP) probably should be in excess to avoid confounding. The approach can be simplified if using a standardized control with consistent metabolizability (Graulet et al., 2005). Again, adequate blood sampling intervals (those authors used at least 4 that were pooled) are needed to obtain an accurate response function with increasing continuous infusion or semi-continuous feeding of the RPM into the rumen to account for normal variation in plasma Met concentration.

We recommend to avoid representing an AA as a proportion of total AA to standardize for variation among blood draws or among animals. Ratios (or percentages) can cause non-normal distributions, whereas using total AA concentration as a covariate helps to center the data to the overall total AA mean and can actually provide a more appropriate distribution of data. Ratios of individual AA/total AA also sometimes introduce spurious correlations that would not necessarily be introduced if total AA was used as a covariate rather than reporting the individual AA as a percentage of total AA. Moreover, ratios can introduce confusion in interpretation. For example, a blood AA concentration can decrease in a sample but increase if represented as a percentage of total AA. Total AA concentration can vary for reasons independent of the indivudal AA being addressed (e.g., blood glutamine or alanine could increase just because there was a less efficient usage of MP).

Bach and Stern (2000) bolus-dosed two RPM sources one of which was more protected and was evaluated at two doses. They measured plasma Met concentrations every 6 h after dosing until 36 h. Their moderately protected Met source peaked in plasma Met concentration prior to the one that had greater protection. They discussed this consistency among other studies. Their data for control, 60 g of moderate protected Met, and 30 and 60 g of well protected Met (688, 1658, 1906, and 2744 μM·h, respectively) also document concerns with using raw areas under the curve (AUC) produced from Met concentration over time after the bolus dose. Their 0-h concentrations among treatments were not statistically different but actually ranged from 15.5 to 22.5 μM (a standardized deviation > 33%). Because the basal concentration should be subtracted to calculate an incremental AUC (i.e., that reflects the dosed RPM, not Met from other dietary sources), such basal variation has large changes on the AUC calculation factored over the entire time span. They noted that the more poorly protected, the faster the peak blood concentration, which could be a result of perturbation (described above). Thus, time points should reflect the changing shapes of the curves and might not be expected to be the same points for all Met sources. What if the baseline drifts? Should you subtract the control values for different cows on the same day or subtract some basal period for the same cow on a different day? For these reasons, we cannot recommend using bolus-dosing and AUC, although it might be less problematic for RPM sources or high metabolizability.

Milk Protein Response

When using milk protein as an index, we have moved into the camp that updates the barrel and stave theory regarding how essential AAs limit milk production. Armentano (2017) captured major issues of an evolving concept that AAs shouldn't just be evaluated for dose responses without consideration of interactions with other AA. Although lower MP supply might improve efficiency of usage of a RPM, it still might not maximize milk protein compared with if a higher MP diet is fed. Certainly some of this response is a result of moderate increases in dry matter intake with increasing MP supply, correction of a limiting AA's supply, or both factors interacting. As he pointed out, more studies need a true positive control in which RPM (or other AA) is fed with high MP (i.e., you can't assume a lower MP diet will give you the best response to an RPM). Because of the complexity in explaining milk protein response, particularly when working with cows fed diets in which an AA must be limiting milk protein production, we caution against over-extrapolating milk protein yields to address the metabolizability of RPM sources.

A way to use milk protein is to feed selenized yeast and to measure the dilution of basal selenomethionine in milk protein by RPM sources (Weiss and St-Pierre, 2009). This technique was made more practical by assuming that Se concentration could be a proxy for the more complicated assay for selenomethionine by requiring basal Se to be practically identical among treatments. The approach also was simplified by assuming a standard RPM against which other RPM sources could be calibrated. Because it is based on Se-Met, not milk protein yield per se, its calculation is more independent of other factors that influence milk protein yield. To use this procedure, a strict feeding protocol is needed, as outlined in their paper. The Se concentration in milk must be high enough to be above background and therefore provide a useful response criterion, and this concentration must be reflective of

differing sampling periods (adequate adjustment time). Therefore, it is too cumbersome for screening but does yield accurate metabolizability of RPM sources in lactating dairy cows.

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EMILY ANDREINI

Effects of feeding level on performance and feed efficiency of high and low RFI beef steers

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ABSTRACT

Feed cost is the leading expense in most beef operations, and genetic selection can be used to improve feed efficiency without reducing performance. Residual feed intake (RFI) is a beef cattle efficiency measure used to identify animals that consume less feed than others for a given rate of gain. Previous research has addressed the hypothesis that low RFI steers remain more efficient during feed restriction based on body weight (Dykier, 2016). Dykier (2016) showed that high and low RFI steers performed similarly under feed restriction, which suggested variation in RFI might be explained by behavior and appetite rather than metabolic efficiency. However, Dykier (2016) suggested that it was unclear if the results of restricted feeding were related to RFI or different levels of restriction (i.e., 68% of *ad lib* intake for high RFI, and 80% of *ad lib* intake for low RFI steers). Therefore, the objective of this study was to explain efficiency changes of high and low RFI steers during feed restriction based on previous *ad libitum* intake. To identify RFI classification, 56 Angus-cross steers were individually housed, offered *ad libitum* access to a total mixed ration, and individual intakes were recorded daily for 56 days. Shrunk BW's (12-16 hours of feed restriction) were taken every 14 days, and average daily gain (ADG) was estimated as the slope of the regression of BW vs. time. Residual feed intake was defined as the residual of the regression of DMI on mid-test $BW_{0.75}$ and ADG, and high and low RFI groups were defined as > 0.5 standard deviation above or below the mean of zero, respectively. During the restricted feeding period, feed intake was restricted to 75% of previous *ad libitum* DMI as a percent of $BW_{0.75}$. During *ad libitum* feeding, ADG did not differ (P=0.99) between the low and high RFI group, and during restricted feeding, ADG did not differ (P=0.50) between the low and high RFI group. For the high RFI group, ADG differed (P<0.05) between *ad libitum* (1.75 ± 0.13) and restricted (1.07 ± 0.13) feeding periods but did not differ (P=0.17) for the low RFI group between *ad libitum* (1.74 ± 0.13) and restricted (1.34 ± 0.13) feeding periods. During *ad libitum* feeding, gain to feed ratios (G:F) did not differ (P=0.67) between the low and high RFI group, and G:F did not differ (P=0.13) between the low and high RFI group during restricted feeding. For the high RFI group, G:F did not differ between *ad libitum* (0.17 ± 0.01) and restricted (0.11 ± 0.01) feeding periods (P=0.07). For the low RFI group, G:F did not differ between *ad libitum* (0.19 ± 0.01) and restricted (0.16 ± 0.01) feeding periods (P=0.49). Results suggests low RFI cattle remain efficient under feed restriction.

KEY WORDS: Residual feed intake, beef cattle, efficiency

REDUCED LIGNIN ALFALFA: Can new genetics impact yield and quality?

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INTRODUCTION

Alfalfa (Medicago sativa L.) is the most important forage crop in North America, and is third in on-farm value (ranking: corn, soy, alfalfa, wheat--USDA-NASS, 2017). It is the key forage for the US dairy industry, and is frequently considered a core feed for dairy rations. The value of alfalfa is generally attributed to the combination of yield and quality and its wide agronomic adaptation, with quality defined by several important attributes (Putnam et al., 2008). These include high protein content, energy (TDN or ME) content, relatively low fiber content, and high intake rates. But as dairy rations have changed to include ever-lower forage component, energy and protein from alfalfa may take on a reduced role in compared with the value of the fiber itself to rumen function and health. Long fiber in alfalfa may limit energy if too great in quantity, but is also critical for proper rumen function, rumen mat formation, maintenance of pH, and animal health (Mertens, 2011, Robinson, 2014).

However, we know that not all 'fiber' is created equal, with digestibility rates varying greatly across feeds and within feeds. Cell wall (NDF) digestibility may be a key indicator of intake potential, which in turn impacts milk production. Approximately 20-25% of the energy for milk production is thought to originate in the digested fiber fraction. Some nutritionists have estimated that increasing the fiber digestibility (from say 30% to 60% total track NDF digestibility) would increase digestible energy to support up to 8-10 lbs./ more milk per day (Combs, 2016).

DOES ALFALFA DIGESTIBILITY VARY?

Yes. The digestibility of the NDF fraction of alfalfa varies from a low of about 35% to a high of about 55%, even within what would be considered 'Supreme' or 'Premium' dairy hays (Figure 1). Why is this important? It's important for both the marketing of hays and for dairy rations. While hay brokers and dairy buyers may argue about the price difference between (say) a 54% TDN hay and 56% TDN hay (or 165 vs. 175 RFV) –based upon a difference of a few points of NDF or ADF - these are ONLY differences in fiber

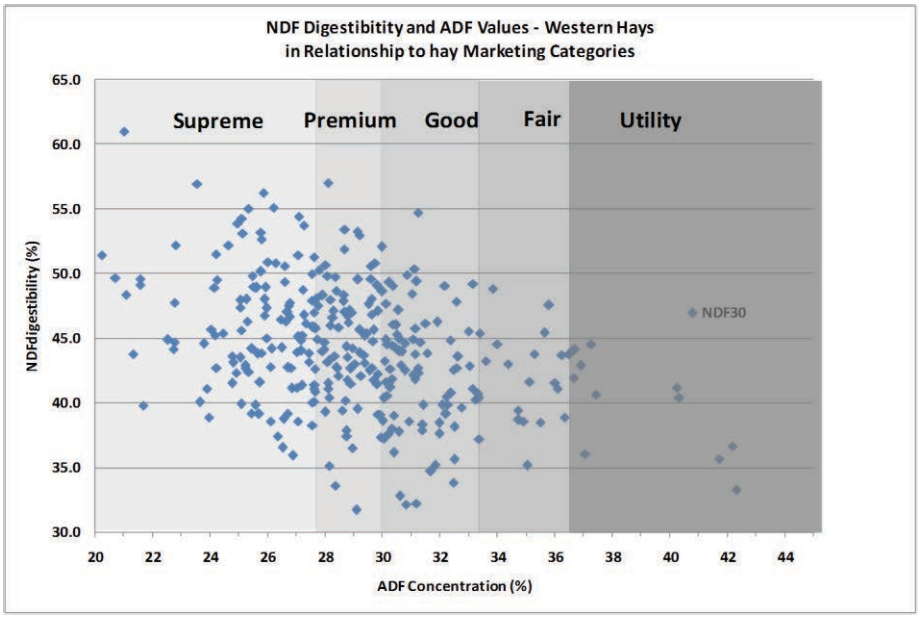

Figure 1. Fiber digestibility (NDFD30 hr.) in western hays varies widely and has little relationship with ADF or NDF content. NDFD may be more important for milk production, but is not reflected in current markets.

[1] California Animal Nutrition Conference, May, 2018, Fresno, CA

content, not the level of digestibility, or energy yield per unit kg of hay or NDF, which may vary by as much as 10-20 points! On the ration side, it stands to reason that a digestibility of 55% (30 h) of the NDF fraction is likely to yield more energy to the ruminant than a feed with only 35% of the fiber being digested in 30 hours. The indigestible portion of forages (iNDF) is a defining characteristic that limits forage utilization (Mertens, 2011).

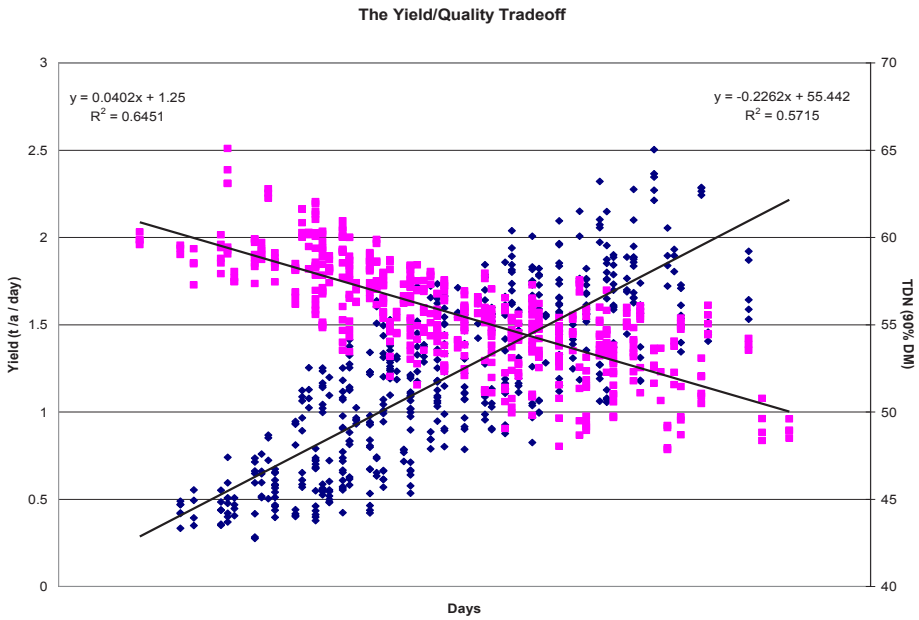

Figure 2. Relationship between yield and quality-Yolo County. Maximum quality rarely occurs at maximum yield and growers must compromise.

AGRONOMIC PENALTIES FOR HIGH QUALITY-Links to Harvest Schedule.

Since growers routinely cut early to attain quality (e.g. low fiber) hay, they typically compromise significant yield, which is an important issue for dairies and forage growers since it influences price and cost. Figure 2 is data from Yolo County from many cuts, showing that yield maximizes at about 2.5 tons/acre per cutting at about 37-40 days, but is approximately ½ ton/acre when harvested at about 21 days. Conversely, quality is lowest at the late cutting schedules and highest at a short cutting schedule, a phenomenon known as the 'yield-quality tradeoff'. Typically, growers strike a compromise between yield and quality, cutting at about 28 days – unfortunately this often misses 'dairy quality' designation, resulting in both lower yields and lower quality. This is because stem quality rapidly declines after about 22-26 days (Figure 3), dramatically lowering quality. Most of this decline is due to lignification of the cell walls in the stems. The secondary cell wall continues to develop in mature alfalfa, accompanied by lignification of the cell wall xylem after elongation of internodes is complete. If the decline in stem quality can be delayed, even by a matter of 4-7 days, this could be a significant.

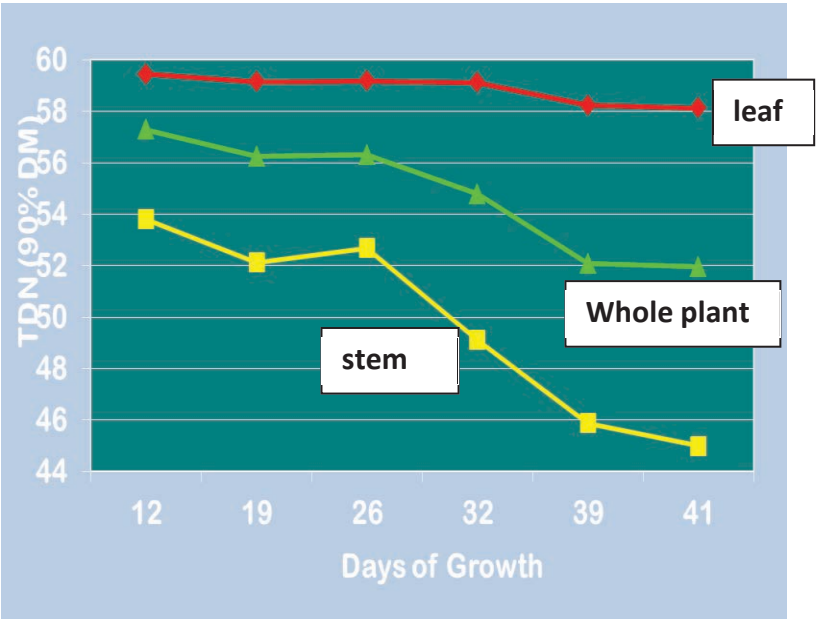

Figure 3. Stem quality rapidly declines in alfalfa typically between 25 and 35 days of growth significantly reducing quality of the whole plant

The Low-Lignin Concept. In 2014, two varieties were introduced by competing companies that purported to have reduced lignin and higher

fiber digestibility. These were the 'HarvXtra' trait by Forage Genetics International (Land O Lakes) and the 'HiGest' trait introduced by Alforex (Dow Dupont). The HarvXtra trait was produced via genetically-engineered down-regulation of the lignin pathway in alfalfa, after research by Nobel Institute and USDA-ARS and FGI identifying the role of various enzymes involved with lignin biosynthesis. The lignification of the cell wall is modified so that lignin content is decreased, and the decline in NDFD is less as the plant matures. The HiGest trait was produced via traditional plant breeding methods and selection for high forage quality. These two products have now been commercialized. Seed is available for some, but not all dormancy groups. Because it is well known that harvest schedule impacts quality, reduced lignin lines could improve quality at a given harvest schedule (e.g. 28 days) or alternatively, produce the higher yield at a later cutting schedule (e.g. 35 days) while maintaining quality.

Experimental Evidence. University researchers have been examining this concept over the past 5-6 years. In addition to company research, independent field research is still ongoing – especially on non-dormant lines—but some of the university data that has been collected across a range of land-grant colleges can be summarized. In data presented by Dr. Mark Sulc at the 2016 California Alfalfa Symposium, a six location average response of HarvXtra lines compared with control lines can be seen in Figure 4. Here, we can see that all varieties decline in NDF digestibility over time, but the HarvXtra variety was always higher in NDFD at any harvest timing. These were dormant lines (FD 3-4). In a separate comparison of conventional lines and HiGest and HarvXtra at Tulelake, CA in 2016, HarvXtra line was significantly higher in NDFD and lower in lignin (ADL) than conventional lines or the HiGest lines tested (data not shown).

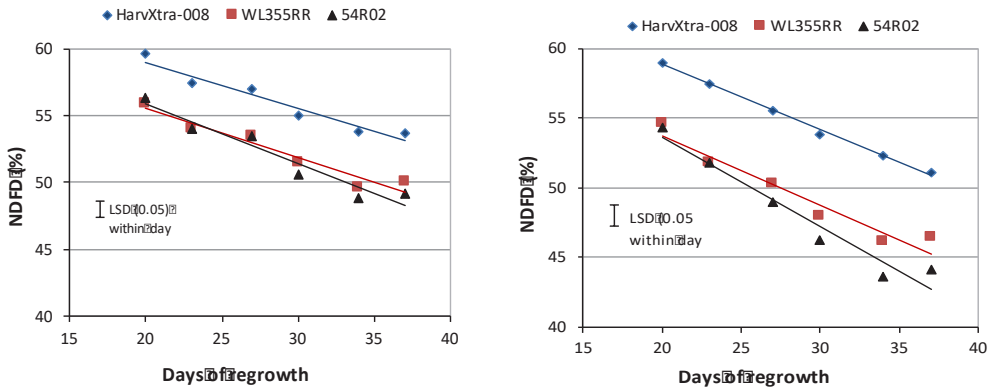

Figure 4. NDF Digestibility (30 hr) response of HarvXtra lines to growth of the crop (average of 6 locations). Sulc et al., 2016. (2nd cut left, 3th cut right)

In a study at UC Davis, 4 HarXtra lines were compared with controls under two cutting regimes (28 days and 35 days) over a 2-year period. The HarvXtra lines averaged lower lignin and higher levels of NDFD than controls, but also exhibited slightly lower NDF and ADF levels, but no change in protein concentration harvested at 35 days (Table 1). Similar trends were seen at 28 days. Yields were slightly lower in the HarvXtra lines compared with the controls at Davis. Similarly in multi-state trials, HarvXtra lines at the same harvest schedule exhibited slightly lower yields – however, since delayed harvests significantly improve yields in alfalfa, HarvXtra lines harvested at 35 days yielded more than conventional lines harvested at 28 days.

Table 1. Quality response of 4 HarvXtra down-regulated lignin lines compared with controls, UC Davis trial (2 years, 5 cuts/year, 35 day schedule). These were lines of Fall Dormancies of approximately FD 4.0 (dormant lines).

Variety	ADF	NDF	CP	ADL	NDFD(30)
			----------%----------		
54R01 (control)	26.2	30.8	23.4	5.0	48.8
Am405TRR (Control)	27.9	32.6	22.8	5.3	47.3
Liberator (Control)	26.8	31.5	23.0	5.1	48.3
WL355RR (control)	27.6	32.2	22.8	5.3	47.4
RL-1	25.6	30.1	22.9	4.5	51.3
RL-2	26.3	31.2	23.0	4.5	51.3
RL-3	25.4	30.3	23.2	4.4	51.8
RL-4	26.0	31.0	22.9	4.6	51.1
Significance (F test):	***	**	n.s.	***	***

SUMMARY

In virtually all the university data we have examined to date, the down-regulated HarvXtra lines have exhibited superior fiber digestibility to control lines of the same fall dormancy level and lower lignin concentrations at the same harvest schedule. Additionally, cutting schedule experiments showed that HarvXtra lines harvested late exhibited similar nutritive value to forage harvested 5-10 days earlier. Although we have limited data on HiGest, in most experiments these lines were closer to high quality conventional alfalfa lines and distinct from HarvXtra lines. Further field trials, particularly on non-dormant lines (which were only recently released) and the interactions with harvest schedule as well as feeding trials are being conducted. Yield, stand persistence, lodging resistance, pest resistance, cost and economic value are all important for producers and require further scrutiny.

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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 (January, 2018), 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

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major concern for Cr). Although we can get accurate total mineral concentrations data for basal ingredients, you must be careful 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).

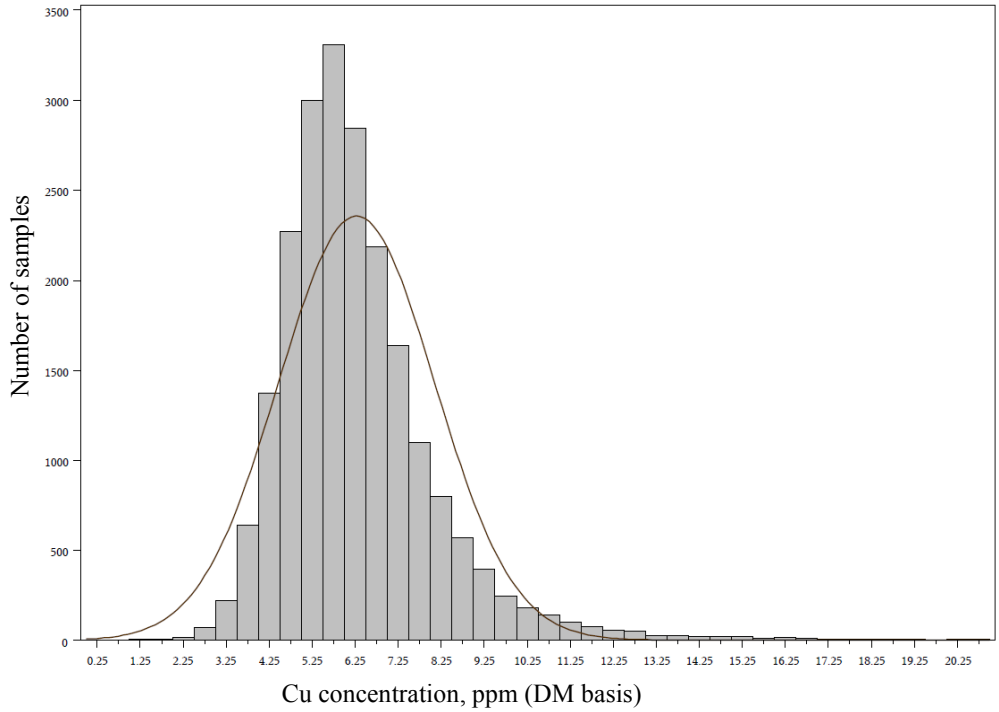

Figure 1. Distribution of Cu concentrations in corn silage grown throughout the U.S. The smooth line indicates a normal distribution would while the bars indicate the actual distribution. (Knapp et al., 2015).

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 $\pm$ 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.

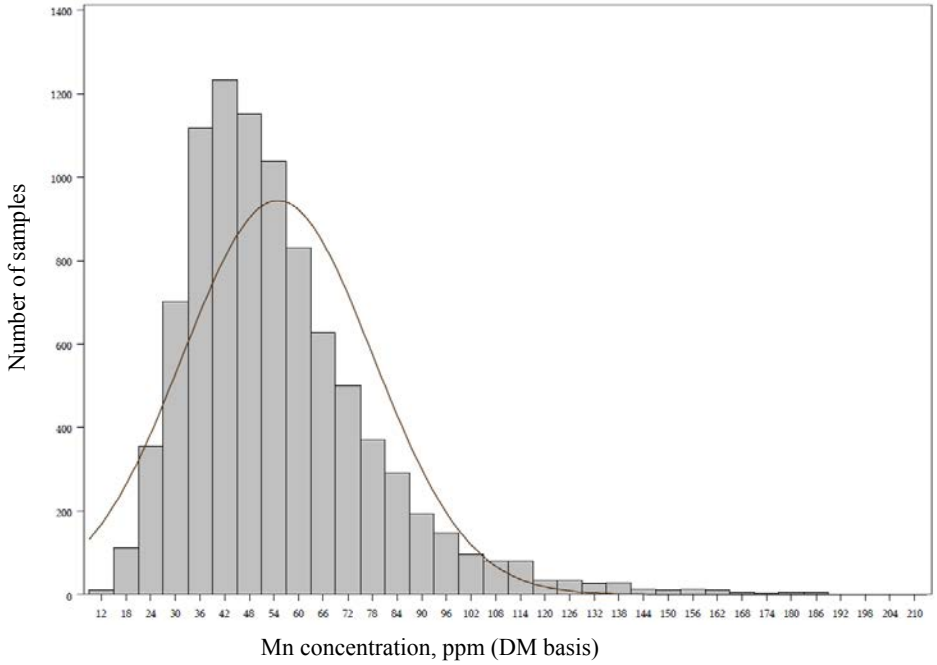

Figure 2. Distribution of Mn concentrations in mixed, mostly legume silage grown throughout the U.S. The smooth line indicates a normal distribution would while the bars indicate the actual distribution (Knapp et al., 2015).

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.

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; 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 >35 kg of milk, feeding an additional 1000 IU of vitamin A/d per kg of milk >35 kg will replace what is normally secreted in milk (about 450 IU/lb of milk above 77 lbs). 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)*1000] = 93,000 IU/day. No data are available showing NRC (2001) vitamin A recommendations for dry and prefresh cows are not adequate.

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 (30 IU of supplemental vitamin D/kg 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 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 to 2000 IU/d during

the last 14 to 21 d of gestation. This rate of supplementation has reduced early lactation mastitis and metritis.

Vitamin A and E recommendations when supplies are extremely tight

1. Stop over supplementing. There are no data showing vitamin A and E supplementation exceeding 2001 NRC has any benefit (except for prefresh cows and vitamin E)

2. If necessary to prioritize:
   -maintain prefresh supplementation at NRC recommendations
   -far-off dry cows should be next. Basal diets fed to dry cows are generally low in B-carotene so basal intake of retinol precursor is low compared with lactating cows
   -many lactating cow diets have substantial basal vitamin E and B-carotene and supplemental A and E can probably be reduced in the short term

3. Fresh green forage and even well-made hay crop silage can be good sources of B-carotene and vitamin E. If diets contain these feeds, supplemental vitamin can be reduced

4. Because of unknown effects, all cows(except for those consuming substantial pasture) should be provided with some supplemental vitamins A and E but supplementation rates can likely be reduced substantially in the short term. Generally vitamin A was over supplemented and the excess is stored in the liver so reducing supplemental vitamin A should not be problematic for a period of several months

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 usually increases milk yield in early lactation (Sales et al., 2010) and may help reduce fatty liver. 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 may often be 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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How Much Supplemental Vitamins do Cows Really Need ?[1]

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Summary

Because of major production problems, vitamin A and to a lesser extent vitamin E are in very limited supply and prices have increased markedly. Because of price and scarcity, many nutritionists are re-evaluating vitamin supplementation strategies. Based on current information, the NRC requirements for vitamin A (approximately 75,000 IU/day for all cows) and vitamin E (500 IU/day for lactating cows and 1000 IU/day for dry cows) are adequate. However, feeding an additional 1000 IU of vitamin E per day during the prefresh period (2 or 3 weeks prepartum) can improve cow health post partum. More data are needed but limited information suggest that for lactating cows, supplementation rates for vitamin D should be increased to about 1.5 X NRC (about 30,000 IU/day). Because vitamin A is in very limited supply, supplementation may need to be prioritized. Because of low expected intakes of basal B-carotene and the high requirement of vitamin A for colostrum synthesis, prefresh cows should be fed at NRC rates at the expense of other cows. Next highest priority is dry cows, followed by lactating cows. Some supplemental vitamin A should be provided to all types of cows if possible; however if necessary the liver can supply adequate vitamin for several weeks and perhaps up to a few months without adversely affecting lactating cow health or productivity.

Introduction

Historically, most nutritionists have given little consideration to the cost of vitamins A, D, E. Cows needed them and even at high supplementation rates, cost per cow per day was reasonable. However because of a fire at a chemical factory in late 2017, worldwide production of feed grade vitamin A has been reduced by more than 40%. Production of vitamin E has also been reduced because an intermediate that was produced at the factory with the fire cannot be produced right now. Because of other productions issues, vitamin D supply is also tighter than normal. These production problems have led to major increases in vitamin prices. Compared to historic norms, vitamin A price at wholesale level has increased about 10 times (local and spot markets may differ markedly), vitamin E price has increased 3 to 4 times and vitamin D price has increased less than 2X. Approximate cost of supplementing vitamins A, D, and E at NRC recommended levels would cost about

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10 to 12 cents per day (there will be a very wide range on this value because of margins and local markets). Using historically typical prices, it costs 3 or 4 cents per day to provide supplemental vitamins A, D, and E. Although this is a very substantial increase in cost, it is still a very small portion of the total feed bill (about 3% of total feed costs). A bigger problem than increased cost is limited supply. In some markets vitamin A simply is not available at any price or supplies are being rationed. This paper will review current research and recommendations regarding vitamins A, D, and E and strategies to use when supplies are inadequate.

Vitamin A

The common form of supplemental vitamin A is all-trans retinyl palmitate with some retinyl acetate also being used. Based on current standards, 1000 IU of vitamin A is equal to 0.55 mg of retinyl palmitate or 0.35 mg of retinyl acetate. Based on a survey of nutritionists we conducted about 20 years ago (Weiss, 1998), average supplementation rates ranged from 100,000 to 150,000 IU/d depending on the type of cow. On a mass basis that is only about 80 mg/day of supplemental vitamin. The current NRC recommendation for supplemental vitamin A (all NRC 2001 vitamin recommendations are for supplemental, not total vitamins) is 50 IU/lb. of body weight (BW) or about 75,000 IU/day for an average Holstein cow (Table 1). That recommendation is for all classes of dairy cattle. Although vitamin A is not an active area of research there is little data indicating that the NRC (2001) recommendation is inadequate for lactating cows fed a typical diet. A recent study evaluated feeding 2X NRC (95 IU/lb BW) and reported some small increases in various measures of immune function, but no effects on production and clinical responses (e.g., mastitis) were not measured (Jin et al., 2014).

The NRC recommendation is based on several assumptions:

1. The diet is approximately 60% forage
2. The cow is consuming little or no fresh forage
3. Milk yield is approximately 75 lbs/day
3. Basal diet provides typical amounts of B-carotene

The conditions stated above either effect vitamin A supply or vitamin A requirements. Based on in vitro rumen studies (Rode et al., 1990; Weiss et al., 1995) a substantial amount of vitamin A is destroyed in the rumen and destruction rate increases with the amount of concentrate in the diet (in those studies the concentrate was predominantly starch-based). In vitro ruminal destruction of vitamin A was 20 to 25% when the substrate was 90 to 100% forage and 70 to 75% was destroyed with diets containing 50 or 30% forage, respectively. In studies evaluating responses to vitamin A, diets were around 60% forage; ruminal destruction was assumed to be about 50% via extrapolation. Therefore, a diet with 50% forage may need about 17% more vitamin A(~84,000 IU/day) than recommended by NRC (2001); however a cow fed an 80% forage diet may need only 0.65 X NRC requirements (~47,000 IU/day).

Higher forage diets are also typically higher in B-carotene which can be converted into vitamin A by the cow. Once a forage plant is cut, B-carotene starts being oxidized (destroyed). Losses during silage making can be greater than 50% and for hay losses can exceed 80% as compared to fresh forage (Noziere et al., 2006). Corn silage is a poor source of B-carotene and usually has about 50% of the concentration found in haycrop silages. However since most of the experiments evaluating responses to vitamin A fed corn silage, this effect is already incorporated into requirements. Most concentrates are poor sources of B-carotene. Straw, a common ingredient for dry cows has virtually no B-carotene. The take home from this is:

- Cows that are grazing fresh, green forage with pasture providing at least 40% of diet dry matter probably need very little, if any, supplemental vitamin A because pasture is probably providing 70,000 to 100,000 more IU of vitamin A equivalents/day than a cow consuming silage.
- Hay-based diets will need more supplemental vitamin A than silage based diets. If you change from a diet in which the forage was 50% hay and 50% silage (similar to many of the studies) to a diet with all the forage as hay, intake of basal vitamin A equivalents would be reduced by 15,000 IU/d. Most diets in the Midwest do not have that much hay so the adjustment will be smaller.
- Straw-based dry cow diets will require more supplemental vitamin A than hay or silage based diets. Replacing 8 lbs of haycrop silage dry matter with straw will reduce intake of basal vitamin A equivalents by about 45,000 IU/day. You probably do not need to increase supplementation that much, because efficiency of conversion of B-carotene to vitamin A is likely lower than anticipated, but a substantial increase in supplementation is likely necessary with straw based diets

Milk contains about 7 mg of retinol/kg of fat or about 0.11 mg/lb. of milk (assumed 3.7% fat). The average milk yield by cows in studies evaluating responses to vitamin a was about 75 lbs./day. Therefore the current NRC recommendation should be adequate for cows producing 75 lbs of milk. For every additional pound of milk above 75 lbs, vitamin A supplementation should be increased by about 450 IU to cover losses in milk (for a Jersey cow it would be about 580 IU/lb of milk). In a pen situation, if the average cow is milking 75 lbs. and needs 75,000 IU of vitamin A, the diet needs to contain about 1400 IU/lbs of DM. If a cow was milking 100 lbs, she would need an additional 11,000 IU of vitamin A to cover milk losses but because she would be expected to eat about 10 lbs more DM, her intake of A would be adequate. In other words, the concentration of vitamin A (IU/lb. of DM) does not have to be increased for high producing cows).

Substantial amounts of feed grade vitamin A (retinyl palmitate) can be destroyed during storage and this potential loss should be considered when developing formulating strategies. If vitamin A is blended in a premix with inorganic zinc and copper, vitamin A activity decreased by about 9% per month (compared to about 3% for other vitamins) (Shurson et al., 2011). Pelleting and excess heat, humidity and sun exposure during storage will greatly

increase losses in activity. If feed mixes are stored for long period of time especially if it contains inorganic trace metal or is stored under poor conditions, supplementation should be increased to cover losses in activity.

How Low Can You Go ?

Cows are efficient storers of retinol when fed in excess or when large amounts are injected. Excess retinol is stored in the liver and liver retinol concentrations are a good indicator of status. It changes rapidly (days to weeks) in response to changes in supply. Hepatic retinol concentrations less than 30 mg/kg (dry basis; all liver concentrations in this paper are on a mg/kg dry weight basis) is considered indicative of a vitamin A deficiency and values less than about 100 mg/kg are considered suboptimal. Beef cattle fed a high concentrate diet (so ruminal destruction of vitamin A was likely high) and approximately 40 or 80 IU of vitamin A/lb. of bodyweight for 140 days (Figure 1) had liver concentrations ranging from about 500 mg/kg dry weight to more than 800 mg/kg (Bryant et al., 2010). It is very likely that dairy cows fed ~100,000 IU of vitamin A/day probably have liver concentrations in excess of 400 mg/kg. Liver retinol concentrations in beef heifers and steers fed no supplemental vitamin A and a basal diet void of B-carotene diet (Figure 2) dropped from about 474 mg/kg (dry basis) to 210 mg/kg over 84 days (Alosilla et al., 2007). If fed the same diet, depletion will occur more rapidly in a dairy cow than a beef animal because of secretion of retinol in milk, however, typical dairy cow diets contain more B-carotene than feedlot diets. Liver depletions rates have not been determined in lactating cows fed typical diets but based on beef data, liver retinol will remain in the adequate range for several weeks to a few months when all supplemental vitamin A is removed from the diet. I am not advocating removing all supplemental vitamin A from lactating cow diets; however, feeding no supplemental vitamin A for a month or so likely will have no negative impacts.

Dry cows and Prefresh Cows

The 2001 NRC has the same supplemental vitamin A requirements for all dairy cattle (50 IU/lb BW) and data generally support that. However, with the widespread application of straw-based dry cows diets (i.e., low B-carotene diets) increased supplemental vitamin A may be warranted (discussed above). Independent of basal diet, the prefresh cow may need increased vitamin A supplementation. As with vitamin E, plasma concentrations of retinol and B-carotene drop markedly starting about 2 weeks prepartum even when cows are fed diets adequate in supplemental vitamin A (Goff and Stabel, 1990; Weiss et al., 1994) . What is unusual is that plasma retinol concentration is a very poor indicator of vitamin A status or vitamin A intake. When fed deficient diets, animals mobilize retinol from the liver and plasma levels are maintained until liver concentration drop below about 30 mg/kg (clinical deficient state). But in the prepartum dairy cow, plasma concentrations decrease even though the liver likely has more than adequate stores. The decrease in plasma retinol is caused entirely by secretion of retinol into colostrum starting about 7 d before calving because mastectomized cows

experienced no decrease in serum retinol at calving (Goff et al., 2002). It is not known whether additional vitamin during the prefresh period will prevent the decrease in plasma vitamin A or whether the decrease is even a problem. However, when supplemental vitamin E is added and the decrease in plasma tocopherol is prevented, improved mammary gland health is observed.

Prioritizing When Vitamin A Supplies are Limited

If vitamin A supplies are limited or price is a major factor, the first step is to feed supplemental vitamin A at NRC recommendations. Based on survey data this will reduce supplementation by about 50% on average. If additional cuts are needed, the dry cow and prefresh cow should be fed at NRC levels if possible. They have low intakes of basal B-carotene, several studies have shown increased retained placenta and mastitis when dry cows are not fed adequate vitamin A, and the newborn calf will need retinol-rich colostrum since calves are born with almost no circulating retinol. The last priority is lactating cows. Intakes are very high and the basal diet generally has substantial B-carotene (all hay diets are an exception). In addition most cows are probably in excellent vitamin A status (large liver stores of retinol) and it is acceptable for the cow to mobilize that as long as liver concentrations of retinol stay above 30 mg/kg and ideally above about 100 mg/kg.

Vitamin E

The standard form of supplemental vitamin E used in the feed industry is all-*rac* α-tocopheryl acetate. By definition, 1 IU of vitamin E equals 1 mg of all-*rac* α-tocopheryl acetate. Based largely on reduction in incidence of mastitis and retained placenta, the 2001 NRC set the supplemental vitamin E requirement at 0.36 IU/lbs. BW for lactating cows and 0.73 IU/lb. BW for dry cows. This equates to about 500 IU/day for lactating cows and 1000 IU/day for dry cows (Table 1). Basal diets can provide substantial amounts of tocopherol but the same factors that affect B-carotene concentrations (discussed above) affect tocopherol concentrations. Diets used in the studies evaluating supplemental vitamin E were largely hay-based for dry cows and silage based for lactating cows. The only major adjustment to vitamin E supplementation needed because of basal diet is for grazing cows. Fresh pasture can have 2 to 10 times more tocopherol than silage or hay (respectively), and plasma concentration of tocopherol in grazing cattle (with no vitamin E supplementation) are usually much higher than what we observe in confinement cattle fed supplemental vitamin E per NRC. If the diet is composed of 50% or more of pasture DM, no supplemental vitamin E is needed. Based on average tocopherol concentrations in fresh pasture and corn silage and alfalfa silage and assuming pasture replaces silages, a diet with about 30% fresh pasture (DM basis) will need about 50% of NRC supplementation. Another type of basal diet that needs to be considered with respect to vitamin E supplementation is straw-based dry cow diets. Straw is essentially void of tocopherol but it often replaces hay which is low in tocopherol. If 8 lbs of straw replaced 8 lbs of hay, basal intake of tocopherol likely did not decrease very much.

However, if the straw replaced hay silage, intake of basal tocopherol could decrease by 100 to 150 IU/day.

Current data support the 2001 NRC requirement for dry and lactating cows. One study suggested that excess vitamin E during the dry period (3X NRC) may actually be detrimental to cow health (Bouwstra et al., 2010). Since 2001, several studies have evaluated the effect of additional vitamin E during the prefresh period and in general positive response on immune function or clinical measures have been reported (Politis et al., 2001; Politis et al., 2004; Chandra et al., 2014). Supplementation rates during the last 2 to 3 weeks of gestation ranged from 2000 IU/d to 4000 IU/d. Because of cost, providing prefresh cows (not grazing) with about 2000 IU/d will likely improve immune function and cow health.

Vitamin E supplies have been reduced and prices have increased 3 to 4 times over historic prices but true shortages have not been reported. Considering the benefits of adequate vitamin E relative to its cost, NRC supplementation rates should be maintained and if a prefresh diet is fed, consider increasing vitamin E to 2000 IU/day.

Vitamin D

The primary form of supplemental vitamin D fed to livestock is vitamin D_3. Vitamin D_2 may be available but it is vastly inferior to D_3 and probably should not be fed. If it is used, supplementation rates should be about double those for vitamin D_3. For this paper recommendations are appropriate for vitamin D_3. Cows and other animals can synthesize vitamin D when the skin obtains adequate UV irradiation from the sun. The amount of vitamin D synthesized depends intensity of the sunlight which depends on season (summer >> winter) and time of day (noon > morning or evening), cloud cover, and duration of exposure. Cows exposed to 90 minutes of intense sun (centered around noon) maintained serum concentrations of 25-OH vitamin D in the adequate range (Hymoller and Jensen, 2012). Based on human synthesis rates, cows in winter in the tristate area cannot synthesize adequate vitamin D regardless of how long they are outside, and during spring and fall may need more than 5 hours of sun exposure to synthesize adequate vitamin D.

After decades of almost no research on vitamin D for dairy cows, it is starting to receive substantial interest. This is probably caused by the data showing relationships between low vitamin D status and increased risk for numerous diseases in humans. Previously vitamin D was considered only with respect to calcium metabolism and current requirements (14 IU/lb of BW or about 20,000 IU/d; Table 1) are adequate to maintain normal calcium metabolism. New data suggests a role of vitamin D in immune function and more general health responses (Lippolis, 2011) and supplementation rates may need to be higher to see this responses. Based more on data from human subjects than cattle, blood concentrations of 25-OH vitamin D (an excellent status indicator of vitamin D) below 30 ng/ml are associated with increased health problems. Concentrations of 8 to 10 ng/ml are probably adequate for Ca metabolism. From a survey of commercial and university dairy herds, feeding 30,000 to 50,000 (1.5 to 2.5 X current NRC recommendation) maintained serum

25-OH vitamin D well above 30 ng/ml. However, one herd was fed 20,000 IU/d (i.e., NRC requirement) and although the average was above 30 ng/ml, several individual cows had concentrations less than 30 ng/ml. This suggests that feeding 20,000 may not be adequate; however data showing improve clinical or production responses with additional vitamin D supplementation are lacking. Based on the limited data available, supplementation rates of 1.5 X NRC are justified (i.e., about 30,000 IU/day for lactating cows). Because calcium metabolism is so important to transition cows, at this time, feeding at NRC (2001) rate is recommended.

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Table 1. Recommended daily intakes (IU/day) of supplemental vitamins A, D, and E for a Holstein cow (multiply values by 0.75 for Jersey cows).

Vitamin	Type of Cow			Adjustments
	Dry	Prefresh	Lactating	
A	75,000	75,000	75,000	• Increase when feeding straw-based diets and consider increasing when feeding hay-based diets. • For grazing cattle, these should be reduced substantially (sometimes to 0). • Prefresh cows may benefit from higher intakes because of colostrum synthesis • For lactating cows producing more than 75 lbs. of milk increase by 450 IU/d per pound of milk greater than 75 lbs.
D	20,000	20,000	30,000	• Cows grazing at least 2 hours per day in the summer probably do not need supplemental D. • Increase substantially if using vitamin D_2.
E	1000	2000	500	• Increase by about 100 IU/d with straw based diets. • Hay based diets may need slightly more vitamin E. • For grazing cows, reduce supplementation substantially (sometimes to 0)

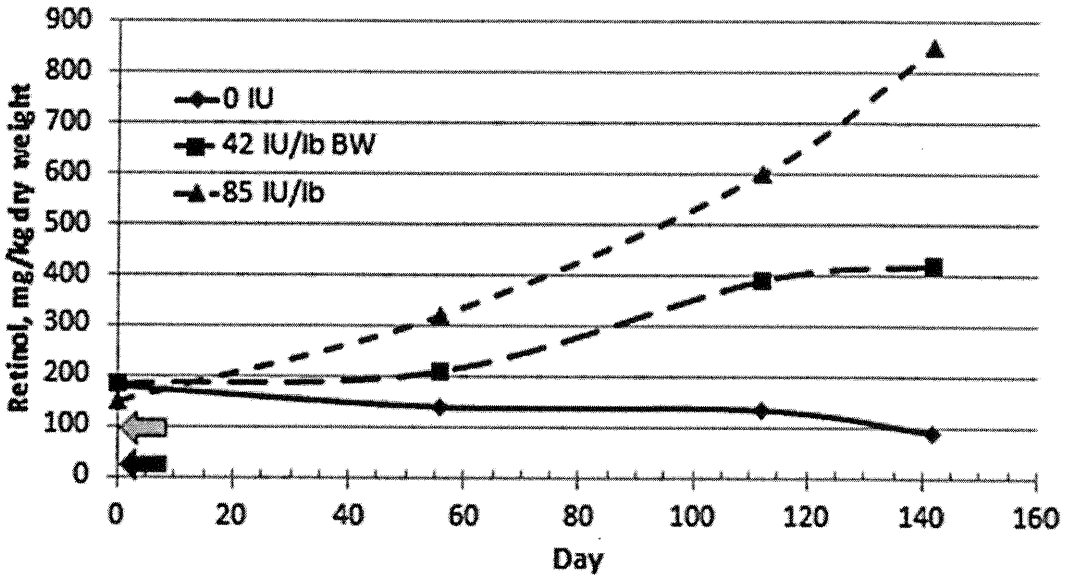

Figure 1. Concentrations of retinol (vitamin A) in liver of beef steers that were fed no supplemental vitamin A or approximately 40 or 80 IU/lbs of bodyweight (the NRC requirement for dairy cattle is 50 IU/lb. BW). The basal diet likely provided some B-carotene (not measured). The black arrow marks the clinical deficient concentration and the grey arrow indicates marginal deficiency (Figured derived from Bryant et al., 2010).

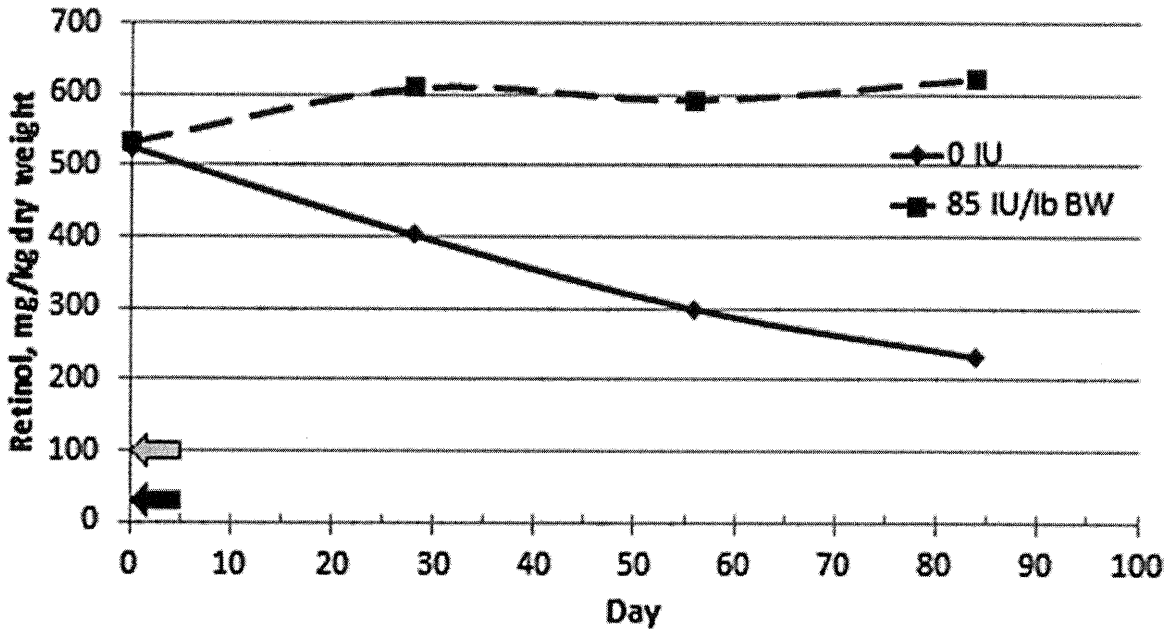

Figure 2. Concentrations of retinol (vitamin A) in liver of growing beef steers and heifers fed no supplemental vitamin A or approximately 85 IU/lb of body weight (1.7 X NRC requirement for dairy cows). The basal diet likely provided no B-carotene. The black arrow marks the clinical deficient concentration and the grey arrow indicates marginal deficiency (Figured derived from Alosilla et al., 2007).

Supplemental Fatty Acids in Lactating Cow Diets: Myth and Reality

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Introduction

Recently, the effects of individual fatty acids (FA) on digestibility, metabolism, and production responses of dairy cows has received renewed attention. The addition of supplemental FA sources to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. The ability to understand and model FA, the effects of individual FA, and different FA supplements on production parameters has direct impact on dairy industry recommendations and the usefulness of FA supplementation strategies. In fresh cows, the high metabolic demand of lactation and reduced DMI during the immediate postpartum period result in a state of negative energy balance. Approaches to increasing energy intake of postpartum cows include increasing starch content of the diet and supplementing FA to increase the energy density of the diet. However, feeding high starch diets that promote greater ruminal propionate production during early lactation could be hypophagic and therefore further reduce DMI and increase the risk of ruminal acidosis and displaced abomasum (Allen and Piantoni, 2013). Regarding supplemental FA, some authors suggest that caution should be exercised when using dietary FA to increase the caloric density of diets in early lactation dairy cows, since a high lipid load may affect the endocrine system, feed intake, and increases the risk for metabolic disorders (Kuhla et al., 2016). However, just as we recognize that not all protein sources are the same it is important to remember that not all FA or FA supplements are the same. We will briefly review the biological processes and quantitative changes during the metabolism of FA, the digestibility of these FA, and their overall impact on performance. Our emphasis in the current paper is on recent research supplementing palmitic (C16:0), stearic (C18:0), oleic (*cis*-9 C18:1), omega-3, and omega-6 acids on feed intake, nutrient digestibility, milk production and milk composition, health, and reproduction.

C16:0, C18:0, and *Cis*-9 C18:1 Effects on FA Digestibility

Our recent FA digestibility research has utilized and focused on C16:0, C18:0, *cis*-9 C18:1. Of particular importance, Boerman et al. (2017) fed increasing levels of a C18:0-enriched supplement (93% C18:0) to mid-lactation dairy cows and observed no positive effect on production responses, which was likely associated with the pronounced decrease in total FA digestibility as FA intake increased (Figure 1A). Similarly, Rico et al. (2017) fed increasing levels of a C16:0-enriched supplement (87% C16:0) to mid-lactation dairy cows and even though a positive effect was observed on production response up to 1.5% diet DM, a decrease in total FA digestibility with increasing FA intake was observed (Figure 1B). However, considering that the range in FA intake was similar across both studies, the decrease in total FA digestibility was more pronounced when there was increased intake/rumen outflow of C18:0 rather than C16:0. This is supported by our meta-analysis, in which a negative relationship between the total flow and digestibility of FA was observed, with the decrease in total FA digestibility driven by the digestibility of C18:0 because of the negative relationship between duodenal flow and digestibility of C18:0 (Boerman et al., 2015). The exact mechanisms for these differences in digestibility are not understood; however, potential causes include the lower solubility of C18:0 compared to C16:0, which would be more dependent of emulsification for absorption (Drackey, 2000). Additionally, results have shown that *cis*-9 C18:1 has greater digestibility than C16:0 and C18:0 (Boerman et al., 2015). Freeman (1969) examined

the amphiphilic properties of polar lipid solutes and found that *cis*-9 C18:1 had a positive effect on the micellar solubility of C18:0. To further understand what factors influence FA digestibility, we utilized a random regression model to analyze available individual cow data from 5 studies that fed a C16:0-enriched supplement to dairy cows. We observed that total FA digestibility was negatively impacted by total FA intake, but positively influenced by the intake of *cis*-9 C18:1 (unpublished results). Finally, we recently evaluated the effects of varying the ratio of dietary C16:0, C18:0, and *cis*-9 C18:1 in basal diets containing soyhulls or whole cottonseed on FA digestibility (Table 1). We observed that feeding a supplement containing C16:0 and *cis*-9 C18:1 increased FA digestibility compared with a supplement containing C16:0, a mixture C16:0 and C18:0, and a non-fat control diet. The supplement containing a mixture C16:0 and C18:0 reduced 16-, 18-carbon, and total FA digestibility compared with the other treatments (Table 1). This is demonstrated in Figure 2 by using a Lucas test to estimate the apparent digestibility of the supplemental FA blends. The slopes (i.e., digestibility of the supplemental FA blends) in soyhulls based diets were 0.64, 0.55 and 0.75 and in cottonseed diets were 0.70, 0.56 and 0.81 for supplements containing C16:0, a mixture C16:0 and C18:0, and a mixture of C16:0 and *cis*-9 C18:1, respectively. This supports the concept that a combination of 16-carbon and unsaturated 18-carbon FA may improve FA digestibility, but reasons for this need to be determined.

In fresh cows, there is scarce information about the effects of supplemental FA on FA digestibility. We recently conducted a study to evaluate the effects of timing of C16:0 supplementation on performance of early lactation dairy cows (de Souza and Lock, 2017b). We observed a treatment by time interaction for C16:0 supplementation during the fresh period (1 – 24 DIM); although C16:0 reduced total FA digestibility compared with control, the magnitude of difference reduced over time (Figure 3). Interestingly, we also observed an interaction between time of supplementation and C16:0 supplementation during the peak period (25 – 67 DIM), due to C16:0 only reducing FA digestibility in cows that received the control diet in the fresh period. This may suggest an adaptive mechanism in the intestine when C16:0 is fed long-term. Understanding the mechanisms responsible for this effect deserves future attention, as does the impact of other supplemental FA during early post-partum on FA digestibility and nutrient digestibility.

Effect of Fatty Acids on NDF Digestibility

Changes in intake and digestibility of other nutrients, such as NDF, due to FA supplementation may affect positively or negatively the digestible energy value of any FA supplement. Weld and Armentano (2017) performed a meta-analysis to evaluate the effects of FA supplementation on DMI and NDF digestibility of dairy cows. Supplementation of supplements high in medium chain FA (12 and 14-carbons) decreased both DMI and NDF digestibility. Addition of vegetable oil decreased NDF digestibility by 2.1 percentage units, but did not affect DMI. Also, feeding saturated prilled supplements (combinations of C16:0 and C18:0) did not affect DMI, but increased NDF digestibility by 0.22 percentage units. Overall, the authors concluded that the addition of a fat supplement, in which the FA are 16-carbon or greater in length, has minimal effects on NDF digestibility, but the effect of C16:0-enriched supplements were not evaluated.

We recently utilized a random regression model to analyze available individual cow data from 6 studies that fed C16:0-enriched supplements to dairy cows (de Souza et al., 2016). We observed that NDF digestibility was positively impacted by total C16:0 intake (Figure 4A) and DMI was not affected. This suggests that that the increase in NDF digestibility when C16:0-enriched supplements are fed to dairy cows is not explained through a decrease in DMI. Additionally, when comparing combinations of C16:0, C18:0, and *cis*-9 C18:1 in supplemental fat, we observed that feeding supplements containing C16:0 or C16:0 and *cis*-9 C18:1 increased NDF digestibility compared with a supplement containing C16:0 and C18:0 (Table 1).

With early lactation cows, Piantoni et al. (2015b) fed a saturated fat supplement (~ 40% C16:0 and 40% C18:0) and observed that fat supplementation increased NDF digestibility by 3.9% units in the low forage diet (20% fNDF), but had no effect in the high forage diet (26% fNDF). In our recent

study that evaluated the effects of timing of C16:0 supplementation (PA) on performance of early lactation dairy cows (de Souza and Lock, 2017b), we observed that C16:0 supplementation consistently increased NDF digestibility ~ 5% units over the 10 weeks of treatment compared with control (Figure 4B).

Effects of C16:0, C18:0, and *Cis*-9 C18:1 on Production Responses

We recently carried out a series of studies examining the effect of individual saturated FA on production and metabolic responses of post-peak lactating cows (Table 2). Piantoni et al. (2015a) reported that C18:0 increased DMI and yields of milk and milk components, with increases more evident in cows with higher milk yields, but the response occurred only in one of the two periods of the crossover design. Reasons why only higher yielding cows responded more positively to C18:0 supplementation and only in one period remains to be determined. Additionally, in a recent dose response study with mid lactation cows, feeding a C18:0-enriched supplement (93% C18:0) increased DMI but had no effect on the yields of milk or milk components when compared to a non-FA supplemented control diet, which was probably associated with the decrease in FA digestibility (Figure 1A). Our results, and those of others, indicate that C16:0 supplementation has the potential to increase yields of ECM and milk fat as well as the conversion of feed to milk, independent of production level when it was included in the diet for soyhulls or C18:0 (Table 2). We recently utilized a random regression model to analyze available individual cow data from 10 studies that fed C16:0-enriched supplements to post peak dairy cows (de Souza et al., 2016). We observed that energy partitioning toward milk was increased linearly with C16:0 intake, as a result of a linear increase in milk fat yield and ECM with increasing intake of C16:0.

When we compared combinations of C16:0, C18:0, and *cis*-9 C18:1 in FA supplements, a supplement containing more C16:0 increased energy partitioning toward milk due to the greater milk fat yield response compared with the other treatments (Table 1). In contrast, a FA supplement containing C16:0 and *cis*-9 C18:1 increased energy allocated to body reserves compared with other treatments. The FA supplement containing a combination of C16:0 and C18:0 reduced nutrient digestibility, which most likely explains the lower production responses observed compared with the other treatments (Table 1). Interestingly, in a follow up study we compared different ratios of C16:0 and *cis*-9 C18:1 in FA supplements fed to post-peak cows, and observed that supplements with more C16:0 favored energy partitioning to milk in cows producing less than 45 kg/d, while supplements with more *cis*-9 C18:1 favored energy partitioning to milk in cows producing great than 60 kg/d (de Souza and Lock, 2017a). Also, regardless of production level, supplements with more *cis*-9 C18:1 increased BW change. This may suggest that C16:0 and *cis*-9 C18:1 are able to alter energy partitioning between the mammary gland and adipose tissue, which may allow for different FA supplements to be fed in specific situations according to the metabolic priority and needs of dairy cows. Further research is needed to confirm these results in cows at different stages of lactation or other physiological conditions.

In early lactation cows, Beam and Butler (1998) fed a saturated FA supplement (~ 40% C16:0 and 40% C18:0) and observed that FA supplementation decreased DMI and did not affect yields of milk and ECM in the first 4 weeks after calving. Piantoni et al. (2015b) fed a similar saturated FA supplement (~ 40% C16:0 and 40% C18:0) and observed that FA supplementation during the immediate postpartum (1-29 DIM) favored energy partitioning to body reserves rather than milk yield, especially in the lower forage diet. The high forage diet with supplemental FA increased DMI and tended to decrease BCS loss compared with the same diet without FA supplementation. Also, regardless of forage level, feeding supplemental FA increased DMI, decreased BCS loss, but tended to decrease milk yield. When cows were fed a common diet during the carryover period, the low forage diet with FA supplementation fed during the immediate postpartum continued to decrease milk yield and maintained higher BCS compared with the other treatments. On the other hand, Weiss and Pinos-Rodriguez (2009) fed a similar saturated FA supplement (~ 40% C16:0 and 40% C18:0) to early-lactation cows (21 to 126 DIM) and observed that when high-forage diets were supplemented with FA, the increased NE_L intake went toward body energy reserves as measured

by higher BCS with no change in milk yield. However, when low-forage diets were supplemented with FA, milk yield increased (2.6 kg/d) with no change in BCS.

In a recent study, we evaluated the effects of timing of C16:0 supplementation on performance of early lactation dairy cows (de Souza and Lock, 2017b). During the fresh period (1-24 DIM), we did not observe treatment differences for DMI or milk yield (Figure 5A), but compared with control, C16:0 increased the yield of ECM by 4.70 kg/d consistently over time (Figure 5B). However, C16:0 reduced body weight by 21 kg (Figure 6), and body condition score by 0.09 units and tended to increase body weight loss by 0.76 kg/d compared with CON. Feeding C16:0 during the peak period (25 to 67 DIM) increased the yield of milk by 3.45 kg/d, ECM yield by 4.60 kg/d (Figure 5), and tended to reduce body weight by 10 kg compared with control (Figure 6).

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

Conclusion

The addition of supplemental FA to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. Although in general FA supplementation has been shown to increase milk yield, milk fat yield, and improve reproduction performance, great variation has been reported in production performance for different FA supplements, and indeed the same supplement across different diets and studies. Results are contradictory about the benefits of FA supplementation to early lactation dairy cows. We propose that this is a result of differences in FA profile of supplements used and the time at which FA supplementation starts. Further work is required to characterize the sources of variation in response to FA supplementation. Just as we recognize that not all protein sources are the same it is important to remember that not all FA sources and FA supplements are the same. The key is to know what FA are present in the supplement, particularly FA chain length and their degree of unsaturation. Once this information is known it is important to consider the possible effects of these FA on DMI, rumen metabolism, small intestine digestibility, milk component synthesis in the mammary gland, energy partitioning between the mammary gland and other tissues, body condition, and their effects on immune and reproductive function. The extent of these simultaneous changes along with the goal of the nutritional strategy employed will ultimately determine the overall effect of the FA supplementation, and the associated decision regarding their inclusion in diets for lactating dairy cows.

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Table 1. Nutrient digestibility, milk production, and body weight for cows fed diets containing fat supplements with different fatty acid profiles.[1] Data from de Souza et al. (2018).

	FA treatment[1]				
Variable	CON	PA	PA+SA	PA+OA	SEM
Digestibility, %					
DM	67.9^{ab}	68.6^{a}	67.6^{b}	68.5^{a}	0.44
NDF	44.2^{b}	45.1^{a}	42.9^{c}	45.0^{a}	0.36
Total FA	78.6^{ab}	77.4^{b}	68.2^{c}	79.4^{a}	1.52
Yield, kg/d					
Milk	44.6^{b}	46.9^{a}	46.3^{a}	46.5^{a}	1.01
Energy-corrected milk	45.3^{b}	47.7^{a}	46.7^{ab}	46.5^{ab}	0.82
Fat, kg/d	1.60^{b}	1.70^{a}	1.64^{b}	1.64^{b}	0.04
Protein, kg/d	1.42	1.47	1.45	1.43	0.02
BW change, kg/d	0.82^{b}	0.84^{b}	0.70^{b}	1.05^{a}	0.05

$^{a-c}$ Main effects of FA treatments, means in a row without a common letter differ (P<0.05).

[1] CON (Control diet, no fatty acid supplementation); PA (1.5% of FA supplement blend to provide ~ 80% of C16:0); PA+SA (1.5% of FA supplement blend to provide ~ 40% of C16:0 + 40% of C18:0); and PA+OA (1.5% of FA supplement blend to provide ~ 45% of C16:0 + 35% of *cis*-9 C18:1).

Table 2. Summary of DMI, milk production and composition, body weight, and BCS for cows supplemented with C16:0 and C18:0 supplements. The C16:0 supplement contained ~ 99% C16:0 and the C18:0 supplement contained ~ 98% C18:0.

	Piantoni et al. (2013)[1]			**Piantoni et al. (2015)[2]**			**Rico et al. (2014)[3]**		
Variable	**Control**	**C16:0**	**SEM**	**Control**	**C18:0**	**SEM**	**C16:0**	**C18:0**	**SEM**
DMI, kg/d	27.8	27.8	0.54	25.2^{n}	26.1^{m}	0.42	32.1	32.3	0.44
Milk yield, kg/d	44.9^{b}	46.0^{a}	1.7	38.5^{n}	40.2^{m}	0.71	46.6	45.8	2.02
Fat yield, kg/d	1.45^{b}	1.53^{a}	0.05	1.35^{n}	1.42^{m}	0.03	1.68^{y}	1.59^{z}	0.05
Milk fat, %	3.29^{b}	3.40^{a}	0.11	3.60	3.59	0.12	3.66^{y}	3.55^{z}	0.09
Protein yield, kg/d	1.38	1.41	0.04	1.14^{n}	1.19^{m}	0.02	1.50	1.49	0.05
Milk Protein %	3.11	3.09	0.05	3.00	2.99	0.05	3.24	3.29	0.05
3.5% FCM	42.9^{b}	44.6^{a}	1.35	38.6^{n}	40.5^{m}	0.76	47.5^{y}	45.6^{z}	1.64
3.5% FCM/DMI	1.54^{b}	1.60^{a}	0.03	1.53	1.55	0.04	1.48^{y}	1.40^{z}	0.05
Body weight, kg	722	723	14.7	727	730	12.8	720	723	13.6
BCS	2.99	2.93	0.15	2.67	2.67	0.11	2.93^{z}	2.99^{y}	0.11

[1]Treatments were either a control diet (with 2% of diet DM as added soyhulls) or a C16:0-supplemented diet (with 2% of diet DM as C16:0). Means within a row with different superscripts ($^{a, b}$) differ ($P < 0.05$).

[2]Treatments were either a control diet (with 2% of diet DM as added soyhulls) or a C18:0-supplemented diet (with 2% of diet DM as C18:0). Means within a row with different superscripts ($^{m, n}$) differ ($P < 0.05$).

[3]Treatments were either a C16:0-supplemented diet (with 2% of diet DM as C16:0) or a C18:0-supplemented diet (with 2% of diet DM as C18:0). Means within a row with different superscripts ($^{y, z}$) differ ($P < 0.05$).

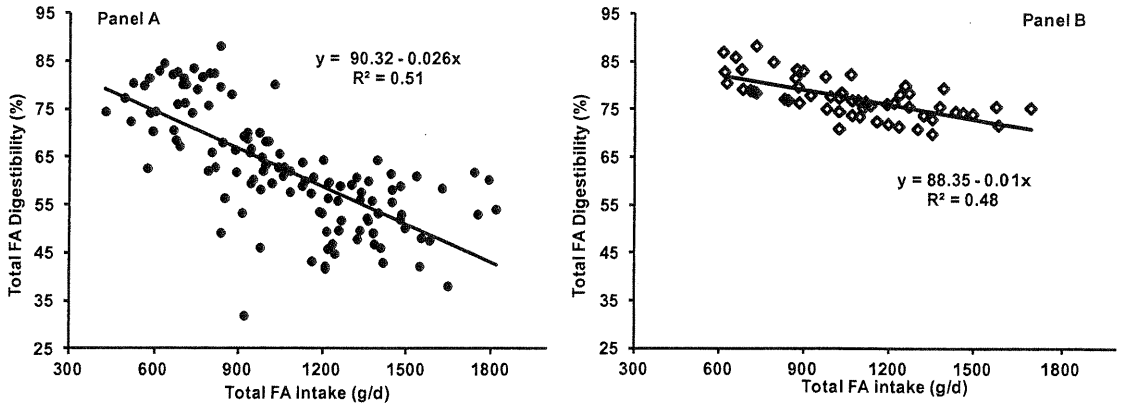

Figure 1. Relationship between total FA intake and apparent total-tract FA digestibility of dairy cows supplemented with either a C18:0-enriched supplement (Panel A) or a C16:0-enriched supplement (Panel B). Results in Panel A utilized 32 mid-lactation cows receiving diets with increasing levels (0 to 2.3% dry matter) of a C18:0-enriched supplement (93% C18:0) in a 4 X 4 Latin square design with 21-d periods (Boerman et al., 2017). Results in Panel B utilized 16 mid-lactation cows receiving diets with increasing levels (0 to 2.25% dry matter) of a C16:0-enriched supplement (87% C16:0) in a 4 X 4 Latin square design with 14-d periods (Rico et al., 2017).

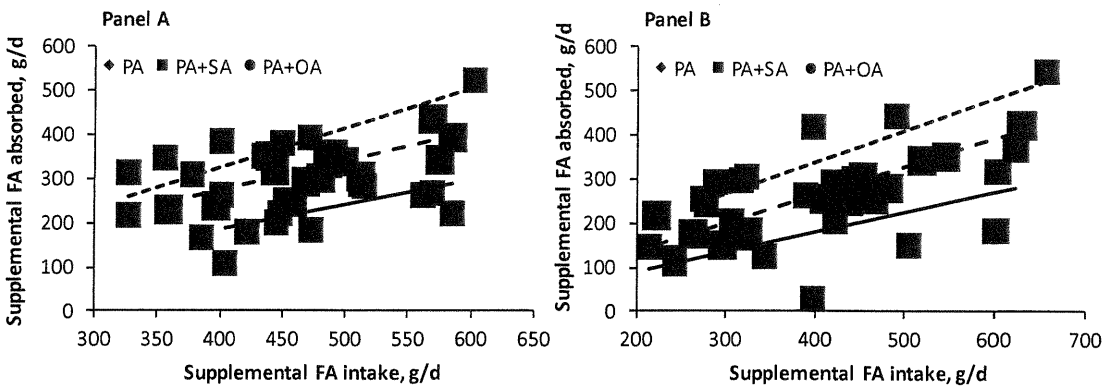

Figure 2. Lucas test to estimate total FA digestibility of supplemental FA treatments when cows received either a soyhulls basal diet (Panel A) or a cottonseed basal diet (Panel B). PA long-dashed line (1.5% of FA supplement blend to provide ~ 80% of C16:0); PA+SA solid line (1.5% of FA supplement blend to provide ~ 40% of C16:0 + 40% of C18:0); and PA+OA short-dashed line (1.5% of FA supplement blend to provide ~ 45% of C16:0 + 35% of C18:1 cis-9). Digestibility of supplemental FA was estimated by regressing intake of supplemental FA on intake of digestible supplemental FA. The mean intakes of FA and digestible FA when cows were fed the control diet were subtracted from the actual intakes of total FA and digestible FA for each observation. From de Souza et al. (2018).

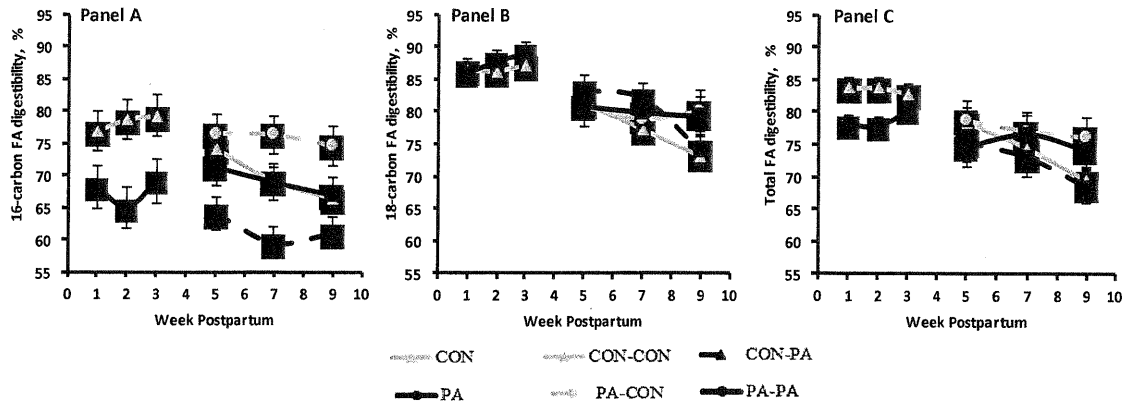

Figure 3. The effects of C16:0-enriched supplementation for early lactation cows on digestibility of 16-carbon (Panel A), 18-carbon (Panel B), and total FA (Panel C). Results utilized 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period FR) or from 25 to 67 DIM (peak period). From de Souza and Lock (2017b).

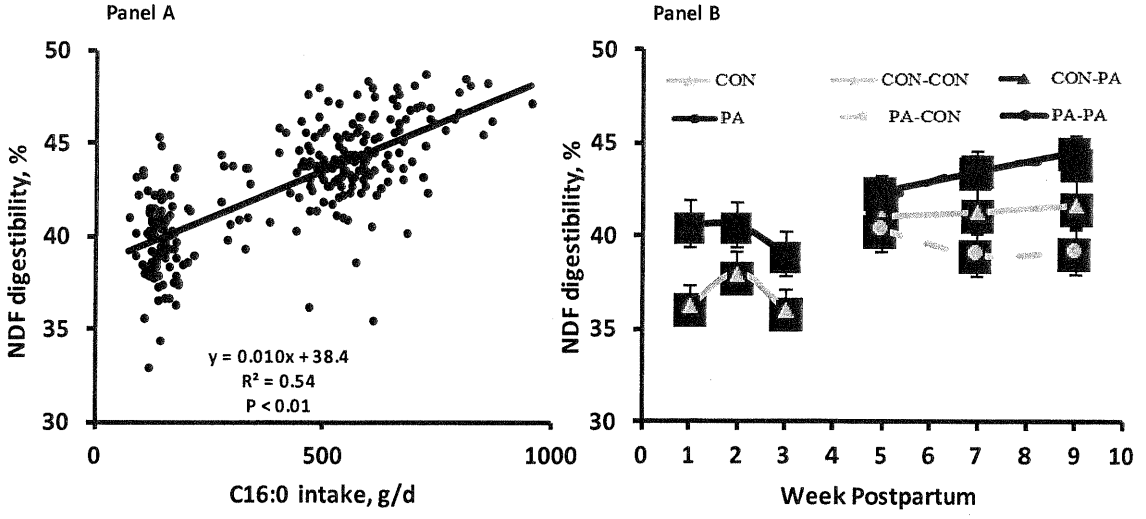

Figure 4. Panel A: Relationship between C16:0 intake and NDF digestibility of dairy cows fed C16:0-enriched FA supplements. Panel B: The effects of C16:0-enriched supplementation in early lactation cows on NDF digestibility. Results in Panel A represent a combined data set evaluated using a random regression model from 6 studies feeding C16:0-enriched supplements on NDF digestibility of post-peak cows (de Souza et al., 2016). Results in Panel B utilized 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period) or from 25 to 67 DIM (peak period). From de Souza and Lock (2017b).

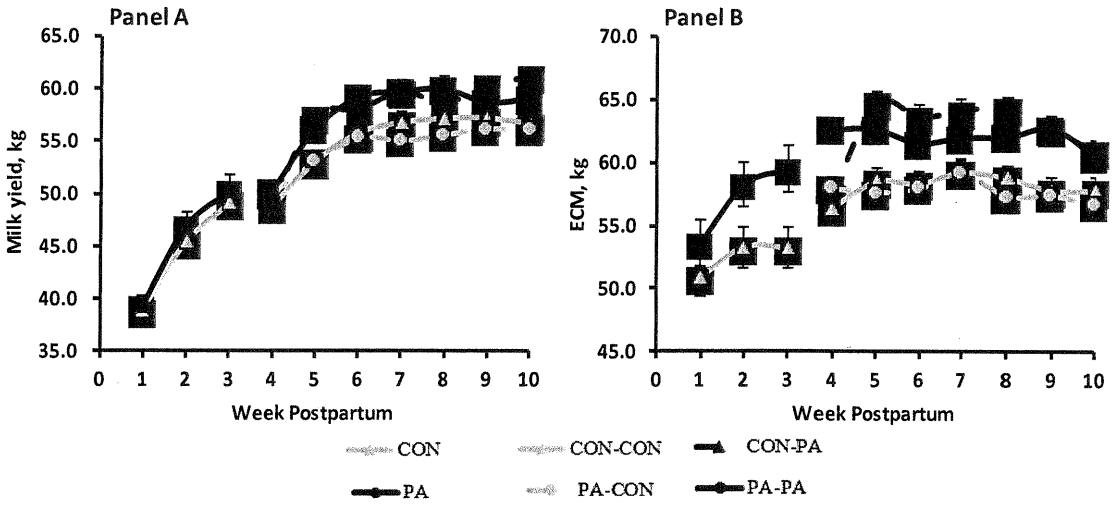

Figure 5. The effects of C16:0-enriched supplementation in early lactation cows on the yield of milk (Panel A) and ECM (Panel B). Results from 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period FR) or from 25 to 67 DIM (peak period). From de Souza and Lock (2017b).

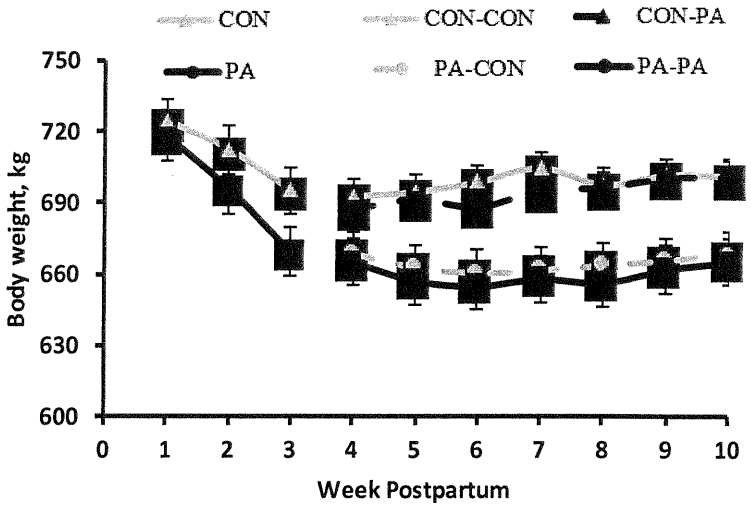

Figure 6. The effects of C16:0-enriched supplementation in early lactation cows on body weight. Results from 52 early-lactation cows receiving the following diets: no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving (1 to 24 DIM; fresh period) or from 25 to 67 DIM (peak period). From de Souza and Lock (2017b).

New Milk Composition Metrics for Dairy Herd Management: Milk Fatty Acids and Milk Estimated Blood NEFA.

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Summary

Partial least square (**PLS**) models were developed from Fourier transform mid-infrared (MIR) spectra, externally validated, and are being used commercially in the US for direct measurement of: 1) groups of milk fatty acids [i.e., de novo (**DN**), mixed origin (**MO**), and preformed fatty (**PF**) acids], 2) fatty acid (**FA**) chain length (expressed as carbon number), 3) FA unsaturation (expressed as double bonds per FA) and 4) estimated blood non-esterified FA (**NEFA**). Six laboratories in different regions of the US are routinely using the models for bulk tank bovine milk analysis simultaneously with payment testing for individual farms on almost every milk pick up basis. Two research laboratories are testing both bulk tank and individual cow milk samples, while one is also testing sheep and goat milk. There is a high correlation in bulk tank milk of DN (C4 to C15) FA concentration (g/100 g milk) with increased bulk tank milk fat and milk protein percentage. The DN FA are made in the mammary cells from acetate and butyrate produced by the microbial fermentation of carbohydrates in the rumen. The changes in concentration of DN FA in milk reflect efficiency of rumen fermentation and the microbial biomass load (i.e., essential amino acid production) in the rumen. Seasonal variation in bulk tank milk fat and protein content are highly correlated with seasonal variation in milk DN FA. As milk FA chain length and double bonds per FA increase, milk fat decreases, and DN and MO FA synthesis and output per cow per day decreases. Farms with high bulk tank milk double bonds per FA, where the average days in milk of the herd is >120 d, have a much higher incidence of trans FA induced milk fat depression. These FA metrics in combination with milk fat and protein concentration, plus milk weight, MUN, and milk SCC have been used to make decisions to adjust feeding to increase production of grams of fat and protein per cow per day and net income from milk minus feed cost. The estimated blood NEFA and DN FA (expressed as DN as a percentage of total FA) are used in combination to monitor fresh cow metabolic status for early detection of individual cows that will develop clinical ketosis or displaced abomasum. These milk-based transition cow analytical tools provide an opportunity to intervene earlier thereby improving recovery while reducing the negative impact of these adverse metabolic health events on animal welfare and lactation performance.

INTRODUCTION

In 2014 (Barbano et al., 2014), the application of mid-infrared (**MIR**) for rapid milk fatty acid (**FA**) analysis was introduced in a commercial laboratory and positive correlations of bulk tank milk fat test with a higher proportion and concentration of de novo FA in bulk tank milk were reported. The analytical aspects of reference milk FA analysis, PLS model development, and validation statistics were reported by Wojciechowski and Barbano (2016) and Woolpert et al. (2016). Briefly, partial least squares (**PLS**) chemometric prediction models for FA were developed from MIR spectra in the Cornell University laboratory using a Delta Instruments Lactoscope (Delta Instruments, Drachten, Netherlands). The form of the FA data from the MIR was structured to report fatty acid values for DM, MO, and PF fatty acids in g/100 g milk and calculated values as the relative proportions of de novo (C4 to C15), mixed origin (C16:0, C16:1, C17:0), and preformed (C18:0 and longer) FA in milk were also provided. The mean FA chain length (carbon number) and degree of unsaturation (double

bonds/fatty acid) are chemical structure metrics, not concentration metrics. The ratio of SD of reference values for the modeling set divided by standard error of cross validation (RPD) for DN, MO, and PF are 10.4, 6.2, and 7.3, while the RPD FA chain length and degree of unsaturation are 2.1 and 3.3. Manley (2014) indicated that RPD values greater than 3 are useful for screening, values greater than 5 can be used for quality control, and values greater than 8 for any application. With field experience in testing bulk tank milk from commercial farms, we found that providing this FA information in units of grams per 100 grams of milk was more useful when making feeding and management decisions on whole herd or feeding group basis, while the relative percentages of DN, MO, and PF fatty acids are more useful for transition cow metabolic health diagnostics in combination with the results of PLS model for milk estimated blood non-esterified fatty acids (**NEFA**). This paper will focus on the use of the milk FA information for management of dairy cows at the bulk tank level and report the status of our work on individual cow data with respect to how these milk composition and production parameters change with stage of lactation for primiparous and multiparous cows.

Woolpert et al. (2016, 2017) reported the results of two studies to determine feeding and farm management factors influencing milk FA composition and their relationship to bulk tank milk fat and protein content and yield per cow per day. The first study (Woolpert et al., 2016) used 44 commercial dairies that were identified as either predominantly Holstein or Jersey in Vermont and northeastern New York. The yields of milk fat, true protein, and de novo FA per cow per day were higher for high de novo (**HDN**) versus low de novo (**LDN**) farms. The HDN farms had lower free-stall stocking density (cows/stall) than LDN farms. Additionally, tie-stall feeding frequency was higher for HDN than LDN farms. No differences between HDN and LDN farms were detected for dietary dry matter, crude protein, neutral detergent fiber, starch, or percentage of forage in the diet. However, dietary ether extract was lower for HDN than LDN farms. Overall, overcrowded free-stalls, reduced feeding frequency, and greater dietary ether extract content were associated with lower de novo FA synthesis and reduced milk fat and true protein yields on commercial dairy farms in this study.

The difference in income per cow depends on the actual milk price at any point in time. The average fat and protein price for the USDA Federal Milk Order No. 1 for March and April 2014 was $2.10 and $4.62 per lb ($4.62 and $10.17 per kg), respectively. Therefore, at 55 lb (25 kg) of milk per cow per day, the average HDN farm earned a gross of $5.50 and $7.72 per cow for fat and protein, respectively. The average LDN farm at 55 lb (25 kg) milk per cow per day earned a gross of $5.26 and $7.29 per cow for fat and protein, respectively. These differences for fat and protein between HDN and LDN herds at 55 lb (25 kg) of milk per 100 cows per year would result in a gross income difference of $8,544 for fat and $15,695 for protein.

Woolpert et al. (2017) conducted a second study with 39 commercial Holstein herds in Vermont and northeastern NY. No differences in milk (about 70.5 lb (32 kg) /cow/d), fat (2.73 lb (1.24 kg)/cow/d), and true protein (2.2 lb (1.0 kg)/cow/d) yields were detected between HDN and LDN farms, but the percentage of milk fat (3.98 vs 3.78%) and true protein (3.19 vs 3.08%) were both higher on HDN farms. The HDN farms had higher de novo FA, a trend for higher mixed origin FA, and no difference in preformed milk FA daily yield per cow per day. This positive relationship between de novo FA and milk fat and true protein percentage agrees with previous results of Barbano et al. (2014) on bulk tank milk composition from 400 commercial dairy farms. The average fat and protein price for USDA Federal Milk Order No. 1 for February through April 2015 (US Department of Agriculture, 2015) was $1.90 and $2.61 per lb ($4.19 and $5.74 per kg), respectively. Therefore, at 66.1 lb (30 kg) of milk per cow per day, the average HDN farm earned a gross of $5.00 and $5.49 per cow for fat and protein, respectively. The average LDN farm at 30 kg of milk per cow per day earned a gross of $4.75 and $5.30 per cow for fat and protein, respectively. These differences for fat and true protein between HDN

and LDN herds at 66.1lb (30 kg) of milk would result in gross income differences of $9,125 for fat and $6,935 for true protein per 100 milking cows per year. Management (i.e., frequent feed delivery and increased feed bunk space per cow) and dietary (i.e., adequate physically effective fiber and lower ether extract) factors that differed between these HDN and LDN farms have been shown in earlier studies to affect ruminal function.

Based on data from these studies the following graphs (Figures 1 to 4) for Holstein farms were developed to help farms understand the relationships between bulk tank milk FA composition and bulk tank fat and protein tests.

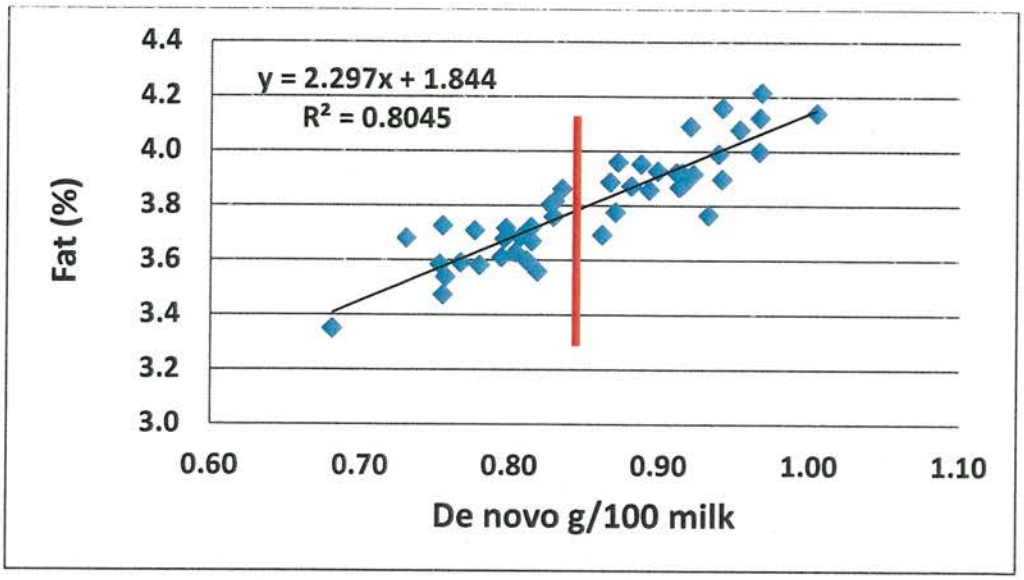

Figure 1. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of de novo FA in milk. In general, a farm needs to have a concentration of de novo FA higher than 0.85 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

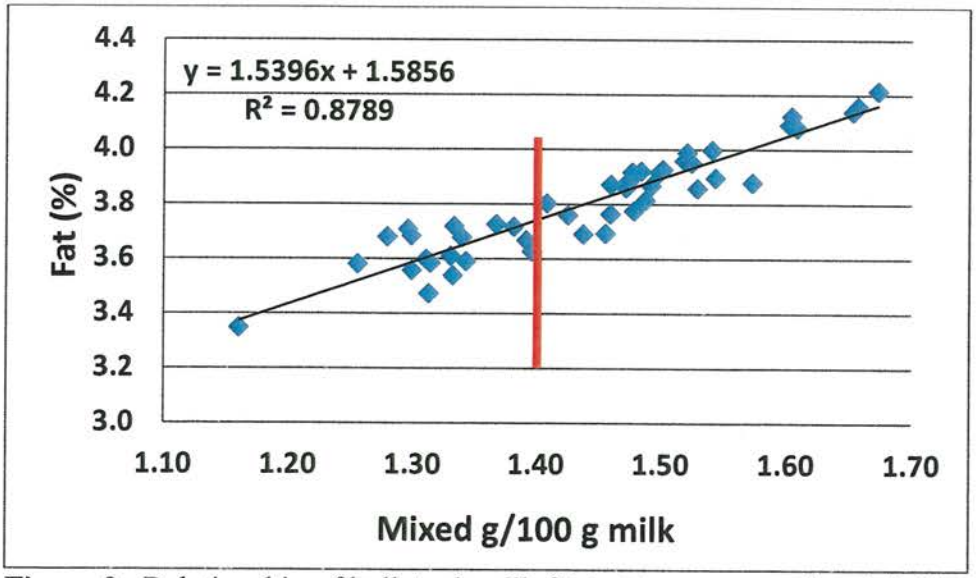

Figure 2. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of mixed origin FA in milk. In general, a farm needs to have a concentration of mixed origin FA higher than 1.40 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

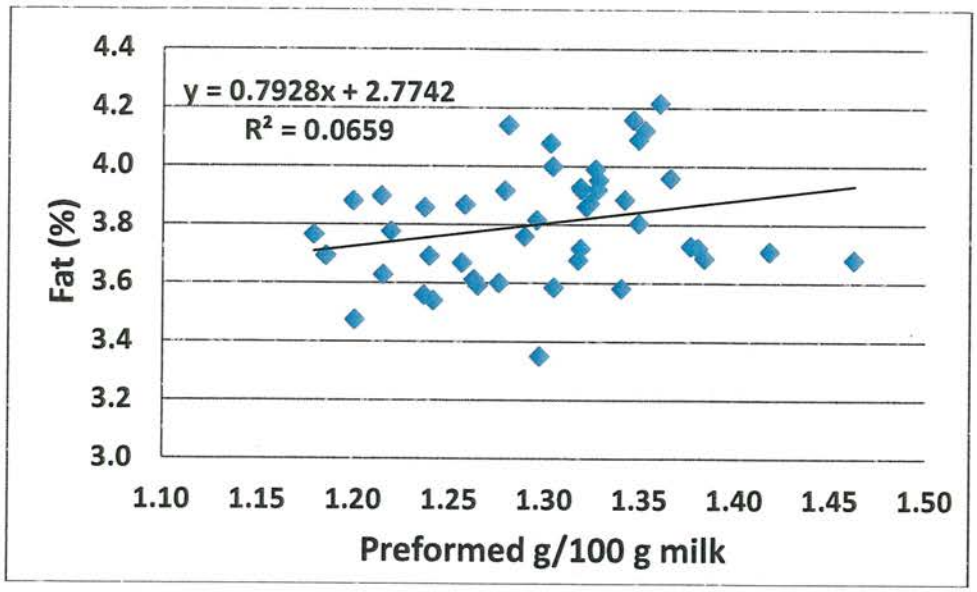

Figure 3. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of preformed FA in milk. In general, the variation in preformed FA concentration in Holstein herds is less than de novo and mixed origin FA and is not well correlated with bulk tank milk fat test.

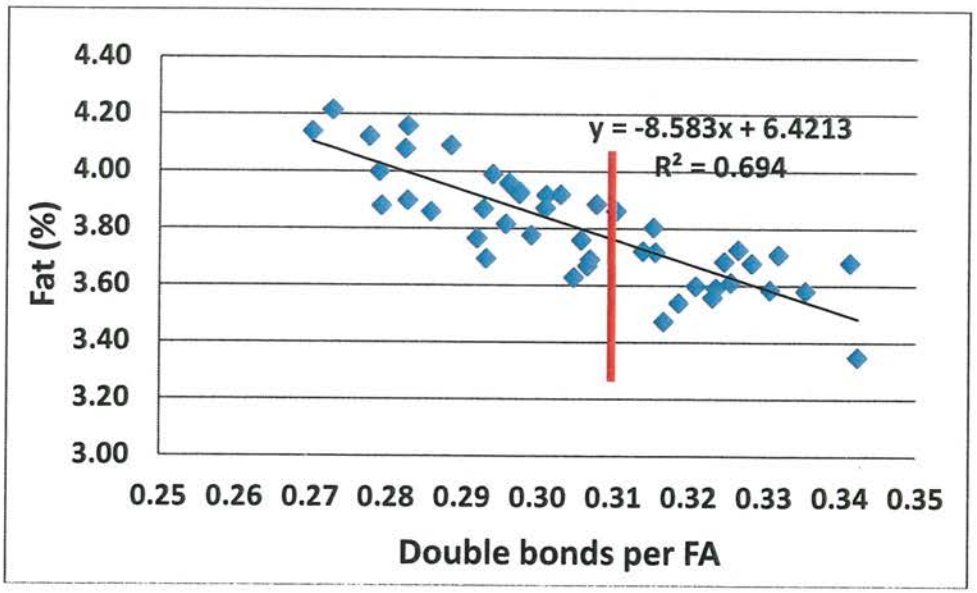

Figure 4. Relationship of bulk tank milk fat FA unsaturation with bulk tank milk fat test. As double bonds per FA increases the bulk tank milk fat test decreases. To achieve a 3.75 % fat test a farm needs to have a double bond per FA of less than 0.31.

FIELD ADOPTION OF ROUTINE MILK FATTY ACID ANALYSIS

Starting in February of 2016, information on FA composition of bulk tank milk was provided to the individual producers of the St Albans Cooperative (Vermont) along with their payment test data on the same milk samples and in the summer of 2017 Agrimark Cooperative (Springfield, MA) and Cayuga

Milk Ingredients (Auburn, NY) have started providing similar data to their producers on the official bulk tank milk samples that are used for milk payment testing. For producers that are not members of those cooperatives, milk samples can be analyzed at commercial laborates (i.e., Zumbrota and Stern County DHIA Labs in Minnesota and the ADM Lab in Clovis, NM) and our research laboratories at Cornell University and Miner Institute. The MIR milk analysis models used for measuring de novo, mixed origin and performed FA and fatty acid chain length (i.e., carbon number) and unsaturation (double bonds per fatty acid) are specific PLS models designed to measure these milk parameters using the MIR equipment produced by Delta Instruments. These values cannot be accurately calculated from other measured fatty acid parameters. The procedures used for development of these herd management MIR PLS models, external validation of the PLS models, and performance statistics for the models have been published (Wojciechowski and Barbano, 2016; Woolpert et al., 2016). Other MIR milk analysis equipment manufacturers may develop similar PLS models to measure these parameters, validate the models and make them available to their customers in the future.

SEASONALITY OF BULK TANK MILK

Over the past 3 to 4 years we have followed the pattern of seasonality of milk fat and protein in relation to milk FA composition on a group of 40 farms with the St. Albans Cooperative. The data January 2014 through July 2017 are from the routine testing results using MIR-analysis in the St. Albans Cooperative on fresh bulk tank milk samples used for payment testing (Figures 5-8).

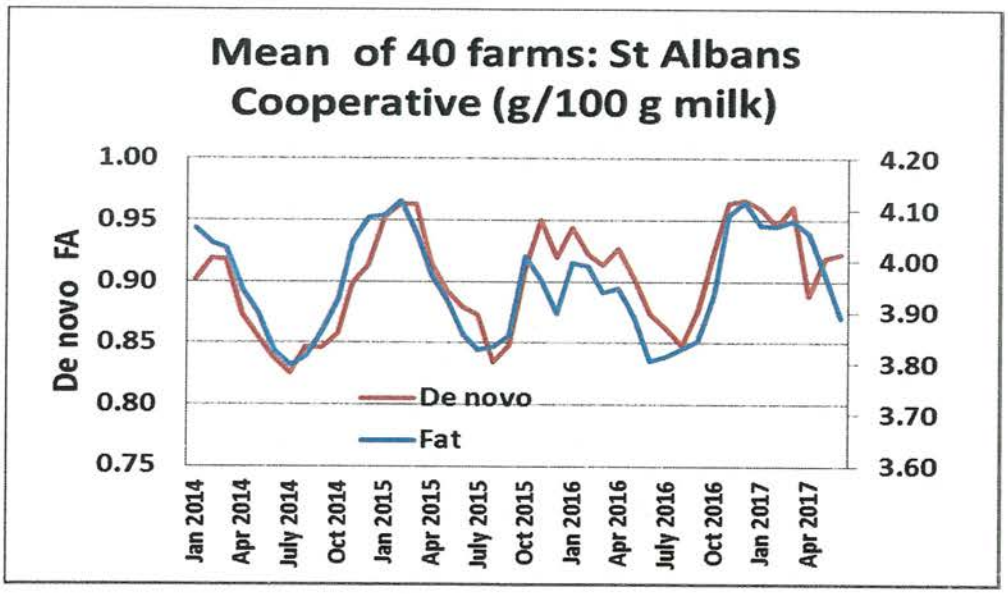

Figure 5. Seasonality of milk fat and de novo FA in milk.

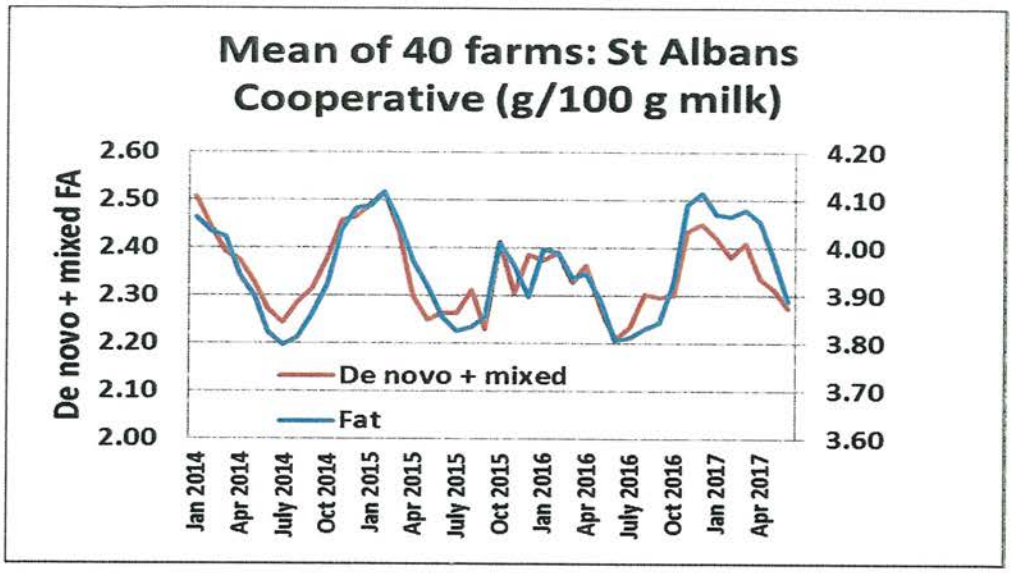

Figure 6. Seasonality of milk fat and de novo + mixed origin FA in milk

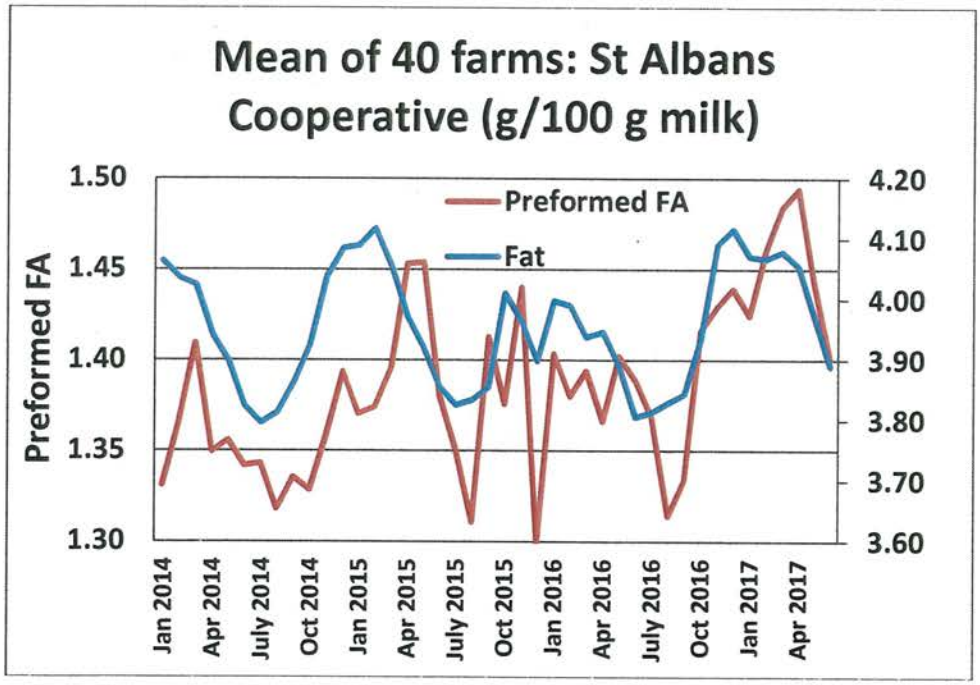

Figure 7. Seasonality of milk fat and preformed FA in milk.

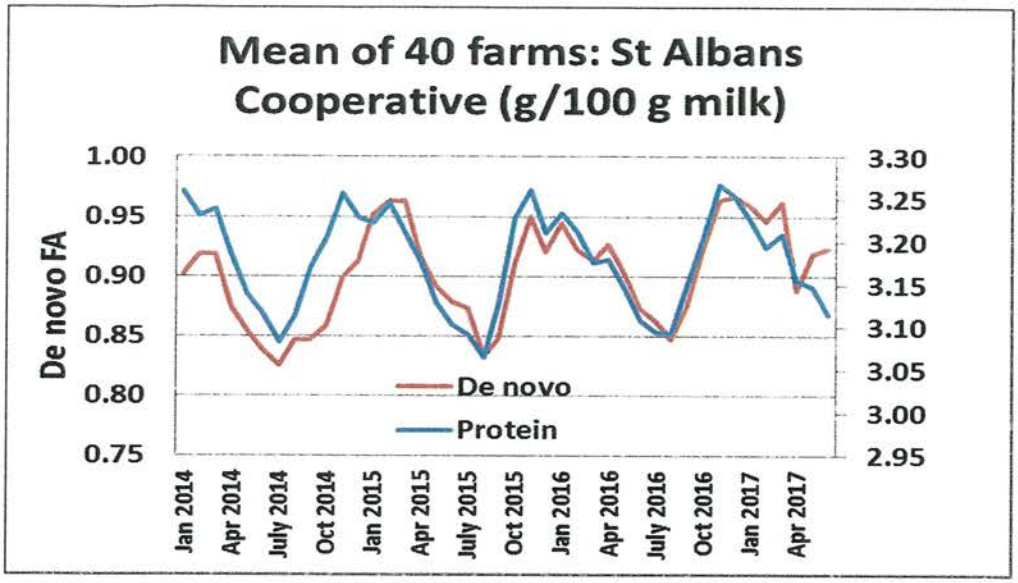

Figure 8. Seasonality of milk protein and de novo FA in milk.

The seasonality of de novo and mixed origin milk FA concentration follows the seasonal pattern of milk fat (Figure 5) and protein (Figure 6) variation while variation in preformed fatty FA in milk does not (Figure 7). Much of the variation in the mixed origin FA concentration is probably due to variation in the portion of the mixed origin FA produced by de novo synthesis from acetate and butyrate from forage digestion in the rumen. These seasonal changes may be related to time and temperature induced changes in the fermentation of corn silage, starch degradability, forage quality and heat stress on the cows.

HERD TO HERD VARIATION IN MILK COMPOSITION IN NORTH AMERICA

Over the past year, bulk tank milk sampling has been done on a wide range of farms from various regions of the US to confirm if the same milk fat, protein and milk FA composition relationships are observed in bulk tank milks from different regions of the US. These samples were collected daily for 5 to 7 days on each farm, preserved and refrigerated. At the end of the collection period, the milk samples were shipped on ice to Cornell University for MIR analysis and spot-checking FA composition with GLC analysis, particularly to obtain more detail about milk trans FA levels at each farm. There were some grazing herds, organic herds, and very large conventional herds in the population with a wide range of milk production per cow and milk composition.

The findings from these 167 farms were reported at the 2017 Cornell Nutrition Conference (Barbano et. al., 2017). The behavior of bulk tank milk fatty acid composition as it related to bulk tank fat and protein test is shown in Figures 9 and 10 for herds managed and fed over a wider range of types of feeds and management systems than we encountered in our studies of farms in the Northeast US. The relationship between de novo and de novo plus mixed origin observed in bulk tanks milk produced by farms from across the US were similar those found for Holstein herds in the Northeast. A level of about 0.85 g de novo FA per 100 g of milk will achieve about a 3.75% fat test (as seen by comparison of Figure 1 versus Figure 9).This indicated that the milk fatty acid metrics (de novo, mixed, preformed, fatty acid chain length, and double bonds per fatty acid) are robust indices for use for herd management and apply over the wide range of condition found across North America. A discussion of interpretation of bulk tank milk fatty acid composition was reported previously (Barbano et al., 2017).

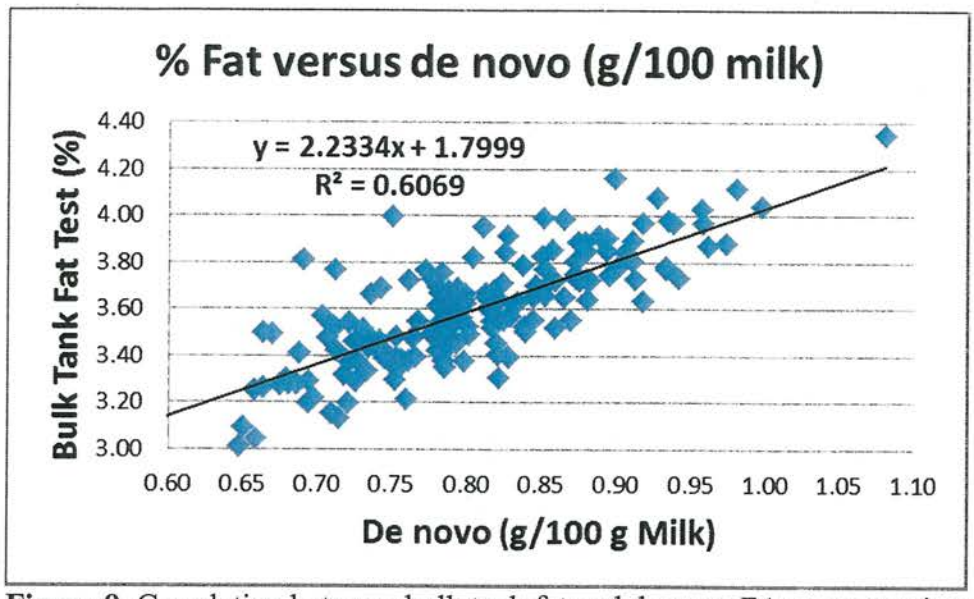

Figure 9. Correlation between bulk tank fat and de novo FA concentration (167 farms).

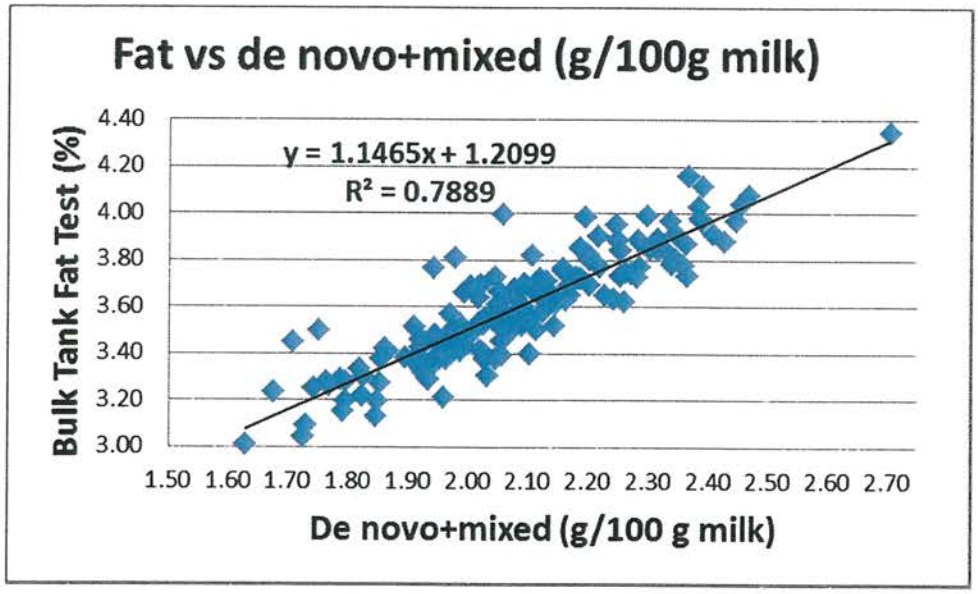

Figure 10. Correlation between bulk tank fat and de novo + mixed origin FA (167 farms).

Milk fat and protein output per cow per day are also strongly correlated with total weight of milk produced per day. Those relationships for the 167 farms from across North America are shown below in Figures 11 and 12.

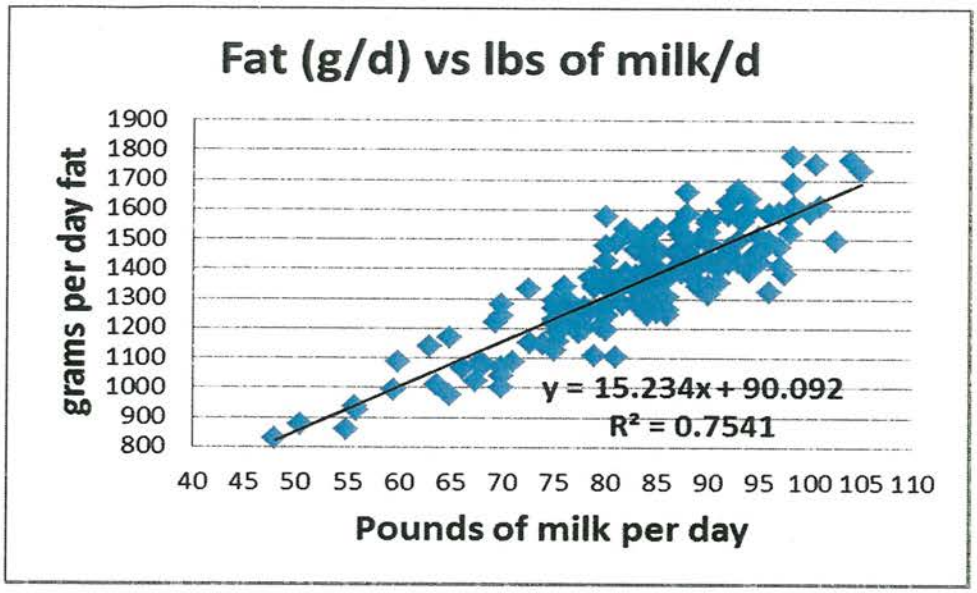

Figure 11. Grams of fat per cow per day and milk production (167 farms).

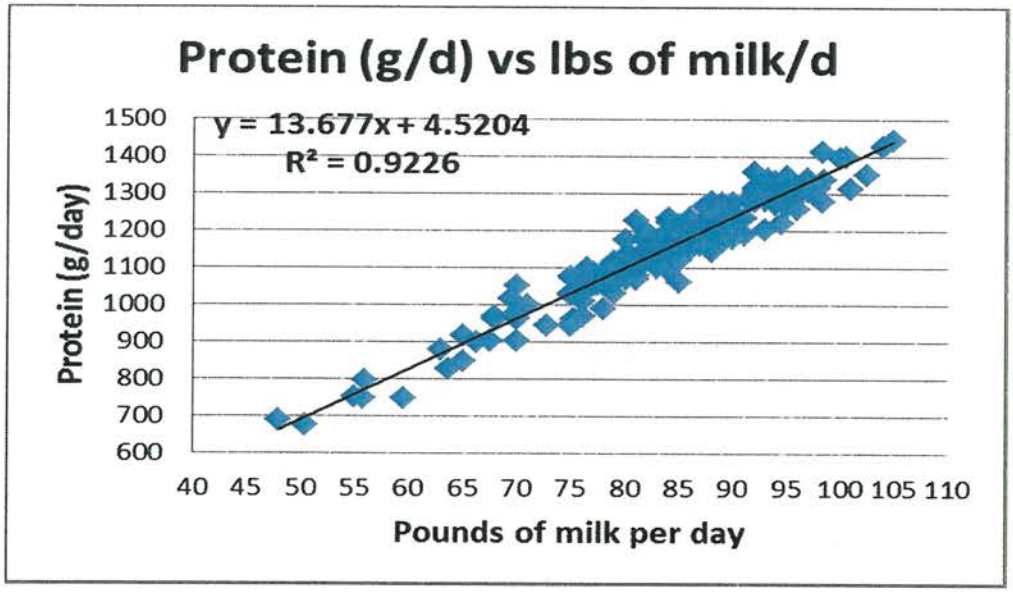

Figure 12. Grams of protein per cow per day and milk production (167 farms).

Overall, dairy cows have the potential to produce more grams of fat and protein per day if they produce more milk. The synthesis of lactose and increasing the grams per day output of lactose is needed to produce more pounds of milk per day. Lactose production is highly dependent on glucose metabolism in the cow. To produce more milk per cow, a cow will need to produce more lactose per day, as shown in Figure 13 below. The correlation is very strong. To achieve 90 to 100 lb (40.9 to 45.4 kg) of milk, the cows need to be producing between 1900 and 2100 grams of lactose per day. A major factor that would compete for use of glucose that could be used in support of milk synthesis is an immune response by the animal because of some adverse health event (e.g., leaky gut, mastitis, lower GI viral infection, etc.). Thus, in our field work we are encountering situations where the fatty acid profile and a high bulk tank fat and protein test indicate that rumen function is good, but the weight of

milk per day is low (i.e., low lactose output per cow per day) and as a result total weight output per cow per day of fat and protein are not as high as they should be. This is a sign that there is some non-feed/non-rumen fermentation problem that is limiting milk production.

As this new milk testing technology becomes more widely available in the dairy industry it is likely to be used as a herd management tool to test milk from different feeding groups of cows that may have a very different number of days of milk (**DIM**) from one group to another or have a different parity status from one group to another. Both DIM and parity influence milk and milk FA composition. There are large changes in milk FA composition with stage of lactation, particularly during the transition period. When looking at milk composition and FA composition, differences in parity or stage of lactation needs to be taken into account when interpreting data. As a result, we have been collecting data at the Miner Institute to produce lactation curves on all of these milk parameters.

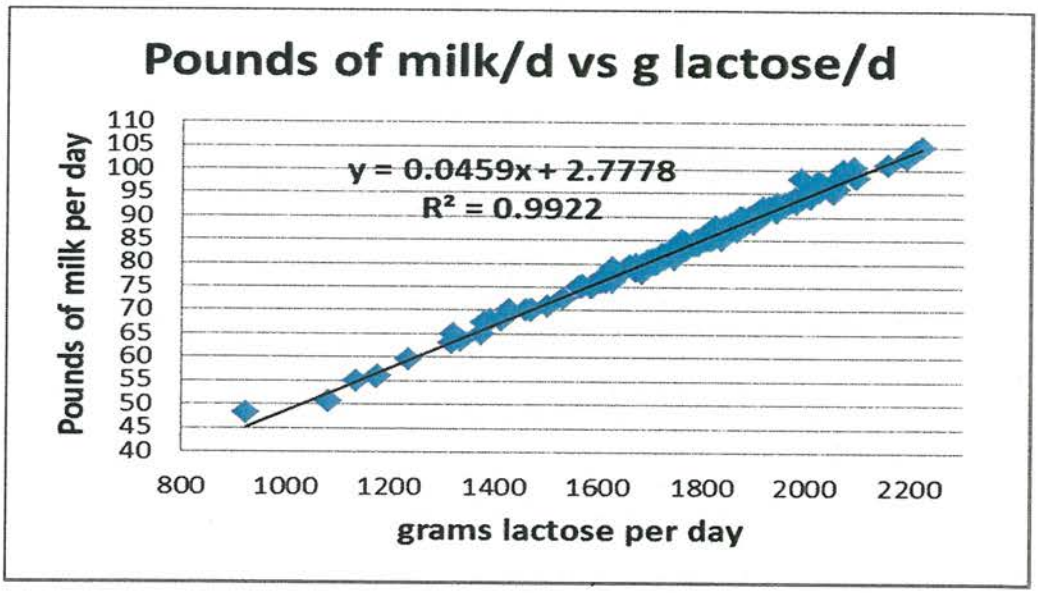

Figure 13. Grams of lactose produced per cow per day and milk production (167 farms).

STAGE OF LACTATION AFFECTS MILK FATTY ACID COMPOSITION

For the past 2 to 3 years, we have been conducting an intensive study at Miner Institute with individual cow milk analysis to better understand changes in milk FA across the lactation cycle. The goal is to build stage of lactation curves for all the new milk analysis parameters on both a concentration basis and a daily output per cow basis. Milk is collected from the entire herd (~400 cows) weekly from 3 milking shifts within the same day and analyzed on-site with a high-speed MIR milk analysis system.

As expected, the concentrations of FA in milk changes with DIM. The changes are particularly large in early lactation (i.e., the transition period) when the cow is in negative energy balance. During this period it is normal for the preformed FA to be high and the mixed and de novo FA to be low. However as dry matter intake increases after calving, the milk FA composition should change quickly if the cow's blood NEFA concentration decreases normally. If milk sampling and testing for FA is being done on different groups of cows within a herd, then these stage of lactation changes need to be considered to properly interpret that data. The graphs below (Figures 14 to 17) are stage of lactation

data collected from Holstein cows over a period of 2 to 3 years that were milked 3 times per day, had a rolling herd average of ~30,000 lb (12,636 kg) and were fed total mixed rations based on stage of lactation (i.e., fresh, 1st lactation, high and low groups). In general, the diets were typically 50 to 60% forage with at least 2/3 of forage coming from corn silage. Grain mixes typically contained corn grain, soybean meal, commercial soy/canola products, byproducts, rumen inert fat, plus mineral and vitamin supplements. Diets were balanced for lysine and methionine. The change in g/100 g milk of de novo, mixed, and preformed FA with week of lactation is shown in Figure 14 and the relative percentages are shown in Figure 15.

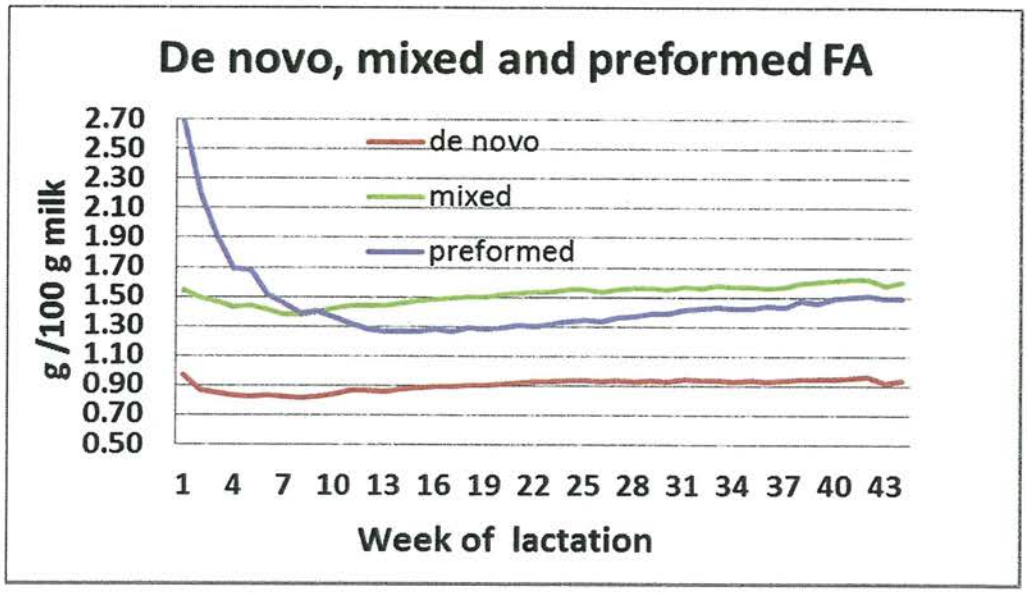

Figure 14. De novo, mixed, and preformed FA (g/100 g milk) over lactation for all cows.

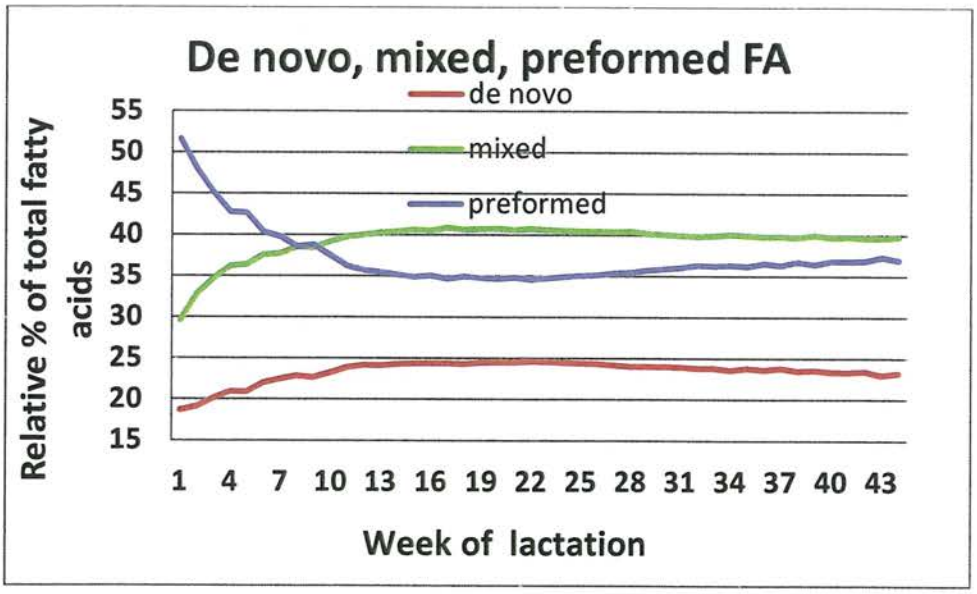

Figure 15. De novo, mixed, and preformed FA (relative %) over lactation for all cows.

There are large changes in milk FA composition during the first 10 weeks of lactation on both a g/100 g milk and relative percentage basis with the preformed FA being high at the beginning of lactation and decreasing to relatively stable levels by about 10 weeks of lactation. When testing milk on larger

farms from groups of cows that differ in stage of lactation, these changes in milk FA composition with stage of lactation need to be considered when interpreting data along with information on milk production per cow per day, cow health, milk SCC, feed composition, and dry matter intake.

Interpretation of results from a management point of view becomes even more interesting when the data are converted to grams per day per cow output. Figure 14 represents the average of all cows in the herd, but the stage of lactation graph for grams per cow per day is very different for first parity versus older cows. When evaluating performance of older versus younger cows, this factor needs to be considered. The difference between multi and primiparous cows for output of de novo and preformed FA per cow per day is shown in Figure 17. The output of all groups of FA in grams per cow per day is much more stable over time for primiparous cows versus older cows. The older cows have much higher preformed FA output per cow per day in early lactation due to body fat mobilization than primiparous cows.

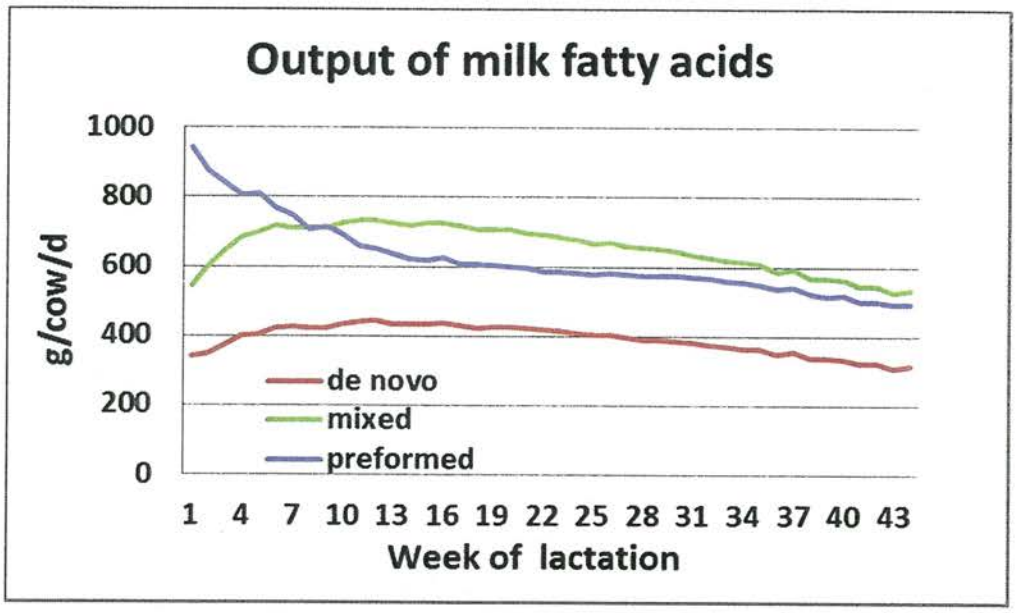

Figure 16. Stage of lactation production graph for all cows (g/cow/day).

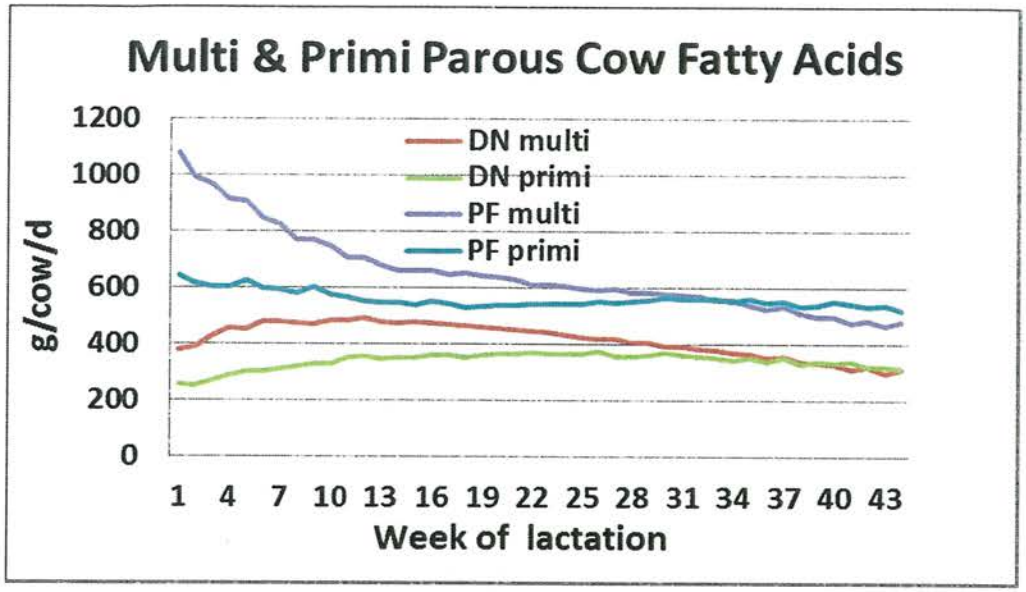

Figure 17. Stage of lactation: de novo (DN) and preformed (PF) fatty acids for primiparous and multiparous cows.

MILK FATTY ACID DATA AND MILK ESTIMATED BLOOD NEFA: TOOLS FOR MANAGEMENT OF METABOLIC HEALTH DURING THE TRANSITION PERIOD

To apply milk analysis to transition cow health management the frequency of individual cow milk testing needs to be much higher than what is currently being done with monthly DHIA milk testing. Our research results indicate that there may be an excellent farm management and individual cow health management opportunity with a higher frequency of milk analysis. As shown above, milk fatty acid composition and yield per cow per day changes rapidly especially during the first 10 weeks of lactation when cows are transitioning from negative to positive energy balance. Separately if blood samples are collected and blood non-esterified fatty acid concentration is measured, cows with a high probability of metabolic related disorders (e.g., ketosis, displaced abomasum, etc.) can be identified. Barbano et al. (2015) reported a method for estimation of blood NEFA (μEq/L) directly from the MIR milk spectra, not by calculation from fatty acid data. Use of milk estimated blood NEFA is faster, less expensive, and timelier than blood analysis. Are there differences in milk fatty acid profile and milk estimated blood NEFA that can be used to better manage transition cow health? To address this question, we did high frequency (1 milking per day) testing of milk from individual fresh cows at Miner Institute to compare data from healthy cows and cows that had clinical diagnoses of DA and/or ketosis. In general, milk was collected before fresh cow exam/check. The milk information was pared with health data collected and stored in a herd management software program.

Typically, blood samples are collected from early lactation cows that may have a high risk of a metabolic disorder. However, when blood NEFA is estimated from the MIR spectra of milk, it becomes relatively easy to produce a milk estimated blood NEFA lactation curve. The change in milk estimated blood NEFA for primiparous and multiparous cows throughout lactation is shown in Figure 18. In early lactation when cows are in negative energy balance, blood NEFA is high as the cows mobilize body fat to help meet energy requirements of milk production in very early lactation when their dry matter intake is increasing. In general, multiparous cows are mobilizing more body fat in the first few weeks of lactation than primiparous cows.

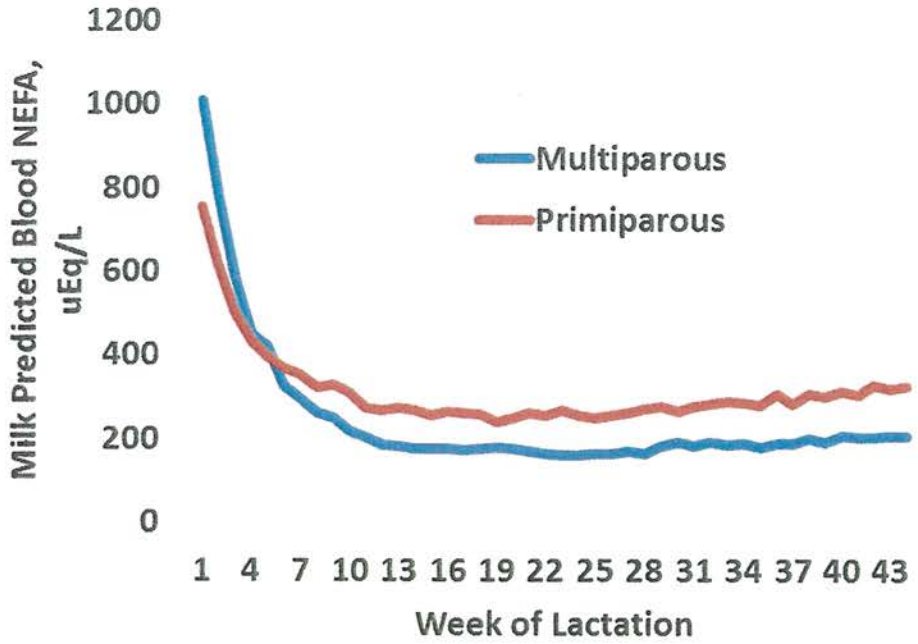

Figure 18. Stage of lactation: milk predicted blood NEFA (μEq/L) for primiparous and multiparous cows.

Displaced Abomasum (DA).

In general the milk estimated blood NEFA was much higher (about 1400 μEq/L) for cows that were clinically diagnosed with a DA than healthy cows (about 800 μEq/L) (Figure 19) and milk de novo fatty acids (Figure 20) were lower for cows with a DA (ca. 13 vs 19% g/100 g fatty acids). Post DA surgery, milk estimated blood NEFA decreased and milk de novo fatty acids increased but did not equal the values for healthy cows at the same days in milk, indicating that there will probably be some longer term negative effect of the DA event on milk production for that cow as lactation continues.

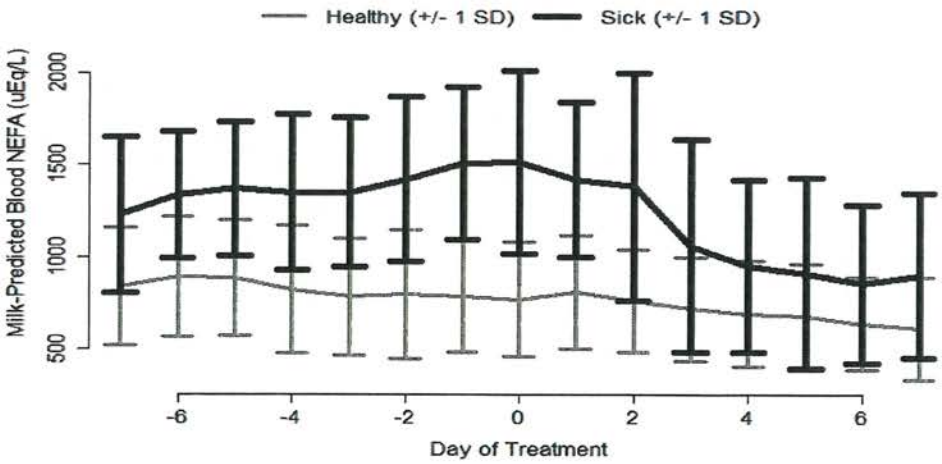

Figure 19. Milk predicted blood NEFA for multiparous cows (n = 47) with a DA versus healthy cows (n = 191) with day zero being the day of treatment. Day 0 = 9.4 days in milk. Healthy cows were matched with DA cows based on days in milk and calving date.

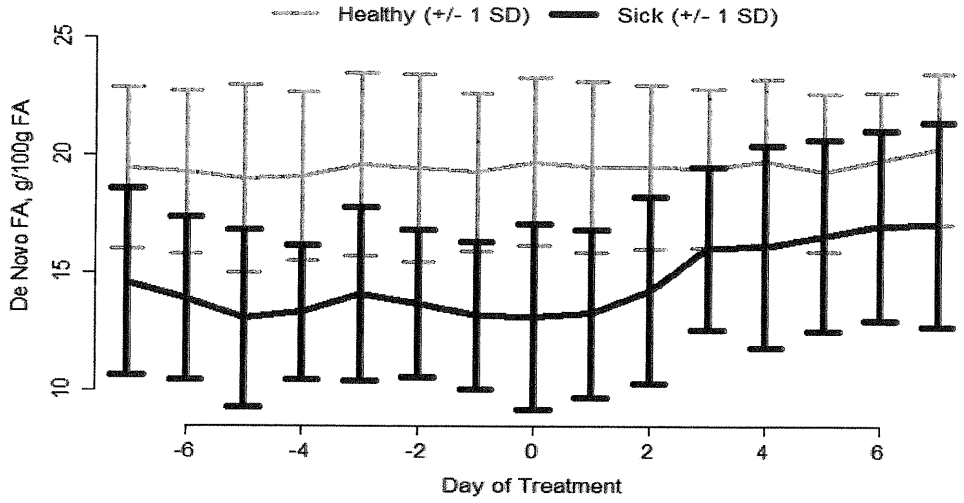

Figure 20. Milk de novo fatty acids relative % (g/100 g fatty acids) for multiparous cows (n = 47) with a DA versus healthy cows (n = 191) with day zero being the day of treatment. Day 0 = 9.4 days in milk. Healthy cows were matched with DA cows based on days in milk and calving date.

Ketosis.

In general the milk estimated blood NEFA was much higher (about 1400 μEq/L) for cows that were clinically diagnosed with ketosis than healthy cows (about 800 μEq/L) (Figure 21) and milk de novo fatty acids (Figure 22) were lower for cows with ketosis (ca. 13 versus 19% g/100 g fatty acids). Post-ketosis treatment with propylene glycol, milk estimated blood NEFA decreased and milk de novo fatty acids increased but did not equal the values for healthy cows at the same days in milk, indicating that there will probably be some longer term negative effect of the ketosis event on milk production for that cow as lactation continues.

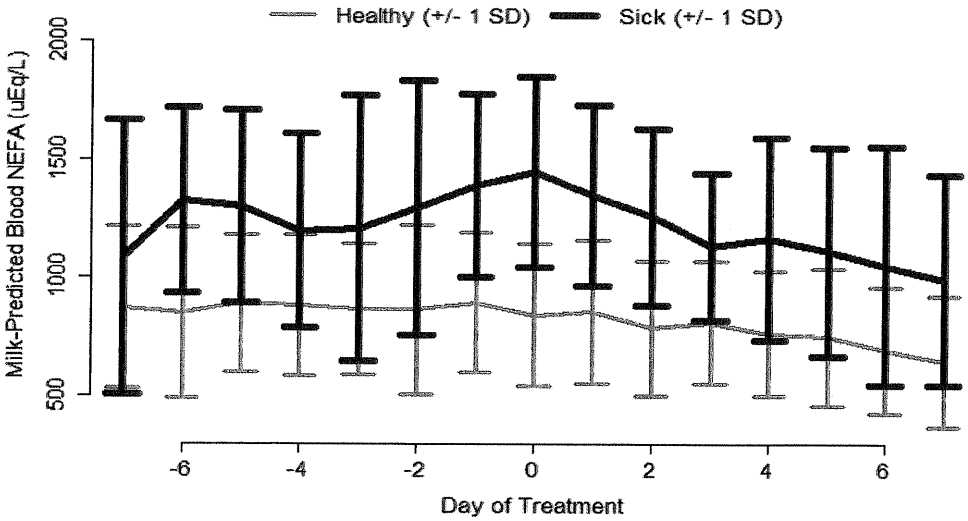

Figure 21. Milk estimated blood NEFA for cows (n = 87) with ketosis versus healthy cows (n = 239) with day zero being the day of treatment. Day 0 = 7.3 days in milk. Healthy cows were matched with ketotic cows based on days in milk and calving date.

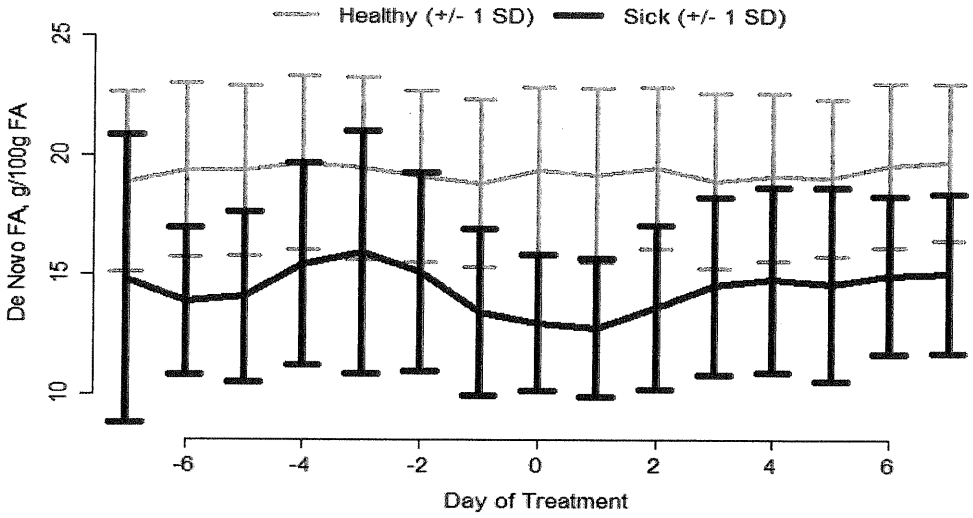

Figure 22. Milk de novo fatty acids relative % (g/100 g fatty acids) for multiparous for cows (n = 87) with ketosis versus healthy cows (n = 239) with day zero being the day of treatment. Day 0 in the graph equals 7.3 days in milk. Healthy cows were matched with ketotic cows based on days in milk and calving date.

CONCLUSIONS

Data from routine high frequency (i.e., daily) bulk tank milk component, SCC, and milk FA testing combined with milk weight per cow for whole herd diagnostic analysis of overall nutritional and management status of dairy herds. The testing was done using MIR as part of the routine milk payment testing. The advantage of this approach is that no additional sampling collection cost is required, the instrument that does the milk FA analysis can be the same instrument that produces the milk fat and protein test result, and it does not take any longer to test each milk sample. There would be additional cost to purchase reference milk samples for calibration of the FA parameters for the MIR milk analyzer. The positive correlation between increased de novo fatty acid synthesis and bulk tank milk fat and protein concentration can be used as an indicator of the quality and balance and the rumen fermentation of carbohydrates and if changes in feeding and management are impacting de novo synthesis of milk fat. Seasonal variation in whole herd milk fat and protein concentration was highly correlated with seasonal variation in de novo FA synthesis. Milk FA composition changes with both DIM and differs between primiparous and multiparous cows. Milk fatty acid testing and this diagnostic approach could be applied to testing milk from large feeding groups of cows within the same farm, if representative feeding group milk samples can be collected and tested and the milk produced per cow is known. For feeding group or individual cow milk testing care must be taken to consider the milk weight per cow per day, diet composition, dry matter intake, DIM and parity into the interpretation of the milk composition data.

Data from high frequency MIR milk testing of individual cow milks, particularly during the transition period can be used to identify quickly cows at high risk for displaced abomasum and ketosis before a clinical diagnosis is made oftentimes. The concentration of milk estimated-blood NEFA (μEq/L) was higher and milk de novo fatty acids as percent of total fatty acids (g/100 g fatty acids) was lower than healthy for cows for cows diagnosed with clinical ketosis or displaced abomasum. At the present time

based on the current milk analysis tools, we were not able to differentiate whether a cow was going to have ketosis or a displaced abomasum in advance, but further research being done to develop milk analysis tools to differentiate these health events in advance of clinical diagnosis. This may allow development of earlier intervention strategies to reduce the severity of these metabolic disorders and their negative impact on milk production. Mid-infrared analysis of milk from transition cows may be an alternative to blood sampling and testing for management of transition cow health.

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A Systematic Approach to Improving Transition Success

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EXECUTIVE SUMMARY

There are many factors that increase the risk for infectious diseases during the transition period. If a cow is able to adapt to all the physiological, environmental, and social changes that occur during the transition period she will be more productive during that lactation and more likely to reach the next one. Therefore, we must take a systematic approach to understand what influences immunity, so that we can increase the odds that cows will successfully navigate this important period, ultimately increasing production efficiency of the dairy.

First, we need to understand what aspects of the immune system are compromised that lead to the increased susceptibility to infectious diseases. There are many components and layers that make up a cow's immune system, including: physical barriers, antimicrobial secretions, and many cellular responses. The immune system is complicated and the competency of the system is a function of many interactions. Infections with environmental microorganisms are common around calving, primarily resulting from holes in the physical barriers of those tissues that are associated with milking and calving. Disease likely will not ensue if other aspects of the immune system can control the growth and ultimately eliminate the microorganism from that tissue. Unfortunately, other immune defenses are compromised or what I consider dysfunctional, which increases the risk the infection will develop into disease.

Cows are exposed to many potential stressors around calving, some physiological (i.e. associated with metabolic demands of lactation) and others social. If cows are overwhelmed by the number and/(or) severity of the stressor(s), various leukocyte responses may become compromised. Stressed animals were shown to have altered leukocyte responses. Cows stress about change and situations that create competition among cows can also create winners and losers, which can increase the risk for disease among some animals. Parturition and subsequent lactation are abrupt; therefore, management strategies need to try and make that change less dramatic and limit additional stressors that may interfere with the ability of the cow to adapt.

Additionally, metabolic demands of leukocytes may not be prioritized or sufficiently met around calving. Neutrophil functions of sub-clinically hypocalemic cows were reduced during early

lactation. There also is evidence that elevated NEFA and BHBA concentrations have a negative impact on immunity. Therefore, management strategies that can improve both calcium and energy homeostasis will improve the success rate of cows during the transition period.

Keywords: Health, Nutrition, Transition Cow

INTRODUCTION

The immune system is made up of many components including: various physical barriers, antimicrobial secretions, and cellular responses. A breakdown in any aspect of the immune system in the presence of a pathogen may increase the likelihood of infectious disease. It is well established that dairy cattle are highly susceptible to infectious diseases affecting many tissues during the transition period, and stress is often considered in the etiology. The word stress is commonly used, but the term itself can take on many different meanings and therefore the use is often vague or an over-generalization. As we alluded to in the previous sentence, stress is commonly referred to in the etiology of infectious disease in cows; therefore, stress is considered negative in the context of dairy cattle health. In contrast, stress is a natural, physiological response that is important in promoting a response to a threat or adaptation to a change (e.g. the transition period). Therefore, the paradox is that stress is likely both crucial for the adaptation to lactation to occur, but potentially damaging to the transition dairy cow.

This presentation will consider what are potential sources of stress for transition cows and will further describe how these potential stressor(s) influence immune defenses and risk for infectious diseases. To address this topic we will first describe the basic framework of the immune system and will briefly describe how the immune system of many transition cows differs from that of either a non-lactating or mid to late lactating cows. Then, we will attempt to define stress, describe possible stressors a transition cow may encounter, and investigate the potential role that various stressors alone or in combination influence immune defenses and risk for infectious diseases.

PERIPARURIENT COW IMMUNE SYSTEM

The principle role of the immune system is to recognize self from non-self, and in doing so protect the cow's organs against infectious, non-self microorganisms such as bacteria, virus, parasites, and fungi. The immune system is exactly that, a system, which is made up of many components. Understanding how any single measurement of the immune system influences the risk for disease is complicated because a break down in any component of the immune system may either increase the risk for disease or have no affect at all under the specific circumstances. The immune system has various layers ranging from the physical barriers to very specialized leukocyte functions. The physical barriers are often compromised (e.g. an open teat end and microbial contamination of the uterus), which contributes to the susceptibility to mastitis and uterine diseases in early lactation. However, if other components of immune system are functioning properly a mild infection is eliminated without any clinical signs of disease. An example of this

was observed when Shuster et al. (1996) challenged cows intra-mammary in either early lactation, 6 to 10 DIM, or in mid lactation with the same strain and dose of *E. coli* and observed that the cows in early lactation had greater replication of the *E. coli* and developed more severe mastitis. Therefore, it is important to understand what other aspects of the immune system are compromised during the transition period that increases the risk for infectious disease.

Ballou (2012) described the immune system of many transition cows as dysfunctional. The compromised physical barriers when coupled with suppressed ability to control the growth of microorganisms in tissues increase the risk of clinical and sub-clinical diseases. Suppressed lymphocyte and neutrophil functions during the transition period are well documented in many studies from different lab groups (Guidry et al., 1976; Mallard et al., 1998; Burvenich et al., 2003). Additionally, other aspects of the innate immune system beyond lymphocyte and neutrophil functions appear to be suppressed during the transition period. Shuster et al. (1996) reported a reduced ability to control the growth of the *E. coli* in the mammary gland when the cows were challenged in early lactation, and this occurred before the recruitment of neutrophils. Additionally, Ballou et al. (2009) reported that whole blood bactericidal capacities against both an environmental *E. coli* and a pathogenic *Salmonella typhimurium* were reduced the day after calving and returned to prepartum levels by 21 DIM. In contrast, one aspect of the immune system that does not appear to be suppressed during the transition period is the inflammatory response. Lehtolainen et al. (2003) reported that cows in early lactation had greater local and systemic signs of inflammation after they were challenged with a large dose, 100 μg, of lipopolysaccharide intra-mammary. In agreement, Sordillo et al. (1995) reported greater *ex vivo* secretion of tumor necrosis factor-α when stimulated with lipopolysaccharide. These studies are important pieces of evidence for an increased propensity to produce inflammation in early lactation because both of these models did not use live microorganism challenges, but instead challenged with a fixed dose of an agonist that activates the inflammatory responses. Therefore, the immune system of a transition cow is dysfunctional because some responses are suppressed, whereas the inflammatory response appears elevated. This immunological phenotype is in contrast to a generalized immunosuppression (Ballou, 2012). This distinction is important when evaluating the role that transition period stress plays in the increased risk for disease.

ROLE OF STRESS IN PERIPARTURIENT DISEASE

Stress has for a long time been implicated in the etiology of many infectious diseases both in humans and dairy cattle, but how much does stress during the transition period actually play in the elevated risk for disease? Is the importance of stress in transition cows exaggerated? In order to establish a causal link between stress and impaired health during the transition period, there are a series of assumptions or steps that must be met, including: (1) the cow is stressed, (2) the classical stress response is elicited, (3) the duration or magnitude is sufficient to alter various immune functions, and (4) that increases the risk of getting an infectious disease, either clinical or sub-clinical. Further, alternative pathways from the first to the last step need to be considered. We will use this framework to investigate the role that stress may play in increasing the risk for infectious disease during the periparturient period.

Therefore, the first step is to understand what stresses a cow. In order to address this question we need to define stress, which is not as straightforward as one might expect. Stress is referred to in many contexts and the meaning is often subjective. Hans Selye was the first to coin the term stress in 1936 and he defined it as, "the non-specific response of the body to any demand for change". Selye performed experiments in laboratory animals and observed consistent pathological changes in animals, lymphoid atrophy, stomach ulcers, and enlargement of the adrenal glands, in response to various psychological and physiological challenges. We will accept his original definition to evaluate what are potential stressors that a cow is exposed to during the transition period. The commonly used term, transition period, already appears to validate the first assumption that cows are exposed to stress during this time. Any transition involves change, so the next question is, what changes are taking place during this period?

Cows are not that different from humans in what causes stress. Have you ever wondered why cows are creatures of habit? It is the same reason that humans are creatures of habit or that humans are most comfortable when they are in a routine. It basically boils down to control. Change or uncertainty causes a loss of control, whether you are a human or a dairy cow. Common laboratory models of stress involve taking control away from the subject. An example would be put a loud alarm in a room housing animals that goes off randomly throughout the day. The alarm must be set to go off randomly so the subjects cannot adapt to the alarm. By setting the alarm to go off at random, the animals have no sense of control. In contrast, if the alarm goes off at a regular interval, the subjects regain control of the situation and therefore will adapt. Other common laboratory stress models include physical restrain, social re-organization, or inability to move away from a painful stimulus. If we apply the principle that a significant change can cause a loss of control and the stress response is the physiological response to help the animal regain control, then we must understand what changes are potentially stressful to transition dairy cows. Generally, the changes that occur during the transition period are primarily either psychological or physiological.

Let's first consider the psychological or social stressors that a dairy cow may encounter during the transition period. Many potential pen moves occur throughout the transition period. Cook and Nordlund (2004) described these pen moves and how after every pen change there is the potential for social re-organizing that persists for 3 to 7 days. von Keyserlingk et al. (2008) reported increased competition at the feed bunk, decreased lying bouts, and reduced allogrooming events the day after a single lactating cow was introduced into a stable population of 11 lactating cows. The same group also observed a 9% decrease in DMI on the day that a dry cow was moved to a new pen when compared to baseline values (Schirmann et al., 2011). Further, they reported that the new cows displaced other cows at the feed bunk twice as much as they did before they were moved. The displacement behaviors are noteworthy because they indicate competition or aggressive/submissive actions. Dominant lactating cows when moved to a new pen did not change their behavior or drop milk production; however, intermediate and subordinate cows produced 3.8 and 5.5% less milk, respectively during the 2nd week after pen moves (Hasegawa et al., 1997). In contrast, Chebel et al. (2016) reported that highly dominant cows with multiple interactions at the feed bunk throughout the day were more likely to have uterine disease and be culled from the herd.

Most dairy cows are raised in confinement and the temptation to maximize facility space can result in overstocking. We'll define overstocking as the number of cows per pen exceeds available resources (i.e. access to feed and/(or) a comfortable place to rest), which creates unnecessary competition. When management creates competition among cows, there are winners, but there are also losers. This will likely increase the risk that the subordinate or overly dominant cows will be stressed and/or have other negative health and productive outcomes.

In addition to the psychological stressors, there are many physical changes taking place in the cow during the transition period that can elicit a stress response. Nutrient and energy demands increase during lactation. Additionally, energetic demands approximately double during lactation and most early lactation cows will be in some degree of negative energy balance. Antioxidants are also used at a greater rate and can cause depletion of antioxidant stores (Weiss et al., 1997). Similarly, increased calcium output in colostrum and milk can cause a rapid drop in ionized calcium in blood, until allosteric mechanisms activate osteoclasts to mobilize calcium from bone. There is evidence that these metabolic changes during the transition period have both direct and indirect effects on leukocyte responses (Lacetera et al., 2004; Moyes et al., 2009; Zarrin et al., 2014).

It is evident from the previous discussions that dairy cows during the transition period are exposed to many changes, both psychologically and physically that are potentially stressful. However, just because something is potentially stressful or even that behaviors of cows change does not necessarily mean that cows are stressed and further that immune function is altered and disease risk increased. Silva et al. (2016) reported an increased frequency of adverse behaviors when stocking density increased; however, they did not observe any differences in leukocyte function or incidence of periparturient disease. The author's suggested that although increasing stocking density may have been a mild stressor in this population, the overall good management of this herd did not make this stressor overwhelm the ability of the cows to cope with additional stressors associated with the periparturient period.

There is good evidence that cows are exposed to many changes that are potential stressors during the transition period. Additionally, activation of the stress response occurs around parturition; however, the impacts on immunity are not completely clear. Increased risk for disease persists past the period of elevated plasma cortisol in cows as well as leukocytes may be less responsive to glucocorticoids around calving because the glucocorticoid receptor is down regulated in those cells. Therefore, future research will need to delineate between stress and alternative routes that result in increased disease risk.

ALTERNATIVE ROUTES OF INCREASED PERIPARTURIENT DISEASE SUSCEPTIBILITY

Immune responses can be metabolically expensive and requirements may be limiting for optimal function during the periparturient period. Evidence from Martinez et al. (2013) indicated that cows classified as subclinical hypocalcemic (total serum calcium less than 8.59 mg/dL) had less reactive neutrophil oxidative burst when compared to normocalcemic cows. Further, they reported increased incidences of metritis and extended days to confirmed pregnancy in the subclinical hypocalcemic cows. These data indicate that even among subclinical hypocalcemic

cows, leukocyte function may be impaired and increase the risk for periparturient disease. Interestingly, in our lab if we collect blood using an anticoagulant that chelates calcium, we are unable to activate neutrophils in whole blood.

There is also evidence that elevated NEFA and BHBA concentrations have a negative impact on immunity. Lacetera et al. (2004) reported that inclusion of NEFA in cell culture media as low as 0.25 mM reduced IgM secretion. Further, they reported attenuated mitogen induced interferon-γ secretion when the cell culture media included as low as 0.125 mM NEFA. Moyes et al. (2009) induced negative energy into post-peak cows by partially restricting feed intake. The feed restriction increased plasma NEFA and BHBA to levels common among periparturient cows. The cows were challenged intramammary with an environmental *Streptococcus uberis* and pathophysiological response determined. The cows in negative energy balance had reduced neutrophil phagocytosis immediately before the mastitis challenge and had elevated acute phase protein concentrations after the challenge. This indicated that the cows in NEB had greater inflammatory response to the mastitis challenge. More recently, Zarrin et al. (2014) infused cows with BHBA to induce hyperketonemia and infused control cows with normal saline. All cows were challenged intramammary with lipopolysaccharide in order to evaluate the intensity of the acute phase response. The hyperketonemia cows had elevated acute phase protein secretion and reduced the influx of somatic cells into the mammary gland after the lipopolysaccharide challenge. The reduced influx of somatic cells into the mammary gland after an infection would increase the risk for development of mastitis and as well as the severity of mastitis.

Lastly, increased metabolic activity and leukocyte derived oxidant production during early lactation can accelerate the use of antioxidants, and if it exceeds the supply of antioxidants can result in some degree of oxidative stress. The implications of this oxidative stress can have negative impacts on the immune responses of cows and ultimately disease resistance. This will be covered in more detail by Dr. Weiss, so I'll limit my discussion of the antioxidant systems.

IMPLICATIONS

The immune dysfunction of periparturient dairy cows is complex, and appears to involve many layers of the immune system. Increased exposure of microorganisms occurs from both calving and milking; however, a competent immune system should be able to eliminate most infections without any clinical disease. Many psychological and physiological stressors appear to be involved in increasing the risk for infectious disease; however, it appears to be somewhat of a cumulative effect. Some stress is unavoidable, but the goal should be to limit additional stressors that may ultimately impair the ability of a cow to cope with the change from non-lactating to lactating. In addition to stress, changes in nutrient supply or use may impair leukocyte function and ultimately increase the risk for infectious disease. Therefore, there is not a single source of immune dysfunction during the periparurient period. Management must look at each production system separately and take a systematic approach to improving transition success.

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TECHNICAL SYMPOSIUM SPEAKERS

J. R. Loften, Ph.D. is Dr. Jim Loften received his BS degree from Iowa State University, and his MS and Ph. D at the University of Georgia. He began working in the Dairy Research Department at Ralston Purina Company for 4 years where his duties included accomplishing research on lactating cows and consulting with dairy farms all over the US and Canada. He moved into sales and nutritional consulting in central Minnesota with the company for 15 years. He then began designing, building and managing large dairies in the Midwest for 5 years. He started working for Milk Specialties Global in 2002 as the Director of Technical Services and Sales where his responsibilities include field technical services, research and development, and large commercial dairy sales in the western US. He has published studies involving lactating cow and calf research, as well authoring an invited review on palmitic and stearic acid metabolism.

Kevin Harvatine, Ph.D. is an Associate Professor of Nutritional Physiology at Penn State University. He grew up on a dairy farm in Pennsylvania and received his BS in Animal Science from Penn State. He earned an MS from Michigan State University and Ph.D. from Cornell University. His area of expertise is nutritional regulation of metabolism. Currently he investigates milk fat depression, fat supplements, and daily patterns of intake and milk synthesis. His lab conducts experiments ranging from applied dairy nutrition to mechanistic molecular biology experiments. He has authored or co-authored 50 peer reviewed publications since 2009 and has spoken extensively at regional, national, and international conferences.

Matt Sellers, Ph.D. received his Ph.D. in Animal Science from the Department of Animal and Food Sciences at Texas Tech University with specialization in ruminant nutrition, immunology, and biostatistics. His dissertation work focused on the interaction between plane of nutrition and innate immune system function in dairy calves and identifying sources of variation in metabolic and immune responses in transition dairy cows. Matt has served as southwest National Account Manager for Milk Specialties Global Animal Nutrition for 3 years, with responsibilities in sales, technical service, and research and development. In his free time, Matt enjoys traveling, scuba diving, and college football.

Pete Morrow, DVM, MBA, DACT, National Account Manager at Milk Specialties Global Animal Nutrition

Phil Cardoso, DVM, Ph.D. is an Assistant Professor at the University of Illinois at Urbana-Champaign. He received his D.V.M. and M.S. degrees from the Universidade Federal Do Rio Grande do Sul in Brazil, and his Ph.D. from the University of Illinois. Since 2012, Cardoso has established a unique program that seamlessly blends his teaching, extension, and research efforts using a business model to give students opportunities to evaluate dairy farms. His research builds from questions asked by dairy producers and focuses on the impact of nutrition on metabolism, reproduction and health in dairy cows, as well as mechanisms of metabolic adaptation.

Jeff Firkins, Ph.D. earned his Ph.D. in ruminant nutrition and pursued postdoctoral research in dairy nutrition at the University of Illinois. Starting as an Assistant Professor of dairy nutrition in the Department of Dairy Sciences (now Animal Sciences) at Ohio State University in 1987, he has moved through the ranks to Professor in 2000. Firkins teaches and conducts research interfacing nutrition with rumen function and microbiology. He has advised 12 Ph.D. and 8 M.S. students to completion and served on committees of dozens more, including 5 from other countries. He served for 9 years as OSU's Director of the Interdepartmental Ph.D. program in nutrition, multiple terms as a panelist or manager of USDA competitive grants, and member of planning committees for international conferences in gut microbiology and ruminant physiology. He served 2 terms as a section editor for the Journal of Animal Science, 2 terms for the British Journal of Nutrition, and currently with the Journal of Dairy Science. He is a member of the current update committee for the NRC's Nutrient Requirements of Dairy Cattle. He has published more than 250 articles, including over 125 refereed journal articles, invited reviews, and book chapters and has been awarded more than $3.5 million in research grants. He has over 150 invited presentations in more than 20 countries. He was awarded the ADSA Nutrition Professionals Applied Dairy Nutrition award in 2003 and the ADSA AFIA Dairy Nutrition Research Award in 2012.

CANC SPEAKERS

Jim Morris – "The Rookie" - Keynote Speaker - Jim's Cinderella Story serves as testimony to the power of dreams and their ability to inspire and transform human life. His meteoric rise from 35 year-old high school teacher to flame-throwing major league pitcher in 3 months, made cinematic history with the release of The Rookie starring Dennis Quaid. Jim's rise from obscurity became the feel-good story of 1999. After pitching for the Tampa Bay Devil Rays in 1999 and 2000, Jim Morris signed with the Los Angeles Dodgers and retired from baseball in 2001. Jim lives with his family near San Antonio and travels the world as America's foremost inspirational and motivational speaker. His story has become an inspiration to anyone interested in overcoming life's obstacles and living their dreams. Jim has participated in such prestigious events as The Million Dollar Round Table, numerous speaker showcases, and is the author of "The Rookie." Jim's story has provided a platform to give back, inspire and encourage people of all backgrounds and age to pursue their dreams and never give up! His philanthropic efforts include partnerships with the following organizations: Arms of Hope; BCFS, Community Services Division, Health & Human Services; Boys & Girls Clubs of America; Boy Scouts of America; Play It Forward; and Texas Youth Commission.

David Barbano, Ph.D., Professor of Food Science – Cornell University – 1980 to present and Director, Northeast Dairy Foods Research Center. He received his BS – Biology/Food Science and MS/Ph.D. – Food Science from Cornell University. Dave conducts an applied and basic research program on dairy product manufacturing and milk analysis for dairy herd management. Recently, Dave has focused on developing new milk analysis measures of cow metabolic health for dairy herd management. He has been very active in the analytical groups of International Dairy Federation and the Association of Official Analytical Chemists International for the past 30 years. Honors include: 2018-National Cheese Institute Cheese Laureate Award; 2017-American Dairy Science Association – 100 peer reviewed JDS Publications Award; 2017-American Dairy Science Association De Laval Extension Award; 2015-American Dairy Science Association Elanco Award for Excellence in Dairy Science; 2014-Award of Excellence-International Dairy Federation; 2010-Harvey Wiley Award-Association of Official Analytical Chemists International; 2008-American Dairy Science Association West Agro Award for Milk Quality Research. What is the best part of my job? Working together with students to create new knowledge, technology, and science-based solutions to problems in the dairy industry.

Daniel H. Putnam, Ph.D. is a faculty member at the University of California Davis Department of Plant Sciences where he has conducted research and extension activities on forage crops in California for the past 25 years. He is from southern Ohio, and received his bachelor's degree from Wilmington College, Ohio, and MS & Ph.D. from University of Massachusetts, Amherst. He works closely with farmers and industry members to address agronomic practices, varieties, development of unique traits, harvesting schedules, and economics of alfalfa, sorghum, and switchgrass or other alternative forages. He has active research on irrigation techniques and soil salinity in the San Joaquin Valley and has field plots throughout California from El Centro near Mexico to Tulelake near the Oregon border. He is a board member of the National Alfalfa & Forage Alliance and the California Alfalfa & Forage Association, and chair of the California Alfalfa Workgroup and is chair of the Western & California Alfalfa Symposium.

Anja Raudabaugh is the Chief Executive Officer of Western United Dairymen. Anja came to WUD from the Madera County Farm Bureau where she served as the Executive Director for four years. Prior to her role at the Farm Bureau, she worked as a Project Manager for a Fortune 500 Company, and as the Senior Legislative Assistant for Congressman Doug Ose, representing California's 3rd District in Washington D.C. Previously the Marketing Director for the California Asparagus Commission and District Representative for State Senator Dick Monteith; her extensive experience in the political environment adds tremendous value to the organization. Anja's time in Sacramento working as a project manager and technical adviser handling CEQA and NEPA requirements as they applied to state and federal agencies, has lent her a skill set adept with navigating complex issues.

William P. Weiss, Ph.D. is a Professor of Dairy Cattle Nutrition in the Department of Animal Sciences at The Ohio State University, located at OARDC in Wooster. He earned his BS and MS degrees from Purdue University and his Ph.D. from Ohio State. He has been on the faculty of Ohio State since 1988 with a joint research:extension appointment. His main research areas currently are: 1) importance of variation in cow and diet factors in diet formulation; 2) factors affecting digestibility in dairy cows; 3) nutrition of pre and early post partum dairy cow; and 4) relationships between minerals and vitamins and health of dairy cows. He has authored more than 125 journal articles and more than 350 popular press and proceedings articles and has given invited talks in 40 states and 29 countries. He was a member of the 2001 Dairy National Research Council (NRC) committee and is currently serving as vice chair of the 2016 Dairy NRC committee. He also served as Interim Chair of the Department of Animal Sciences from 2016-2017.

Adam L. Lock, Ph.D. is an associate professor in the Department of Animal Science at Michigan State University. Originally from a dairy farm in the southwest of the United Kingdom, he received his Ph.D. from the University of Nottingham and completed a post-doc at that institution as well as at Cornell University. Dr. Lock had a research and teaching appointment at the University of Vermont from 2006 to 2009 before moving to his current research and extension appointment at Michigan State University in the fall of 2009. His research and extension programs focus on both dairy production and human nutrition and health, and the interface between these two disciplines. The central theme is fatty acid digestion and metabolism in the dairy cow and the impact of bioactive fatty acids on animal production and human health. Current efforts concern the effect of diet on the production of biohydrogenation intermediates in the rumen, dietary strategies for maximizing milk fat systhesis, applying this knowledge to improve our ability to troubleshoot on farm issues related to milk fat depression, fatty acid absorption in the small intestine, fat supplementation opportunities, and the potential for omega-3 fatty acids to promote dairy cattle metabolism and health. The impact of milk and dairy products on human health, in particular the role of milk fat is also of special interest.

David Barbano, Ph.D., Professor of Food Science – Cornell University – 1980 to present and Director, Northeast Dairy Foods Research Center. He received his BS – Biology/Food Science and MS/Ph.D. – Food Science from Cornell University. Dave conducts an applied and basic research program on dairy product manufacturing and milk analysis for dairy herd management. Recently, Dave has focused on developing new milk analysis measures of cow metabolic health for dairy herd management. He has been very active in the analytical groups of International Dairy Federation and the Association of Official Analytical Chemists International for the past 30 years. Honors include: 2018-National Cheese Institute Cheese Laureate Award; 2017-American Dairy Science Association – 100 peer reviewed JDS Publications Award; 2017-American Dairy Science Association De Laval Extension Award; 2015-American Dairy Science Association Elanco Award for Excellence in Dairy Science; 2014-Award of Excellence-International Dairy Federation; 2010-Harvey Wiley Award-Association of Official Analytical Chemists International; 2008-American Dairy Science Association West Agro Award for Milk Quality Research. What is the best part of my job? Working together with students to create new knowledge, technology, and science-based solutions to problems in the dairy industry.

Michael A. Ballou, Ph.D. is an Associate Dean for Research and an Associate Professor of Nutritional Immunology in the College of Agricultural Sciences and Natural Resources at Texas Tech University. He is also serving as the interim chair for the Department of Veterinary Sciences. He completed a Bachelor's degree in Animal Science from the University of California, Davis in 2002. Michael remained at UC Davis and completed a Ph.D. in Nutritional Biology with an emphasis in Immunology in 2007. Michael's research is primarily focused on how nutrition and management influence the health and performance of dairy calves, heifers, and periparturient cows. He has authored or co-authored 57 peer-reviewed articles, 1 book chapter, and 110 scientific meeting abstracts. Michael has received research support from private foundations, industry, the USDA, and the US State Department.

California Animal Nutrition Conference
2018 Steering Committee

Chairperson: Jason Brixey, M.S., P.A.S. - Consulting Animal Nutritionist
Jason D. Brixey was born and raised in Crescent City, CA. He graduated in 2001 from Cal Poly San Luis Obispo with a Dairy Science and Ag Business Undergraduate Degree. He attended the University of Idaho in Moscow under Dr. Mark McGuire, graduated with a Masters of Science in 2003. He started his animal nutrition career with West Milling LLC in Phoenix, AZ as the Nutrition Technical Representative. In April of 2005, Jason was hired by Pine Creek Nutrition Service Inc. out of Denair, CA to work on dairy farms as a Consulting Animal Nutritionist. He became partner at Pine Creek Nutrition Service in January of 2008. Married to my lovely wife Jodie that I share in the duties of raising three wonderful children; Jack (8), Kaydee (5 ½), and Will (3).

Vice Chairperson: David Ledgerwood graduated in 2004 with a BS degree in Animal Science focusing on livestock and dairy cattle from the University of California Davis. Upon graduating he worked in the university ruminant nutrition lab with Dr. Ed DePeters as a lab technician performing feed nutrient and milk analysis while assisting graduate students run various ruminant nutrition focused research trials. In 2007 he graduated with a MS degree in Animal Biology focusing on ruminant nutrition working with Dr. DePeters. After graduation in 2007 he worked as a lab and research program manager in the field of animal behavior/welfare on the UC Davis campus performing various research trials focused on improving cow comfort. He accepted a job with the Veterinary Medicine Teaching and Research Center in Tulare as a research program manager for the clinical department performing various research projects covering cow behavior, calf health, and nutrition. In April of 2011 he was offered a position with Western Milling LLC working as a dairy nutritionist. In 2018 David joined Novita Nutrition as a Technical Service Manager.

Ex Officio: Phillip Jardon, DVM, MPVM, is a Technical Consultant for Elanco Animal Health. He earned his DVM from Iowa State University and MPVM from UC Davis. Dr. Jardon has worked in the dairy industry for 27 years. He has a wide range of experience in research, dairy veterinary practice, nutritional consulting, and in technical service.

Committee Members:

Anthony Allen, Biomin USA Inc., Ruminant Key Account Manager for Western U.S. with Biomin since since May of 2010. He has worked for Nutrius, Foster Farms Commodities Division, and PM Ag Products on the dairy direct side before joining Biomin on the supplier side. Anthony received his B.S., Animal Science degree from Fresno State in 1995 and he is a Certified Dale Carnegie Trainer and teaches a couple of classes each year. He is a lifelong Fresno resident. Anthony and his wife Stephanie have three boys, Nathan, Garrett, and Sam.

Marc Etchebarne, Michel A. Etchebarne, Ph.D. Inc., Independent Dairy Nutritionist since 2010. USMC 2003-2008, Sergeant. Colorado State University graduate emphasis on Sheep, Feedlot, and Dairy Systems in 2010. ARPAS member, PAS.

Jennifer Heguy, is a native of California's San Joaquin Valley. She received her B.S. in Animal Science, with an emphasis in Livestock and Dairy, at the University of Caifornia, Davis. In 2006, she received her M.S. degree at UC Davis, focusing on dairy cattle nutrition. Jennifer currently serves as the University of California Dairy Farm Advisor in Merced, Stanislaus and San Joaquin Counties where milk is a major agricultural commodity. Jennifer's major program focus is improving silage and feeding management practices on California dairies.

Juliana M. Huzzey, Ph.D. is an Assistant Professor, Behavior and Welfare Specialist in the Animal Science Department at Cal Poly, San Luis Obispo. She received her Ph.D. Animal Science from Cornell University Ithaca, NY and M.S. Animal Science and B.S. Agroecology from University of British Columbia, Vancouver, BC, Canada. The overall aim of Julie Huzzey's program is to enhance the welfare of animals in managed systems by teaching classes and conducting research focused in applied animal behavior and welfare. She is particularly interested in cattle feeding and social behavior and the role these behaviors play in biological functioning and performance. By understanding these processes better, Dr. Huzzey aims to identify optimal housing and management practices that can be directly applied on farms to facilitate improved animal health and wellbeing. Dr. Huzzey is a member of the American Association of Dairy Science, the American Society of Animal Science and the International Society of Applied Ethology.

Jim Loften, Ph.D., is Dr. Jim Loften received his BS degree from Iowa State University, and his MS and Ph. D at the University of Georgia. He began working in the Dairy Research Department at Ralston Purina Company for 4 years where his duties included accomplishing research on lactating cows and consulting with dairy farms all over the US and Canada. He moved into sales and nutritional consulting in central Minnesota with the company for 15 years. He then began designing, building and managing large dairies in the Midwest for 5 years. He started working for Milk Specialties Global in 2002 as the Director of Technical Services and Sales where his responsibilities include field technical services, research and development, and large commercial dairy sales in the western US. He has published studies involving lactating cow and calf research, as well authoring an invited review on palmitic and stearic acid metabolism.

Zachery Meyer, was raised in Ixonia, Wisconsin. He grew up immersed in his family's business, Rock River Laboratory. Meyer spent many hours helping in various jobs around the laboratory, seeing first-hand the dedication and commitment his father and the late Twilah Kulow had to the business and their customers. Meyer gathered business experience at Clear Channel and GE Medical while working toward his degree from the University of Wisconsin-Milwaukee. In 2007, Meyer resumed his involvement in Rock River Laboratory, starting as a soil sampler, moving to outside sales and eventually taking on his current role of director of operations.
Meyer still gathers inspiration from the Rock River Laboratory employees and mentors who cultivated his drive for customer satisfaction and service, while continuing to learn and deepen his understanding of animal nutrition and agronomy. When he isn't building relationships with customers or overseeing laboratory operations, Zac spends his time playing or watching sports and sharing in family time with his wife and two young daughters.

Honorary Members:

Amanda Gipe McKeith, Ph.D., is an Assistant Professor in the Department of Animal Sciences & Agricultural Education at Fresno State. She serves as the graduate program coordinator and co-advisor for both the Meat Science Club and Young Cattlemen's Association. Her research focus areas are how nutrition affects meat quality, processed meat ingredients, and food safety in food products. Dr. Amanda Gipe McKeith is originally from Merced, CA where she grew up on a 700-acre farm/ranch. Her family raises purebred registered Shorthorn cattle and grows corn silage, alfalfa, oats, and pasture hay. Amanda grew up showing livestock with her sister, Amy, and parents, Alpha and Sherri. Her family still runs the operation that now includes her brother-in-law, Tim and nephew, TJ as well as her husband Russell McKeith. Amanda serves as President for the National Shorthorn Lassie Association, and Treasurer for the California Shorthorn Breeders Association. Amanda received her A.A. in General Agriculture from Merced Community College, B.S. in Animal

Science and Food Science at Kansas State University, M.S. in Animal Science with an emphasis in Meat Science from Kansas State University, and Ph.D. in Animal Science with an emphasis in Meat Science from The Pennsylvania State University. Amanda judged on both the Meats and Livestock Judging teams at Kansas State University. She coached meats judging teams at Kansas State and Western Kentucky University and livestock judging teams at Penn State and Western Kentucky.

Kyle Thompson, Ph.D. received his B.S. degree in animal science from Fresno State (2006) and his master's and Ph.D. degrees in animal science from Oklahoma State (2011/2015). He joined the Fresno State staff in the fall of 2016 after taking classes and teaching at Oklahoma State from January 2007-June 2016 and serving as the graduate student assistant manager of the campus dairy cattle center. His research included dairy nutrition research trials and lactating cow probiotics. He also assisted in research for bovine respiratory disease, rumen temperature bolus, milk production by weigh-suckle-weigh and swine antimicrobial replacements. He also assisted in 4-H and FFA Field Day dairy judging competitions. While in Stillwater, OK, he owned and operated Wild Acre Farms and Exotics, which raised ewes, game birds, free range hens and other fowl/animals, and produced grasses and winter wheat for grazing and hay production. As a Fresno State student, he worked in the sheep unit three years, served as a campus farm tour guide, and dairy unit herdsman and feed/hospital technician. He also worked as an exotic animal nutrition intern (2009) and global nutrition fellow at the San Diego Zoo (2013).

CALIFORNIA ANIMAL NUTRITION CONFERENCE HISTORY

YEAR	CHAIRPERSON	COMPANY AFFILIATION
2017	Dr. Phillip Jardon, DVM, MPVM	Elanco Animal Health
2016	Dr. Phillip Jardon, DVM, MPVM	Elanco Animal Health
2015	Mr. Ben Tarr	Adisseo USA Inc.
2014	Dr. Jeffrey M. DeFrain	Zinpro Performance Minerals
2013	Mr. Doug DeGroff	Diversified Dairy Solutions, LLC
2012	Mr. Eduardo Galo	Novus International, Inc.
2011	Dr. Michael A. DeGroot	DeGroot Dairy Consulting
2010	Dr. Jim Tully	Pine Creek Nutrition Service, Inc.
2009	Mr. Michael Braun	Phibro Animal Health
2008	Dr. Luis Rodriguez	Zinpro Corporation
2007	Dr. Marit Arana	A.L. Gilbert Company
2006	Mr. Dennis Ervin PAS	Prince Agri Products, Inc.
2005	Dr. Lawson Spicer	Nutri Management Inc.
2004	Dr. Luis Solorzano	Purina Mills, Inc.
2003	Dr. Alfonso Mireles, Jr.	Foster Farms
2002	Mr. Edmund Vieira	Pine Creek Nutrition Service, Inc.
2001	Dr. Melinda Burrill	California State Polytechnic University - Pomona
2000	Mr. Dave Fischer	Foster Farms
1999	Dr. M. Steven Daugherty	California State Polytechnic University - SLO
1998	Dr. Doug Dildey	Alltech, Inc.
1997	Ms. Carla Price	Nutritionist
1996	Dr. H.John Kuhl, Jr.	Nest Egg Nutrition
1995	Mr. Dennis Ralston	M. Rinus Boer Co., Inc.
1994	Dr. Doug Dildey	Alltech, Inc.
1993	Dr. Mark Aseltine	Consulting Animal Nutritionist
1992	Dr. Carl Old	MacGowan-Smith Ltd.
1991	Mr. Nick Ohanesian	Ohanesian & Associates
1990	Mr. Rod Johnson	M. Rinus Boer Co., Inc.
1989	Mr. Timothy Riordan	Nutri-Systems, Inc.
1988	Dr. Russ W. Van Hellen	Great West Analytical
1987	Dr. John E. Trei	California State Polytechnic University, Pomona
1986	Dr. A.A. Jimenez	Ancon, Inc.
1985	Dr. Wm. A. Dudley-Cash	Foster Farms
1984	Dr. Joel Kemper	Penny-Newman Co.
1983	Dr. Alex J. Kutches	O.H. Kruse Grain & Milling Co.
1982	Dr. Howard Waterhouse	Bell Grain & Milling
1981	Mr. Don Ulrich	Diamond Shamrock Chemical Co.
1980	Mr. Tom Geary	PMS-West, Inc.
1979	Dr. Frank Parks	Kemlin Industries
1978	Mr. Fred Pfaff	Zacky Farms
1977	Mr. Rene Lastreto	Diamond Shamrock Chemical Co.
1976	Mr. Rene Lastreto	Diamond Shamrock Chemical Co.
1975	Dr. R.D. Hendershott	Nulaid Foods
1974	Dr. R.D. Hendershott	Nulaid Foods
1973	Dr. Leland Larsen	Nutri-Systems, Inc.
1972	Dr. Leland Larsen	Nutri-Systems, Inc.
1971	Mr. Rene Lastreto	Diamond Shamrock Chemical Co.

CALIFORNIA ANIMAL NUTRITION CONFERENCE HISTORY- Continued

YEAR	CHAIRPERSON	COMPANY AFFILIATION
1970	Mr. Fred Pfaff	Balfour Guthrie
1969	Mr. Fred Pfaff	Balfour Guthrie
1968	Mr. Fred Pfaff	Balfour Guthrie
1967*	Mr. Gary L. Frame	J.G. Boswell Co.
1966*	Mr. Gary L. Frame	J.G. Boswell Co.
1965*	Mr. Arne Jalonen	Topper Feed Mills
1964*	Mr. Arne Jalonen	Topper Feed Mills
1963*	Dr. W.P. Lehrer	Albers Milling Co.
1962*	Dr. H.J. Almquist	The Grange Co.
1961*	Dr. H.S. Wilgus	The Ray Ewing Co.
1960*	Mr. Bert Maxwell	Nulaid Foods
1959*	Mr. Bert Maxwell	Nulaid Foods
1958*	Mr. Robert Caldwell	Anderson Smith Milling Co.
1957*	Mr. Emery Johnson	P.C.A., Los Angeles
1956*	Mr. Emery Johnson	P.C.A., Los Angeles
1955*	Dr. H.J. Almquist	The Grange Co.
1954*	Dr. H.J. Almquist	The Grange Co.
1953*	Mr. Clifford Capps	California Milling Co.
1951*	Mr. Dolph Hill	Golden Eagle Milling Co.
1950*	Dr. H.J. Almquist	The Grange Co.
1949*	Dr. H.J. Almquist	The Grange Co.
1948*	Dr. H.J. Almquist	The Grange Co.

* California Animal Industry Conference

History of the California Animal Nutrition Conference

The California Animal Nutrition Conference (CANC) originated in the 1940's as the California Animal Industry Conference, sponsored by the California Grain & Feed Association (CGFA). CGFA wanted to expand the continuing education program into a forum encompassing animal health, nutrition and management. The expectations were that communications between (nutritionists) industry, educational institutions and regulatory agencies would be improved. In 1972, CGFA discontinued sponsoring the Animal Industry Conference.

After the conference was discontinued, a small group of nutritionists began meeting annually in Fresno. Two or three invited speakers from industry or the universities presented information on nutrition, especially poultry.

In 1975 a set of organizational bylaws were developed by the steering committee. CANC was established and was provided support by CGFA. The CGFA Board of Directors appointed a chairperson annually and approved the steering committee. In 1978, Dr. Frank Parks, the Chairperson, requested that CANC be granted independent status and be established as a self-governing committee of CGFA. This request was granted.

For a few years, meetings were held in Fresno and Corona, California. For a couple of years starting in 1978, CANC published "Nutri-Facts", a "newsletter" consisting of articles in animal production.

In 1979, donations were requested from industry companies to help keep registration fees low. During the 1980's and through the 1990's the attendance at CANC continued to grow as the quality of the conference improved and the conference became known nationwide. In the 1990's a pre-symposium was added. The pre-symposium is sponsored by a company selected by the CANC Steering Committee. This process allows the selected company to showcase its research and products. In the year 2000, posters on research by students were included.

Attendance at the conference has grown from 50 in the 1970's to over 300 attendees. To encourage attendance, different activities have been tried such as keynote speakers, skiing expeditions and a very successful barbeque dinner put on by the Animal Science Department at Fresno State University.

The California Grain & Feed Association has supported and allowed CANC to work and grow. The premise of the CGFA and CANC relationship is to work together to educate the feed industry with information for problem solving and to disseminate valuable research information. CANC is not an industry, university, or government entity, but a committee collectively working together for the good of agriculture in California.

STUDENT ABSTRACTS FOR

POSTER PRESENTATION

AT THE

CALIFORNIA ANIMAL

NUTRITION CONFERENCE

MAY 2 & 3, 2018

Effects of heat stress mitigation on rumination activity and panting in dairy cows measured by SCR technology

Angelica Carrazco, Alycia Drwencke, Grazyne Tresoldi, Cassandra Tucker, and Frank Mitloehner. Department of Animal Science, University of California, Davis, CA, USA; acarrazco@ucdavis.edu

Heat stress is a serious concern to the California dairy industry, as summer months result in elevated temperatures with little to no rainfall. Because the amount of time a cow allocates to rumination can be affected by nutritional factors, by acute stress, and by disease, monitoring rumination time remains vastly important. Cow health and behavior is generally assessed through visual observations which has become labor intensive due to increased herd sizes. Due to this challenge, there has been tremendous commercial interest in data logger technologies such as the SCR collar mounted technology (SCR Engineers Ltd., Netanya, Israel) which gather and store information regarding particular behaviors including rumination, eating, and general activity. The most recent version of SCR collar allows for detection of heavy breathing and panting, which has the potential to function as an indicator of heat stress. The objective of this study was to validate both the rumination and heavy breathing data generated by the SCR collar (SCR HRLDn-Tag). Tested in pairs in a crossover design at the UC Davis Dairy Facility, 32 cows equipped with an SCR HRLDn-Tag were enrolled into one of 8 groups (n=4 per group). Groups were tested under four cooling treatments: 1) baseline, 2) conductive cooling, 3) optimized baseline, and 4) convective cooling. Panting signs and respiration rates were observed via live observations every 30 min from 1000 to 1900h. Panting signs included three parameters: drooling, open-mouth panting, and panting with tongue extended. Rumination behavior was recorded based on video recordings at 3 min scan sampling intervals 24h/d. One minute interval data was extracted from Heatime Pro v.15.0.13.0 (SCR Engineers Ltd., Netanya, Israel) and matched with the minutes observed. Data analysis is ongoing, with SCR HRLDn-Tag data being compared with observer data by Pearson correlation. Initial interpretations of the data indicates a positive correlation between the two for rumination ($r^2 = 0.61$) and for heavy breathing at respiration rates above 80 bpm ($r^2 = 0.17$).

Innovative cooling strategies for dairy cows

Alycia Drwencke[1], Grazyne Tresoldi[1], Matthew Stevens[2], Vinod Narayanan[2], Theresa Pistochini[2], and Cassandra B. Tucker[1] [1]Center for Animal Welfare, [2]Western Cooling Efficiency Center, University of California, Davis, CA, USA; amdrwencke@ucdavis.edu

Producers commonly use spray water at the feed bunk and fans in the lying area to mitigate heat stress in dairy cows. Spray water cycles on and off and fans turn on once a pre-set activation air temperature is reached. While this is an effective method of mitigating heat stress, innovative methods are needed to improve sustainability. Our objective was to evaluate the effectiveness and efficiency of 4 cooling methods on behavioral and physiological responses in dairy cows housed in a free-stall barn. We also measured water and energy use of the technologies. The 4 strategies we tested were: 1) conductive cooling, where water mats cooled by a sub-wet-bulb evaporative chiller were buried under the lying area (Mat; activated at 19°C), 2) targeted convective cooling where cool air from a evaporative cooler was directed toward the cows through fabric ducts at both the feed bunk and lying area (Targeted Air; activated at 22°C), or a combination of spray water and fans described above (3) Baseline, activated at 22°C), and 4) spraying half the amount of water as in Baseline and moving the fan at the feed bunk to improve air flow over cows (Optimized Baseline; activated at 22°C). In a crossover design, groups of cows averaging (±SD) 34.9±5.3kg/d of milk (n=8 groups; 4 cows/group) were tested for 3d/treatment. For ethical reasons, both the Mat and Targeted Air also had spray water beginning at 30°C. Body temperature, posture, and location within the pen were recorded every 3 min 24h/d. Respiration rates were record every 30 min daily from 1000 to 1900h. Pairwise comparison within a mixed model was used to compare each treatment to the Baseline. Average lying time was 51±2.4 %/d and was not affected by treatment ($P>0.1$). Milk production was not affected by treatment ($P>0.5$). Respiration rates did not differ across treatments overall, but on an hourly basis, cows on Mat had a significantly higher rate, compared to Baseline, at h 10 and 11 ($P<0.03$). Body temperature was also higher when on Mat compared to Baseline at h 10, 11, 20, 21, 22 ($P<0.04$). During these 5 hours, average lying time was 56% for the Mats, indicating that they were being used. Taken together, these results indicate that the Mat treatment did not effectively reduce early indicators of heat load, compared to Baseline. In contrast, both Targeted Air and Optimized Baseline were both effective, but differed in other aspects of sustainability. Targeted Air used the least amount of water, but the most energy of all options tested. In conclusion, more efficient heat abetment options can be identified, particularly an Optimized Baseline strategy, which cut water use in half and maintained similar energy use and responses in cows.

Keywords: dairy cows; heat stress; spray water; conductive cooling; targeted convective cooling

Double-blind, block-randomized, placebo-controlled clinical trial on effectiveness of zinc supplementation on diarrhea and average daily gain in pre-weaned dairy calves.

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The objective of this clinical trial was to evaluate the effectiveness of zinc supplementation on decreasing diarrhea and increasing average daily weight gain (ADG) in dairy calves during the pre-weaning period. A total of 1,482 healthy, newborn Holstein heifer and bull calves from a large California dairy in the San Joaquin Valley were studied between December 2015 and June 2016. Each calf was enrolled at 24 to 48 hours of age until exit from the hutch at approximately 90 days of age. Calves were block randomized to one of three treatments: 1) placebo, 2) zinc methionine (ZM), or 3) zinc sulfate (ZS) to be administered once daily for the first 14 days. Serum total protein at enrollment and body weight at birth, end of treatment, and hutch exit were obtained. Fecal consistency was assessed daily for 28 days post-enrollment. In addition, serum zinc concentrations before and after the treatment period and a fecal ELISA on the first day of diarrhea and at diarrhea resolution were performed for a random sample of 127 calves. Bull calves treated with ZM had increased ADG (22 g per day) compared to placebo-treated bulls (P = 0.042). Conversely, ZM-treated heifers had decreased ADG (12 g per day) compared to placebo-treated heifers (P = 0.019). For calves on the study dairy, this corresponds to an additional 1.98 kg of weight gain in ZM-treated bulls and 1.08 kg of weight loss in ZM-treated heifers compared to their placebo-treated counterparts over a 90-day hutch period. Calves treated with ZM and ZS had a 14.7% (P = 0.015) and 13.9% (P = 0.022) reduced hazard of diarrhea, respectively, compared to placebo-treated calves. Given a median age at first diarrhea of 8 days, calves that were treated with ZM and ZS for all or at least the first five days of their diarrhea episode had a 15.6% (P = 0.028) and 8.4% (P = 0.039) increased hazard of clinical cure, respectively, compared to placebo-treated calves. Pathogen-specific models demonstrated that the odds of microbiological cure for rotavirus or *Cryptosporidium parvum* at diarrhea resolution was not different between treatment groups. An overall model of calves positive for any single fecal pathogen (*E. coli* K99, rotavirus, coronavirus, or *Cryptosporidium parvum*) on the first day of diarrhea showed the odds of microbiological cure at diarrhea resolution was not different between treatment groups. The current trial showed a potential role of zinc supplementation for improved weight gain, diarrhea prevention, and faster diarrhea recovery in pre-weaned dairy calves with the need for further research on sex-specific or weight-based dosing.

Relationships between muscle mitochondrial function and residual feed intake in beef steers.

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The objective of this study is to evaluate the relationship between muscle mitochondrial function and residual feed intake (RFI) in growing beef cattle. A 56 day feeding trial was conducted with 81 Angus crossbreed steers (initial BW = 378.4 ± 43.3 kg) from the Sierra Foothill Research Station (University of California). All individuals were fed the same finishing ration (ME = 3.279 Mcal/kg DM). ADG, DMI, and RFI were 1.82 ± 0.27, 8.89 ± 1.06, and 0.00 ± 0.55 kg/d, respectively. After the feeding trial, the steers were categorized into high, medium, and low RFI groups. Low RFI steers consumed 13.6 % less DM ($P < 0.05$) and had a 14.1% higher G:F ratio ($P < 0.05$) than the high RFI group. No differences were found in initial age, ADG, nor BW ($P > 0.10$). The most extreme individuals from the low and high RFI groups were selected to measure mitochondrial function (n = 6 low RFI and n = 6 high RFI). Mitochondrial oxygen measurements were completed after the submission of this abstract and will be presented during the conference.

Enzymatically digested food waste as feed for growing-finishing pigs

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Enzymatic digestion is a technology that can be used to convert food waste from supermarkets into pasteurized liquid pig feed. The objective of this experiment was to examine the growth performance, visceral mass, carcass characteristics, meat quality, and fatty acid profile of growing and finishing pigs fed with enzymatically digested food waste. Fifty-six crossbred pigs (approximately 32.99 kg BW) were randomly assigned to one of 2 dietary treatments with 7 replication pens and 4 pigs per pen. A 3-phase feeding program was used with d 0 to 28 as Phase 1, d 28 to 53 as Phase 2, and d 53 to 79 as Phase 3. The 2 dietary treatments were control diet based on corn-soybean meal diet and a liquid diet produced from enzymatically digested food waste that was only supplemented with vitamin-mineral premix and salt. All diets met the estimates for nutrient requirements of growing-finishing pigs based on the NRC (2012). The pigs were fed control or liquid diet in phases 1 and 2 while all pigs were fed with control diet in phase 3. Bodyweights of all pigs on d 0, 28, 53, and 79; daily feed allotments; and DM of all diets were recorded to calculate ADG, average daily dry matter intake (**ADDMI**), and Gain:Feed. At the end of the feeding program, one pig with the BW closest to the average BW from each pen was slaughtered to measure the viscera mass and carcass characteristics. Longissimus muscle (**LM**) was excised from the posterior of the 10th rib to measure meat quality and back-fat samples were collected for fatty acid profiles. All data were analyzed with PROC MIXED of SAS with pen as experimental unit and the statistical model included diet as fixed effect and block as random effect. Pigs fed with liquid feed had lower ($P < 0.05$) BW on d 28, 53, and 79 and ($P<0.05$) ADG on phase 1 than pigs fed with control feed. This observation was likely due to the reduced ($P < 0.05$) ADDMI on phases 1 and 2. Pigs fed with the liquid diet tended to increase ($P = 0.082$) Gain:Feed by 4.1% on phase 3 and also had heavier ($P < 0.05$) gastrointestinal tract including stomach, small intestine, and large intestine than the pigs fed with the control diet. Hot carcass weight was lower ($P < 0.05$) in pigs fed the liquid diet due to the smaller ending live weight; however, no differences were observed in carcass yield and other carcass measurements. The liquid feed tended to decrease ($P = 0.087$) subjective firmness (2.43 vs. 2.86), but did not impact pH, marbling score, and objective color L*, a*, and b* in the LM. Pigs fed with the liquid feed

contained more ($P < 0.05$) pentadecanoic acid and margaric acid, and greater ($P < 0.05$) myristoleic acid, palmitoleic acid, oleic acid, vaccenic acid, gondoic acid, EPA, and DHA in their back-fat than the pigs fed the control diet. Feeding control diet increased more ($P < 0.05$) palmitic acid, arachidic acid, linoleic acid, linolenic acid, and eicosadienoic acid in the back-fat of the pigs than in the back-fat of the pigs fed with liquid feed. In conclusion, the high moisture content in the enzymatically digested food waste limits the growth performance of growing pigs. However, it is believed that this byproduct could be similar to or even exceed the nutrient contents in corn-soybean meal diet after increasing DM content. Feeding enzymatically digested food waste to growing and early finishing pigs did not affect their meat quality and may benefit pork products by providing more beneficial fatty acids to pork consumers.

Key words: enzymatically digested food waste, growth performance, meat quality, pig

The effects of two equine feed supplements on gastrointestinalhealth as reflected in fecal pH, fecal microbial profiles and digestibility

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Abstract

Gastrointestinal disease is the number one killer of horses (Daly et al., 2001). Little is known about the maintenance of microbes in the equine hindgut and how to distinguish a healthy gut in a live horse. Utilization of internal and external digestibility markers and starch fermentation has been extensively studied in ruminants and is the basis for research conducted on horses. The aims of this study were to investigate the effects of two equine feed digestive aid supplements on hindgut health (HGH) as reflected in fecal pH, fecal microbial profiles and digestibility and to compare and validate DM digestibility measurements through the use of internal and external markers such as chromium oxide (CR), Lignin (Lig), indigestible acid detergent fiber (iADF), indigestible neutral detergent fiber (iNDF) and indigestible lignin (iLig). Nine mature Quarter horses (6 geldings, 3 mares) were used in a cross over design, three feeding periods of 17 d (51 d total), using 3 treatments: control, no feed additive (Con), Smartpak (SP), or Platinum Performance (PP). Within the 17 d period, horses were offered orchard grass hay, sweet cob grain and the assigned treatment daily and 4 CR cookies to deliver 8/g/d of CR for the last 7 d of each period. Total feces were collected from 15 to 17 d of the

trial. Feed and fecal samples were dried, ground and sent to ANALAB (Fulton, IL) for nutrient analysis. Duplicate samples of feed and feces were placed into a ruminally cannulated cows for in situ determination of iADF, iNDF and iLig to estimate digestibility. Estimated CR FO and DM digestibilities were evaluated using the root mean square prediction error percentage of the observed mean (RMSPE), concordance correlation coefficient (CCC) and Nash-Sutcliffe efficiency (NSE) methods. The best internal marker was iNDF with the smallest RMSPE (8%), largest CCC (0.55) and an acceptable NSE value (0.17). Fecal pH was not affected by treatment but mares tended to have lower fecal pH than geldings, 6.8 and 7.1 respectively ($P < 0.09$), and fecal pH decreased with age ($P < 0.15$). Total FO tended to increase with age as young animals (<12 yr) produced 2.4 kg/d and old animals (>12 y) producing 2.8 kg/d ($P < 0.12$). This study is in agreement with previous studies that iNDF is the best internal marker to estimate apparent digestibility in horses. Treatments PP and SP had no effect on fecal pH or DM digestibility. Since there were large differences in the individual responses of horses to treatments, more subjects may be needed or a lower gut acidosis may need to be induced to detect responses to PP and SP.

Supplementation of *Bacillus subtilis* enhanced growth rate and gut barrier function of weanling pigs experimentally infected with F18 *Escherichia coli*

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The objective of this experiment was to investigate dietary supplementation of *Bacillus subtilis* on growth performance, diarrhea, gut permeability and immunity of weaned pigs experimentally infected with a pathogenic F-18 *Escherichia coli* (***E. coli***). Forty-eight pigs (6.73 ± 0.77 kg BW) were individually housed in disease containment rooms and randomly allotted to one of the four treatments with 12 replicate pigs per treatment. Four treatments included negative control (**NC**), positive control (**PC**), single dose probiotics group, and double dose probiotics group. Pigs in the NC and PC groups were fed with basal diet but without or with *E. coli* challenge. Pigs in the probiotics groups were fed the diets either supplemented with 1.28×10^9 CFU or 2.56×10^9 CFU *Bacillus subtilis*/kg feed and challenged with *E. coli*. The experiment lasted 18 days with 7 days before and 11 days after the first inoculation (d 0). The inoculum used in this experiment was F-18 *E. coli*, containing LT, STb, and SLT-2 toxins. The inoculation doses were 10^{10} cfu/3 mL oral dose daily for 3 consecutive days. Growth performance was measured on d -7 to 0 before inoculation, d 0 to 5, 5 to 11, and 0 to 11 post-inoculation (**PI**). Diarrhea score (DS; 1, normal, to 5, watery diarrhea) was daily recorded for each pig. On d 5 and 11 PI, 6 pigs per treatment were euthanized to collect duodenum, middle of jejunum, and ileum to analyze gut morphology. Jejunum were also freshly collected from all treatments (4 pigs/treatment) except the single dose probiotics group for transcellular and paracellular permeability analysis using Ussing Chamber. Intestinal mucosa samples were collected from jejunum and ileum on d 5 and 11 PI and were snap-frozen in liquid nitrogen for gene expression analysis. The mRNA expression of genes related to gut barrier function and nutrients transportation (*CFTR, SGLT1, MUC2, claudin, ZO1*, and *occludin*) in jejunal mucosa and the mRNA expression of genes associated with immune defense (*COX2, TNFA, IL1B, IL-6, MUC2, IFNG, IL7, and IL1A*) in ileal mucosa were analyzed by qRT-PCR. All data were analyzed by ANOVA using the PROC MIXED of SAS with pig as the experimental unit. The *E. coli* infection reduced ($P < 0.05$) growth performance and intestinal villi height, but increased ($P < 0.05$) frequency of diarrhea and transcellular and paracellular permeability in jejunum compared with pigs in the NC. *E. coli* infection up-regulated ($P < 0.05$)

the mRNA expression of *SGLT1* and *MUC2* on d 5 PI, but down-regulated ($P < 0.05$) the gene expression of *SGLT1, MUC2*, and *claudin* on d 11 PI in jujunal mucosa. *E. coli* infection also increased ($P < 0.05$) the mRNA expression of several immune genes (*IL1A, IL1B*, and *IL7* on d 5 PI, and *IL1B, IL6, IL7*, and *TNFA* on d 11 PI) in ileal mucosa of weaned pigs. Supplementation of *Bacillus subtilis* linearly enhanced BW at d 5 PI ($P = 0.086$) and 11 PI ($P = 0.084$), ADG from d 0 to 5 PI ($P < 0.05$) and 0 to 11 PI ($P = 0.058$), compared with the PC. However, no differences were observed in daily diarrhea score and overall frequency of diarrhea among *E. coli* challenge groups. Supplementation of double dose of *Bacillus subtilis* reduced ($P < 0.05$) both transcellular and paracellular permeability on d 5 and d 11 PI compared with the PC. Supplementation of *Bacillus subtilis* linearly up-regulated ($P < 0.05$) the mRNA expression of *CFTR* and *ZO1* on d 5 PI and *SGLT1* and *MUC2* on d 11 PI in jejunal mucosa, compared with the PC. Inclusion of *Bacillus subtilis* down-regulated the gene expression of *IL1A* in ileal mucosa of pigs on d 5 PI (linearly, $P = 0.07$), and the mRNA expression of *IL6* in ileal mucosa on d 11 PI (linearly, $P < 0.05$), compared with the PC. In conclusion, supplementation of *Bacillus subtilis* enhanced growth rate and reduced leaky gut of weaned pigs infected with a pathogenic *E. coli*. Those benefits were probably related to enhanced gut barrier function in jejunum of weaned pigs.

Key words: gut permeability, pathogenic *E. coli*, probiotics, weaned pigs

Effects of Heat Stress Mitigation Techniques on Feedlot Cattle Performance, Environmental, and Economical Outcomes in a Hot Climate

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ABSTRACT

Heat stress in feedlot cattle is a major animal welfare and economic concern, costing the U.S. beef cattle industry up to $369 million annually. To mitigate the deletarious effects of heat stress on animal performance and feedyard productivity, a feedyard in central Mexico compared various cooling methods for finishing cattle. Using the Integraded Farm System Model (IFSM), a partial lifecycle assesment (LCA) was performed to determine economic returns and envionmental footprints of various cooling methods. The two year feedyard study was arranged in a completely randomized design with four treatments and three replications/yr, with time as a block (n = 6). The four treatments included 1) conventional shade (control; SC; steel shade 1.8 m^2 of shade/hd), 2) double conventional shade (DS; steel shade 3.6 m^2 of shade/hd), 3) dome structures without fans (DSA; 8.5 m^2/hd with 98% solar radiation blocked), and 4) domes with fans (DCA; three large sized low-speed fans). Each of the four treatments had 65 *Bos indicus* bulls, in a pen area of 570 m^2. When compared to the control (DC), DCA improved final BW by 25 kg ($P< 0.05$), followed by DSA at 12 kg ($P< 0.05$), and DS at 5 kg ($P> 0.05$). When treatment results were extrapolated to the entire feedyard population (annual turnover of 209,700 hd/yr), cattle in domed structures (DSA and DCA) versus steel shades (SC and DS) had reduced greenhouse gas (GHG) and ammonia (NH_3) emissions on a kg of BW basis. Compared to the control (SC), DSA had the greatest economic return of $13.14/ hd, followed by DCA and DS treatments with a return profit of $7.47 and $7.03, respectively. Overall in this hot climate, the implementation of advanced shade structures improved cattle performance and profitability while reducing environmental impacts of beef production.

Key Words: Heat Stress, Beef Cattle, IFSM, Methane Mitigation

Field refractometry for assessment of passive transfer of immunoglobulin G in kids and colostrum quality in does

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The objective of this study is to evaluate the efficacy of refractometry in assessment of both passive transfer of immunoglobulin G (IgG) in goat kids and goat colostrum quality in regard to IgG concentration. Colostrum samples were obtained from post-parturient does within the first 2 hours after kidding and measured for percent Brix on a digital refractometer. Blood serum samples (n=80) were obtained from kids prior to colostrum feeding, at 24 hours post-feeding, and 48 hours post-feeding. Kids (n=68) were fed a minimum of 240 ml of heat treated colostrum within the first 8 hours after birth. A subset of kids (n=12) were restricted to either 60 ml (n=4), 120 ml (n=4), or 180 ml (n=4) of colostrum, and blood samples were collected at the same specified time points. Serum samples were then measured for total protein concentration (g/dl) on a digital refractometer. The IgG concentrations of both serum and colostrum were quantified via enzyme-linked immunosorbent assay (ELISA). The resulting coefficient of determination (R^2) between colostral IgG and percent Brix was 51.8%. Using 20g/L IgG as a cut point between adequate and inadequate quality colostrum, the sensitivity and specificity of the refractometer were 75.0% and 83.8%, respectively. Likewise, the R^2 value between serum IgG and serum total protein was 0.77, with a sensitivity of 90.8% and a specificity of 84.6% at a serum IgG concentration cut point of 1,200 mg/dl between success and failure of passive transfer. Based on our findings we conclude that refractometry can be used to estimate both serum and colostral IgG with reasonable accuracy, suggesting that dairy goat producers may adopt this inexpensive, durable, easy to use instrument for on-farm evaluation of their colostrum feeding program to ensure adequate passive transfer of IgG in kids.

Forage in close-up rations: type, inclusion rate and dry matter adjustments

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The objective of the present study was to describe the type, inclusion rate (dry matter; DM%), and frequency of DM adjustments of forages incorporated into the close-up recipe (CUR). Records from a 12-mo (n=23) or 5-mo (n =1) consecutive period were extracted from a herd feeding management software (FeedWatch 7.0). Enrolled dairies were located in California and ranged in size from 1,100 to 6,900 cows. The data set included the following variables: date, recipe type, recipe number, ingredient type, ingredient quantity and ingredient DM. Descriptive statistics were conducted with MedCalc (v. 18.) The median number of forages included in CUR ranged from 2 to 4 across dairies. Corn silage was used in 95% of dairies and alfalfa forages in 83% of dairies [hay (low K: 60%, unknown: 23%), haylage (8%), green chop (4%)]. Half of the dairies incorporated a combination of corn silage and alfalfa hay as the sole sources of forage in CUR. Other forages included in CUR were oat hay (25%), wheat straw hay (25%), wheat silage (12%), and sorghum silage (8%). Across dairies, forage inclusion in CUR ranged from 45 to 90%, with most dairies (70%) including at least 65% of forage. Within dairy, forage inclusion varied over time; it increased up to 2 to 38% from Q_{10} to Q_{90}. Corn silage inclusion in CUR was < 40% (8%), 40 to 60% (56%), and > 60% (34%). Within dairy, corn silage inclusion varied, increasing 4 to 77% from Q_{10} to Q_{90}. Alfalfa hay inclusion in CUR averaged < 30% (20%), 30 to 40% (30%), 40 to 50% (40%) and >50% (10%). On dairies with 12-mo data set (n=23), DM adjustments for corn silage were performed 0 (17.3%), 3 to <6 (26.0%), 6 to <12 (30.4%) or ≥ 12 (26.0%) times per

year. For corn silage, adjustments in DM represented a change of 1.5 to 10% in percentage units of DM. DM adjustments for alfalfa hay were observed 0 (85%) or 1 (15%) time per year; the changes made were of 0.3 to 3 percentage units. Overall, in our study forage inclusion rate varied within and across dairies. Alfalfa forages were used on most dairies but only 60% recorded alfalfa as low K. There seems to be an opportunity to increase the frequency of DM adjustments for corn silage on dairies.

Keywords: forage, DM analysis, dairy cow

The effects of different feeding practices on heifer growth and reproduction at five California dairies using single-time-point measurements.

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Feeding for increased growth improves heifer reproductive efficiency and shortens the unproductive period from birth to calving, allowing producers to quickly regain costs associated with raising heifers. The objective of this study was to compare nutrients supplied to growing heifers, heifer weights, and age at first breeding on 5 commercial dairies and form a ranking system for evaluating and improving heifer-raising programs. A rank of 1 indicated the dairy fed DM greater than 10 kg/heifer/d, CP greater than 1.2 kg/heifer/d, and NEg greater than 0.9 Mcal/kg DM, had heifer weights greater than 600 kg at 20-24 mo, and early breeding, i.e., less than 410 d. Each subsequent rank indicated reduced performance from the first in at least one area with the lowest rank being 5. To determine the impact of nutrient supply on heifer growth and reproduction, TMR samples for each heifer diet were collected weekly for 4 wk at each dairy. Heifer weights were estimated using a weigh tape on 10% of the heifers (n=1747). Reproduction records for the previous year (n=4809) were collected from DairyComp305 (Valley Ag. Software, Tulare, CA). Least square means for DM, CP, NEg, weight, and age at first breeding were compared among dairies using the PROC GLM procedure of SAS (v. 9.4, 2014). Dairy E ranked 1 overall due to a NEg of 0.96 Mcal/kg DM ($P \leq 0.05$), weight at 20-24 mo of 602 kg ($P \leq 0.05$), and low SD in age at first breeding (411 ± 16.7 d; $P \leq 0.05$). The other dairies were ranked as follows: dairy D (1.1 Mcal/kg DM, 599 kg, 418 ± 16.9 d, respectively) was ranked second, dairy C (0.84 Mcal/kg DM, 649 kg, 420 ± 27.5 d, respectively) was ranked third, dairy B (0.87 Mcal/kg DM, 589 kg 399 ± 48.3 d, respectively) was ranked fourth, and dairy A (0.82 Mcal/kg DM, 579 kg, 412 ± 17.0 d, respectively) was ranked fifth. Dairies that fed more NEg in the 7 to 12 mo age range had greater heifer weights at parturition and earlier calving compared to the other dairies in this study. These results show that single-time-point measurements can be used to create a benchmarking system to evaluate and improve heifer-raising programs in nutrients supplied, growth, and reproduction.

PBMC mitochondrial enzyme activity in high and low producing Holstein cows during early lactation

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Mitochondria are central to metabolism and the primary energy producers for all biosynthesis, including lactation. The objective of this study was to determine if high and low producing dairy cows exhibit differences in mitochondrial enzyme activities during early lactation. Fifty six Holstein cows (70±11 DIM) were assigned to one of four groups: primiparous high or low milk production and multiparous high or low milk production. Group assignments for each milk production parameter were made after data were collected by averaging each milk production parameter for primiparous cows and then for multiparous cows and assigning below average cows to the low group and above average cows to the high group. Whole blood samples were collected at one time point within early lactation and processed for crude mitochondrial extracts from peripheral blood mononuclear cells (PBMCs). Mitochondrial function of the extracts was assessed by measuring the activity rates of citrate synthase, complex I, complex IV, and complex V using kits from Abcam (Cambridge, MA). Milk samples were collected 9 times within a week of blood collection and analyzed for major components using a MilkoScan FT2 by FOSS (Mulgrave, Australia). Data were analyzed using the Mixed procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) for high or low groups for each production parameter with cow as the experimental unit of interest and dependent variables parity, and DIM as a covariate. There were no interactions between milk yield level (high or low) and parity. All milk production parameters were different by milk yield level and parity ($P < 0.001$) indicating that the method used to group cow data was successful. Proton efficiency, which represents usage of H ions to produce ATP, and Complex V enzyme activities tended to be higher in multiparous than primiparous cows ($P \leq 0.01$). Complex I activity was lower in low producing cows ($P \leq 0.04$) and not affected by parity ($P > 0.1$). Additionally, genetic indices of fluid merit, milk and protein (CLARIFIDE Nelore, Zoetis) were compared to CS, CI, CIV and CV activities with no correlation found ($P > 0.1$). The genetic index for fat (CLARIFIDE Nelore, Zoetis) was associated with CI activity ($P \leq 0.01$, $R^2 = 0.15$) but was not significant for CS, CIV and CV ($P > 0.1$). These findings suggest that complex I enzyme activity may be a marker of ability to produce milk and support previous findings that mitochondrial density (citrate synthase activity) decreases with age in dairy cattle.

CLAW MEASURES OF JERSEY COWS: AN ANATOMY STUDY

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Hoof trimming is a common practice on dairy operations to treat and prevent lameness. However, more information on claw anatomy of Jersey (JE) cows is needed before trimming guidelines can be developed for this dairy breed. The objective of this study was to describe anatomical structures of cadaver claws from JE cows. Rear claws (n =39) from 8 primiparous and 12 multiparous JE cows with 5 to 277 DIM were collected from 3 commercial dairies in California. The following measurements were performed in intact claws: sole width (SW), heel height (HH), dorsal wall length (DWL), sole thickness (ST) and dorsal wall angle (DWA). Measurements were obtained using a precision goniometer (± 0.3°) and caliper (± 0.01 mm). Each toe was divided sagittaly using a band saw to measure ST at the apical margin of distal phalanx. Statistical analysis were performed with the Proc MEANS and UNIVARIATE of SAS (version 9.4). Results (means, range) are shown in Table 1. The lateral toe was 7% wider than the medial toe in primiparous cows whereas in multiparous cows lateral toe was 1.4% longer and 13.5% wider than the medial toe. Based on industry-wide recommendations the DWL should never be trimmed below 75 mm to ensure ST of at least 7 mm and the DWA should be within 45° to 52°. In our study, primiparous cows had toes with DWL < 75 mm (93.8%), ST < 7 mm (46.8%) and DWA within 45° to 52° (59.3%). Multiparous cows had toes with DWL < 75 mm (32.7%), ST < 7 mm (39%) and DWA within 45° to 52° (58.6 %).There are

thin soles in JE. More studies are needed before trimming guidelines are developed for JE cows.

Table 1: Descriptive statistics of measurements from 39 JE claws, mean (range).

	Parity	SW (mm)	DWL (mm)	HH (mm)	ST (mm)	DWA (°)
Lateral	1	46 (40-52)	69 (61-77)	26 (19-33)	6.8 (3.1-10.4)	50 (41-59)
	>1	48 (44-55)	73 (67-80)	27 (20-35)	7.8 (4.8-11.3)	48 (40-58)
Medial	1	43 (37-51)	69 (62-80)	23 (42-58)	6.8 (4.8-9.6)	50 (42-58)
	>1	43 (39-50)	72 (67-77)	22 (15-30)	7.5 (3.6-11)	48 (39-57)

KEYWORDS:

Jersey, claw anatomy, hoof trimming

Effects of a novel additive on gaseous emissions from liquid stored dairy cattle waste

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Nutrition of dairy cattle has a direct effect on the composition of excreted waste. This waste can have detrimental effects on air and environmental quality if not managed properly. With new legislation from the state of California to reduce greenhouse gas emissions, research into mitigation strategies for dairy waste is of utmost importance. A novel product, SOP Lagoon (SOP SLR, 21052 Busto Arsizio VA, Italy) may be a viable means to alleviate the environmental effects of dairy waste. The product is designed to inhibit the production and release of greenhouse gases, such as methane (CH_4) and nitrous oxide (N_2O), as well as other criteria pollutants, such as ammonia (NH_3). The objective of the present study was to evaluate the efficacy of SOP Lagoon as a tool to reduce gaseous emissions from lagoon stored- liquid dairy cattle waste (lagoon water). Lagoon water was collected from a dairy farm near Davis, CA. The facilities nutritional strategy includes total mixed rations based on corn and cereal silages that reflect dairies across California. A completely randomized design was used with treatments that included: control (CONT, no additive), a low dose (LOW; the addition of 2 g of SOP Lagoon), and a high dose (HIGH; the addition of 4 g of SOP Lagoon). Lagoon water was collected and transported to the UC Davis Feedlot where it was allocated into six 208 L steel drums containing 65 kg of lagoon water each. The additive was added to the lagoon water and thoroughly mixed. Each steel drum was covered with an OdoFlux flux chamber (Odotech Inc. Montreal, Quebec, Canada) and connected to a mobile air emissions laboratory. Emissions from each drum were continuously measured over a one week period and measurements included carbon dioxide (CO_2), CH_4, N_2O, and NH_3. Data, including ANOVA and Least Squares Means, were analyzed using the lsmeans statistical package in R. Emissions of CO_2 and NH_3 were significantly decreased with increasing concentrations of SOP Lagoon ($P < 0.001$). Methane and N_2O were significantly decreased with the high dose of SOP Lagoon ($P < 0.001$ and $P < 0.005$, respectively). The SOP Lagoon additive, especially for the high dose, proved to be a viable option for reduction of lagoon emissions. Given that the present study sourced lagoon water from a dairy representative of those across California, it is likely that SOP Lagoon will prove to be a viable additive for other lagoons from California dairies with similar nutrition strategies. Future research will include investigating the efficacy of SOP Lagoon on varying sources of dairy lagoon water as well as on large scale dairy lagoon systems.

Manure Flushing versus Scraping in Dairy Freestall Lanes Reduces Gaseous Emissions

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ABSTRACT

The objective of the present study was to mitigate ammonia (NH_3), methane (CH_4), and air pollutants from lactating dairy cows using different freestall waste removal techniques. Four cows per treatment (n = 4) were housed in an environmental chamber. Waste was removed by either flushing or scraping at two different frequencies. The four treatments arranged as a latin square were: 1) flushing 3 times a day (F3), 2) flushing 6 times a day (F6), 3) scraping 3 times a day (S3), and 4) scraping 6 times a day (S6). Gas concentrations were collected at the inlet and outlet air ducts of the environmental chamber and were transported to a mobile agricultural air quality lab. Gas emission rates were calculated and then analyzed using the lmerTest package in R. Ammonia and hydrogen sulfide (H_2S) emissions decreased ($P < 0.001$ and $P < 0.05$) in the flushing vs. scraping treatments, respectively. Ethanol (EtOH) emissions increased ($P < 0.001$) when the frequency of either scraping or flushing was increased from 3 to 6 times, but remained similar between scraping and flushing treatments. Methane emissions for the F3 were lower than all other treatments ($P < 0.001$). Removal of dairy freestall manure by flushing versus scraping has the potential to decrease gaseous emissions such as NH_3 and CH_4.

Keywords: dairy cow, ammonia emissions, freestall barn, scraping, flushing, methane

Effects of postpartum oral calcium supplementation on productive and reproductive outcomes in Jersey cows

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The effects of postpartum prophylactic oral Ca supplementation on milk yield, milk composition and first service conception were evaluated on 1,095 multiparous Jersey and Jersey × Holstein crossbreed cows from 2 commercial herds. After calving, cows were systematically assigned to control (no oral Ca supplementation; n = 553) or oral Ca supplementation (CaOS; 50 to 60 g of Ca as boluses; QuadricalMINI, Bio-Vet Inc., Barneveld, WI; n = 542) at 0 and 1 days in milk (DIM). Monthly milk yield and composition data (1st, 2nd and 3rd test) from Dairy Herd Improvement Association (DHIA) were evaluated using linear regression with the MIXED procedure. First service conception was analyzed by logistic regression with the GLIMMIX procedure of SAS. Herd was included in the models as a random effect. Variables considered for inclusion in the final model were: lactation number, previous lactation days open, previous lactation 305-d milk yield (Pr305ME) and length, gestation length, dry and close-up periods length, body condition and locomotion scores at calving, calving easiness, calf gender, DIM and month at 1st DHIA test, DIM and month at 1st AI and breeding code (timed AI or heat breeding). For the 1st DHIA test, CaOS cows with a dry period length greater than the 75th percentile of the herd (>Q_3; >58 d Herd 1 and >90 d Herd 2) produced 2.4 kg of milk more than control cows. Also, at 1st DHIA test CaOS cows ranked at >105% of the herd mean for Pr305ME had lower milk fat% compared to control cows (4.6 vs. 4.9%, respectively). Based on the first 3 DHIA tests, CaOS with a previous lactation or dry period length >Q_3 of the herd (>334 d Herd 1 and >355 d Herd 2) produced 1.5 and 1.8 kg of milk more than control cows. Conception at 1st service was 9% lower for CaOS with a previous gestation length longer than the herd median (>279 d Herd 1, >281 d Herd 2). Our results suggest that the response to postpartum oral Ca supplementation may vary according to different peripartum factors.

Keywords: Calcium, hypocalcemia, dairy cow

Response of Holstein dairy cows to a sodium propionate supplement fed postpartum

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Abstract

Subclinical (SCK) and clinical (CK) ketosis is a metabolic disease common in dairy cattle and can decrease milk production, reproductive efficiency, and increase risk of being culled from the herd. Traditionally, cows have been supplemented with glucogenic precursors either by drenching or inclusion in the TMR to reduce the negative impacts of this disease on animal health and milk yield. Providing glucogenic precursors can decrease ketone formation and increase blood glucose levels. The objective of this experiment was to examine the incidence of SCK and CK, levels of ketones and glucose in blood, and milk yield in Holstein dairy cattle fed a molasses based sodium propionate supplement (Innovative Liquids, LLC., Sacramento, CA) for the first 14 DIM. On a commercial dairy in California, 426 cows and 208 heifers were systematically enrolled to either control (C) or glucose precursor treated (GP) group in a switchback design. A total of 226 cows and 102 heifers were enrolled in C with a subset of 74 cows and 39 heifers bled, and 200 cows and 106 heifers were enrolled in GP with a subset of 81cows and 36 heifers bled. Blood glucose and beta-hydroxybutyric acid (BHBA) concentrations were measured on 3, 7, and 14 DIM using NovaMax® meters (Nova Diabetes Care, Inc., Billerica, MA). Ketosis was defined as BHBA levels of 1.0-1.4 mmol/L for SCK or BHBA levels of >1.4 mmol/L for CK. Glucose concentrations were defined as low (≤ 40 mg/dL) or adequate (> 40 mg/dL). Data were analyzed using the Mixed procedure of SAS (v. 9.4, SAS Institute 2015). Average blood BHBA and glucose concentrations did not differ between treatments for primiparous or multiparous cows (C 0.53, GP 0.55 mmol/L BHBA, $P = 0.5$; C 44, GP 43 mg/dL glucose, $P = 0.6$), but there were differences among parities ($P < 0.01$). The incidence of SCK and CK was low during this study (C $n_{SCK}= 9$, $n_{CK}= 6$; GP $n_{SCK}= 7$, $n_{CK}= 9$) therefore there was little improvement in the incidence of CK and SCK with supplementation. Concentration of blood glucose was inversely related to BHBA ($P < 0.01$). Treatment GP did not impact milk yield compared to C for primiparous or multiparous cows during the first 21 DIM (29.5 kg milk /d, GP 30.0 kg milk/d; $P = 0.5$).