SEROTONIN AND THE PHYSIOLOGY OF CALCIUM HOMEOSTASIS DURING THE TRANSITION PERIOD

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INTRODUCTION

Adequate circulating Ca concentrations throughout the transition period are necessary for productive lactation, but large quantities of Ca are lost from maternal Ca pools into milk and colostrum. A rapid, large drop in maternal blood Ca causes 5-10% of cows to be afflicted with clinical hypocalcemia (CH) and an additional 50% to suffer from subclinical hypocalcemia (SCH). SCH and CH are significant risk factors of early lactation culling/premature removal from the herd (DeGaris and Lean, 2008; Reinhardt et al., 2011; Roberts et al., 2012). Furthermore, SCH increases risks of developing ketosis; displaced abomasum; and metritis; SCH depresses immune function; prolongs the interval until pregnancy is achieved; decreases pregnancy rate; and reduces overall productivity (DeGaris and Lean, 2008; Chapinal et al., 2011; Chapinal et al., 2012; Figure 1).

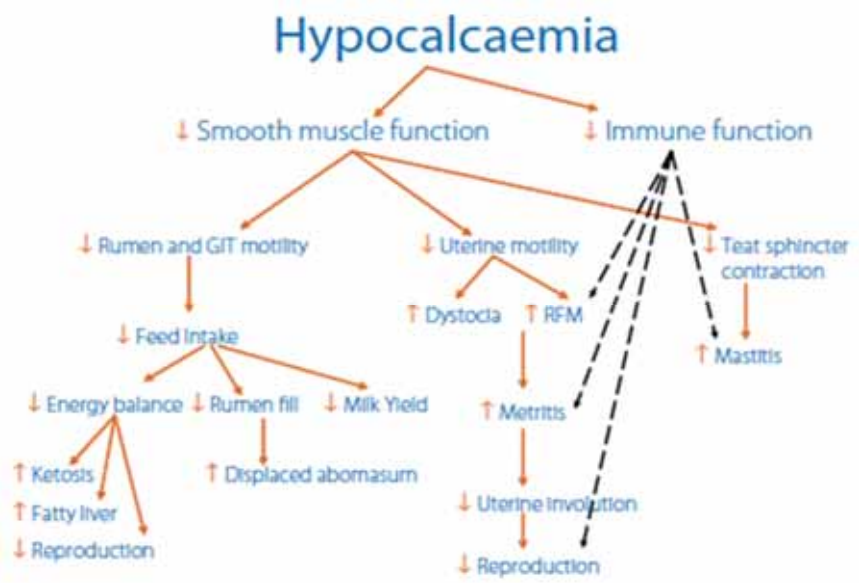

Figure 1. Hypocalcemia is a 'gateway' disease that leads to increased risks of other periparturient diseases. (DeGaris and Lean, 2008).

The transition period (3 weeks pre-calving through 3 weeks post-calving) is an extremely critical time period in the life of the dairy cow. At this time the animals are highly susceptible to a variety of disorders that negatively impact the animal's health, and hence their overall production. Of particular concern during this time is the inability of the animal to maintain adequate blood calcium concentrations due to increased

demand for calcium at the onset of lactation by the mammary gland. This increase in calcium results in decreased circulating calcium levels and can lead to the development of periparturient hypocalcemia (milk fever). Parturient paresis is one of the most common metabolic diseases of dairy cattle, with Jersey cows being more susceptible than Holsteins (Oetzel, 1988; NRC, 2001). Hypocalcemia is associated with numerous other health disorders during this time period (Oetzel, 1988). Due to inadequate blood calcium levels at the onset of lactation, animals experience a range of clinical symptoms, depending on the extent of the decreased calcium levels (Adams et al., 1996). CH is clinically defined as a total blood calcium level of less than 1.4 mmol/L, and subclinical hypocalcemia defined as total blood calcium of 1.4-2.0 mmol/L (DeGaris and Lean, 2008). Approximately 25% of heifers and 50% of older cows will succumb to SCH, and between 5-10% of animals will develop clinical hypocalcemia in the United States (Goff, 2008). Cattle that are afflicted with periparturient hypocalcemia exhibit a 14% decrease in milk production and are more susceptible to other transition disorders such as ketosis, retained placenta, displaced abomasum and muscle weakness, with the average cost of incidence of milk fever being $334/animal (Oetzel, 1988). However, should an animal succumb to additional issues due to suffering from milk fever, costs increase substantially. Subclinical hypocalcemia affects about 50% of second lactation and greater dairy cattle, and costs approximately $125/animal to treat. Overall, prevalence of milk fever and subclinical hypocalcemia are more common in Jersey cattle, likely due to their higher milk production per unit body weight (Oetzel, 1988). Typically, in order to compensate for decreased blood calcium, increased intestinal calcium absorption and/or calcium resorption from the bone must occur, however calcium resorption from the bone is the primary mode used during this time frame. Dairy cattle, in particular, exhibit a delay in calcium resorption from bone.

Parathyroid hormone related-protein (PTHrP) synthesized within the mammary gland has been described as the molecule responsible for mobilization of calcium from bone that occurs at the onset of lactation in mammals (Wysolmerski, 2010). Recently, we have demonstrated that mammary serotonin (5-hydroxytryptamine) regulates induction of PTHrP (Hernandez et al., 2012). Manipulation of serotonin induced PTHrP synthesis near the end of the pregnancy period could be critical in preventing the onset of hypocalcemia during the early lactation period. This is important because the early symptoms of milk fever often go undetected because they are short-lived. Data indicates that prevention of milk fever, rather than treatment, would save the dairy industry approximately $140 million per year (http://www.animate-dairy.com/dcad-nutrition/index.html).

The onset of milk production drains Ca pools in dairy cows.

Colostrum and milk synthesis rapidly depletes Ca from the maternal circulation and therefore Ca must be mobilized from maternal bone to maintain adequate circulating concentrations. Circulating Ca concentrations are tightly regulated and controlled by several hormones including: Vitamin D, calcitonin, parathyroid hormone (PTH) and parathyroid hormone related-protein (PTHrP; Figure 2). Liberation of Ca from bone stores can only be triggered when circulating Ca concentrations dip below

the animal's minimal threshold for Ca, via a classic negative feedback loop. Dietary Ca is insufficient to maintain maternal Ca homeostasis during milk synthesis. This is demonstrated by the fact that a dairy cow will lose 9-13% of her bone mass during the first 30 days of lactation. Bone loss during lactation is an evolutionary strategy of mammals used to support the cow as well as the mammary glands' demand for Ca for milk synthesis (Wysolmerski et al., 1995; Wysolmerksi, 2010; Goff, 2014).

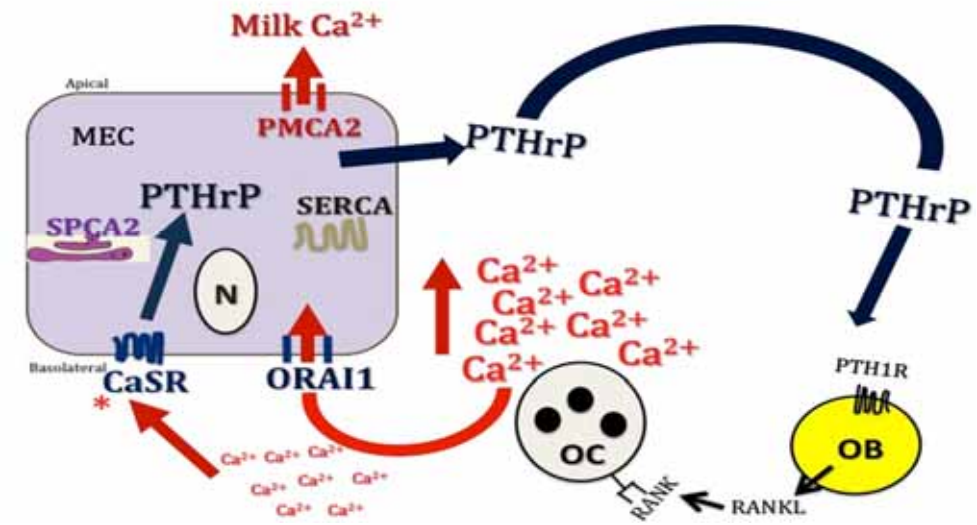

Figure 2. Maternal Ca homeostasis is regulated by the mammary gland-bone axis. During lactation, the Ca sensing receptor (CaSR) on the basolateral side of the mammary epithelial cell (MEC) during lactation detects low blood Ca concentrations due to the increased transport of Ca into the MEC by Ca release-activated Ca channel protein 1 (ORAI1). Ca is either secreted into the milk through the apical plasma membrane Ca ATPase 2 (PMCA2) or sequestered in the Golgi apparatus by secretory pathways Ca ATPase 2 (SPCA2) or endoplasmic reticulum by the sarco(endo)plasmic reticulum Ca ATPase (SERCA). Detection of systemic decreased Ca by CaSR results in parathyroid hormone related-protein (PTHrP) production. PTHrP is secreted into the circulation and will bind its receptor PTH1R on the osteoblast (OB) cell in the bone increasing production of receptor activated nuclear factor kappa B (RANKL), which binds its receptor (RANK) on the osteoclast (OC) cell in the bone tissue, activating Ca liberation from bone.

The mammary gland functions as an "accessory parathyroid gland" during lactation.

The mammary gland produces the hormone PTHrP, which binds to receptors on bone to drive bone resorption and liberate Ca into the systemic circulation (Wysolmerski et al., 1995; Wysolmerski, 2010). PTHrP is only produced by the mammary gland during lactation. The Ca sensing receptor (CaSR) present in the mammary epithelium plays a crucial role in controlling maternal Ca concentrations during lactation. CaSR is highly expressed in the mammary gland during lactation, compared to virgin and pregnant time periods (VanHouten et al., 2003). Mammary PTHrP production is

responsible for the mobilization of Ca from the bone during lactation, rather than the typical endocrine regulator of bone, PTH (Wysolmerski et al., 1995; VanHouten, 2005; Wysolmerski, 2010; Wysolmerski, 2012). Our lab made a novel discovery that serotonin is essential for the liberation of Ca from bone during lactation to sustain maternal Ca homeostasis in rodent models. This occurs through induction of PTHrP by the mammary gland (Hernandez et al., 2012; Laporta et al., 2014a, 2014b). Furthermore, we demonstrated that serotonin is critical for the expression of CaSR. This finding indicates that serotonin is crucial for mammary gland sensing of systemic Ca concentrations.

Mammary gland coordination with the skeletal system liberates Ca during lactation.

The skeletal system maintains its structural and functional roles via communication between two cell types, osteoblasts (OB), which are responsible for bone formation, and osteoclasts (OC), which are responsible for bone resorption, and thus Ca mobilization. PTH regulates this mechanism under non-lactating conditions. Research in humans and rodents has suggested the PTH action on bone is uncoupled during lactation (Wysolmerski, 2010; VanHouten and Wysolmerski, 2013). PTHrP signals through the same G-protein coupled receptor (PTH1R) that PTH does on the OB to decrease OB cell proliferation and up-regulate genes responsible for OC differentiation during lactation. In rodents and humans, the mammary gland is the main source of PTHrP found in the circulation (Thiede, 1994; Wysolmerski et al., 1995; Wysolmerski, 2010; VanHouten and Wysolmerski, 2013). Mammary-derived PTHrP, not PTH, is the critical hormone responsible for induction of bone Ca mobilization during lactation (Wysolmerski et al., 1995).

Serotonin regulates mammary gland physiology during lactation.

Serotonin is synthesized in numerous tissues throughout the body and brain and is incapable of crossing the blood-brain barrier. Serotonin is synthesized from the amino acid L-tryptophan in a two-step process. The first step is production of 5-hydroxytryptophan (5-HTP) via the rate-limiting enzyme, tryptophan hydroxylase (TPH). The second step is the conversion of 5-HTP to serotonin by aromatic amino acid decarboxylase (Wang et al., 2002). TPH1 is the rate-limiting enzyme for serotonin production in non-neuronal tissues, while TPH2 is used to produce serotonin in neuronal tissues. Our laboratory and others have shown that serotonin regulates milk protein gene expression, as well as the disassembly of tight junctions that occurs during the involution process (Matsuda et al., 2004; Stull et al., 2007; Hernandez et al., 2008; Pai and Horseman, 2008). Furthermore, we have shown that the mammary gland expresses a unique pattern of serotonin receptors in rodent, bovine, and human mammary epithelium (Hernandez et al., 2009; Pai et al., 2009). The epithelial component of the bovine mammary gland expresses at least five serotonin receptor isoforms (5-HT1B, 2A, 2B, 4 and 7; Hernandez et al., 2009). Our lab determined that the 5-HT2B receptor subtype modulates serotonin's regulation of PTHrP production within the mammary gland in a rodent model (Hernandez et al., 2012; Laporta et al., 2013a; Laporta et al., 2014a,b). We also confirmed that circulating serotonin

concentrations post-partum are positively correlated with circulating Ca concentrations on the first day of lactation in dairy cows (Laporta et al., 2013b). Furthermore, we showed that serotonin activates expression of various Ca pumps and transporters in the mammary gland to stimulate transport of Ca from blood to milk during mouse lactation (Laporta et al., 2014a). Ca transport into the mammary gland is thought to occur through the Ca^{2+} influx channel (ORAI1) and subsequent pumping into the milk by the apical plasma membrane Ca^{2+} ATPase (PMCA2; Cross et al., 2014).

Current research in humans and rodents implicates PTHrP in the regulation of maternal Ca homeostasis during lactation. Our laboratory has demonstrated the necessity of serotonin for regulation of Ca transport in the mammary gland during lactation. Furthermore, we have demonstrated that serotonin is necessary for the production of mammary PTHrP during lactation. Mammary PTHrP production is critical to the mobilization of Ca from bone tissue to support lactation. Therefore, delineation of the mechanisms regulating the mammary gland serotonin-PTHrP axis in the dairy cow could lead to development of novel therapeutic interventions to reduce the incidence of SCH and CH in the U.S. dairy cow population.

The following model for the regulation of Ca mobilization from bone by the mammary gland during the transition period has been proposed by our laboratory.

New ideas about calcium and serotonin

Our laboratory recently demonstrated that serotonin is necessary for mammary PTHrP synthesis in lactating rodents and mammary epithelial cells grown in lactogenic culture (Hernandez et al., 2012; Laporta et al., 2013a; Horseman and Hernandez, 2014). We also demonstrated that supplementation of a serotonin precursor, 5-HTP, to rats during the transition from pregnancy to lactation increased the post-parturition circulating serotonin, PTHrP, and Ca concentrations, and also increased total Ca content in milk (Laporta et al., 2013a). Furthermore, we observed increased OC numbers in the femurs collected from rats supplemented with 5-HTP, indicating this response was due to bone Ca mobilization. These findings led us to perform several experiments in dairy cows in order to evaluate the utility of these findings in rodents to dairy cows.

In order to evaluate the utility of the mammary serotonin-PTHrP axis in Holstein dairy cows, we performed several observational studies. We have observed that serotonin concentrations are dynamic over the course of a given lactation, and decrease around the time of calving (d 0-2 lactation), rebounding by approximately ten days into lactation (Moore et al., 2015). The overall average serotonin concentration in dairy cows is approximately 1700 ng/ml. However, it should be noted, that the concentrations fluctuate dependent on stage of lactation. These results combined with our rodent data support our hypothesis that serotonin and PTHrP are critical players in the regulation of Ca homeostasis in Holstein dairy cows.

Intravenous (IV) infusion of 5-HTP in late lactation, non-pregnant, multiparous Holstein dairy cows increases circulating serotonin concentrations and alters Ca dynamics.

In order to demonstrate the role of serotonin in Ca homeostasis in dairy cows, we performed a preliminary experiment in which we infused 5-HTP IV for one hour daily for four days in late-lactation dairy cows at varying doses (0, 0.5, 1.0, or 1.5 mg/kg) to determine an optimum dose of 5-HTP necessary to produce significant changes in Ca. All three doses of 5-HTP significantly increased circulating serotonin concentrations (Laporta et al., 2015) to a similar extent in the two hours after dosing, with concentrations returning to baseline concentrations observed in the saline controls by two hours after infusion. In addition to serotonin concentrations, we measured circulating total Ca concentrations following the same time course post infusion. While initially counter-intuitive, our data demonstrated that total Ca concentrations decreased in immediate response to 5-HTP treatments (Laporta et al., 2015). In order to determine where the circulating Ca was going after 5-HTP infusion, we measured urine Ca concentrations prior to the start of infusion and two hours after the end of the infusion. Our results indicate that there was a decrease in urine Ca output with higher doses of 5-HTP treatment. This suggests that Ca is not being lost into the urine. Therefore, we measured total Ca concentrations in the milk during the infusion periods and observed that the highest dose of 5-HTP increased total milk Ca concentrations. This supports the hypothesis that serotonin causes transient hypocalcemia by increased Ca transport into the mammary gland and subsequently into milk. Increased Ca transport into the mammary gland during lactation is critical for the stimulation of bone Ca mobilization by PTHrP because transient systemic hypocalcemia.

Use of 5-HTP before calving to prevent hypocalcemia, is it possible and are breed differences present?

In order to determine if elevating serotonin concentrations in pre-fresh dairy cows would result in increased post-calving Ca concentrations, we treated multiparous Holstein cows with daily IV infusions of 1.0 mg/kg of 5-HTP beginning 7 d before the estimated calving date until calving. Our data demonstrates that IV infusions of 5-HTP pre-calving increased post-calving total Ca concentrations compared to saline treated controls (Weaver et al., 2016). Furthermore, we measured deoxypyridinoline (DPD), a marker of OC activity and therefore bone resorption, in the urine. These data demonstrate that cows receiving 5-HTP before calving have increased bone resorption on at calving. These results support demonstrate that 5-HTP treatment pre-calving can potentially improve post-calving Ca concentrations by increasing bone Ca resorption. Furthermore, we also tested the same hypothesis in multiparous Jersey cows. Interestingly, Jersey cows responded to 5-HTP differently than the Holstein cows. Jersey cows had significantly decreased calcium concentrations prior to parturition, and then began to increase calcium concentrations at calving. This was in contrast to the control Jersey cows who did not reach their total calcium concentration nadir until 1 day post-partum (Weaver et al., 2016). Furthermore, Jersey cows treated with 5-HTP had higher concentrations of calcium in their milk compared to the saline treated cows, which was opposite to what was seen in the Holstein cows. These data indicate that

serotonin positively impacts calcium homeostasis in both Holstein and Jersey cows, but the mechanisms underlying this appear to be different and should be further investigated.

Interrelationship of a negative DCAD and serotonin.

Given that 5-HTP treatment pre- calving was capable of increasing post-calving Ca concentrations, we wanted to determine if a common preventative treatment for SCH and CH, negative DCAD, controls Ca homeostasis via a serotonergic mechanism. To this end, we fed Holstein dairy cows a positive DCAD (+130 mEq/kg) diet for 21 days prior to calving or a negative DCAD (-130 mEq/kg) diet for 21 days prior to calving. Upon analysis of circulating serotonin concentrations from 9 days before calving through 6 days post-calving, we determined that a negative DCAD diet increased circulating serotonin concentrations pre-calving ($P=0.05$). This suggests the resulting improvement in post-calving Ca concentrations in the cows receiving a negative DCAD diet pre-calving could be due to serotonin's control of Ca homeostasis. We have preliminary results from a study testing the hypothesis that 5-HTP and negative DCAD diets have a synergistic effect on post-calving calcium concentrations. Our preliminary results indicate that the combination of 5-HTP treatment with a negative DCAD diet has the largest increase in post-calving ionized calcium concentrations.

CONCLUSION

In conclusion, we have demonstrated that serotonin plays a critical role in regulation of maternal Ca transport, maternal Ca homeostasis and mammary PTHrP production in the rodent. Additionally, our data demonstrate that mammary gland Ca transporter expression and induction of PTHrP production by the mammary gland during lactation are key regulators of maternal Ca homeostasis in rodent models. Furthermore, our rodent models indicate that the mammary gland is a significant source of serotonin during lactation. Our observational data in Holstein cows suggests that serotonin, PTHrP, and Ca are interrelated during the early days post-partum. Furthermore, our initial experiment exploring the effects of 5-HTP on maternal Ca homeostasis in late-lactation dairy cows supports the hypothesis that serotonin induces transient hypocalcemia by shuttling Ca into the mammary gland in order to stimulate mammary production of PTHrP, and the elevated PTHrP is critical to stimulate bone Ca resorption. Treating pre-partum Holstein dairy cows with 5-HTP resulted in improvement of post-partum Ca concentrations. It also appears that Jersey cows respond differently to 5-HTP treatment and further research should be directed to understanding their physiology as compared to Holstein cows. Using a current therapeutic intervention for prevention of SCH and CH in the dairy industry, feeding of a negative DCAD diet pre-partum, resulted in the increase of circulating serotonin concentrations. Our preliminary data examining the interaction of 5-HTP and negative DCAD suggests that two treatments together have a synergistic effect on increasing post-calving ionized calcium concentrations.

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INTERACTIONS BETWEEN THE FEED AND FEEDING ENVIRONMENT

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INTRODUCTION: IMPORTANCE OF FEEDING ENVIRONMENT

The dairy industry continues to sharpen its focus on efficiency of feed use. Providing an optimal feeding environment enhances the cow's response to her diet, in particular ensuring adequate feed availability. For example, herds that routinely feed for refusals and practice consistent feed push-up average about 1.4 to 4.1 kg/d more milk than herds that do not (Bach et al., 2008). Not many management factors elicit this magnitude of production response.

When cattle are grouped, competition at the feed bunk is inevitable. Even with unlimited access to feed, cows will interact in ways that give some an advantage over others (Olofsson, 1999). Consequently, the management goal is not to eliminate competition at the feed bunk, but rather to control it. Key factors that must be optimized to encourage aggressive feeding activity and optimal intake of a well-formulated diet include:

- feed availability and accessibility,
- stocking density that results in a level of competition that doesn't hinder feed access, and
- no restrictions on resting or ruminating activity.

Foremost among the factors that influence feeding behavior and feed intake is stocking density. Overstocking is a too common occurrence in the US dairy industry. A USDA-NAHMS survey of free-stall dairy farms reported that 58% of farms provided less than 0.60 m/cow of bunk space (i.e., current dairy industry recommendations for feeding space; NFACC, 2009) and 43% provided less than one stall per cow (USDA, 2010). In a survey of the northeastern US, feed bunk stocking density averaged 142% with a range of 58 to 228% (von Keyserlingk et al., 2012). The continued prevalence of overstocking reflects its association with maximizing profit per stall (De Vries et al., 2016).

This paper shares recent research conducted at Miner Institute on the influence of stocking density and its interactions with key components of the diet and feeding environment: physically effective and undigested fiber and feed availability. In the future, we intend to explore the interaction of stocking density with feed delivery frequency and push-up strategy. For all of the experiments, the primary focus is on ruminal pH responses and how the interaction of stocking density and feeding environment affect it.

OVERSTOCKING AND ITS EFFECT ON COW RESPONSES

Current economic analysis suggests that some degree of overstocking may be optimal if the focus is solely on profitability. De Vries et al. (2016) used published data to model the relationships among stocking density (stalls and feed bunk), lying time, and profit ($/stall/year). This economic analysis reported that profit per stall actually was maximized around 120% stocking density for prevailing costs of production and milk price in the US. The profitability of overstocking was a function of revenue gained by increasing production per stall, the cost of increasing or decreasing production per cow, variable costs (i.e., costs that vary with changes in milk production), and milk price (De Vries et al., 2016). However, overstocking reduces the cow's ability to practice natural behaviors (Wechsler, 2007) which is a primary factor related to cow well-being.

Overstocking interferes with the cow's ability to practice normal feeding and resting behaviors, which comprise approximately 70% of the cow's day (Grant and Albright, 2001). Cows place priority on resting when forced to choose among resting, eating, and other behaviors (Metz, 1985; Munksgaard et al., 2005) which suggests that overstocking may limit their ability to meet their daily time budget, defined as 3 to 5 h/d of feeding, 10 to 14 h/d of lying, and 7 to 10 h/d of rumination (Grant and Albright, 2001; Gomez and Cook, 2010). Bach et al. (2008) were able to isolate the effect of management environment on cow performance using 47 dairy farms that were members of the same cooperative and fed the same TMR. Despite similar genetics and the same diet, average herd milk production ranged from 20.6 to 33.8 kg/d. The housing environment explained 56% of this variation and free stall stocking density accounted for 32% of the variation among farms by itself.

Higher stocking densities reduce feeding time and increase aggression at the feed bunk (Huzzey et al., 2006), may reduce rumination (Batchelder, 2000), decrease rumination while recumbent (Krawczel et al., 2012a), and reduce lying time (Fregonesi et al., 2007; Hill et al., 2009; Krawczel et al., 2012b). Overstocking also increases rate of feed consumption and meal size (Collings et al., 2011).

Taken together, it is reasonable to predict that higher stocking density will negatively affect ruminal pH, although this has not been measured to-date.

STOCKING DENSITY AS A SUB-CLINICAL STRESSOR

The concept of subclinical stressors suggests that the summation of two stressors, such as housing and feeding management, will be greater than either in isolation. A subclinical stressor depletes the animal's biological resources without generating a detectable change in function, which leaves the animal without the resources to respond to subsequent stressors (Moberg, 2000). Therefore, subdominant animals may exhibit changes in behaviors that do not always result in clinical or visible outcomes such as lower milk production or altered health status. However, the sub-clinical stressor of stocking density would diminish her effectiveness against additional stressors, placing her in a state of distress. Additional stressors are likely to occur due

to constant changes in feeding and cow management. Understanding the effects of stocking density with additional management stressors such as low-fiber diets or feed restriction are the next steps in alleviating stress and improving the well-being and long-term productive efficiency of lactating dairy cows housed in free-stall barns.

EXPERIMENT 1:
OVERSTOCKING AND PHYSICALLY EFFECTIVE FIBER

In our first study, forty-eight multiparous and 20 primiparous Holstein cows were assigned to 1 of 4 pens (n = 17 cows per pen). Pens were assigned to treatments in a 4 x 4 Latin square with 14-d periods using a 2 x 2 factorial arrangement. Two stocking densities (STKD; 100 or 142%) and 2 diets (straw, S and no straw, NS; Table 1) resulted in 4 treatments (100NS, 100S, 142NS, and 142S). Stocking density was achieved through denial of access to both headlocks and free-stalls (100%, 17 free-stalls and headlocks per pen; 142%, 12 free-stalls and headlocks per pen). Pen served as the experimental unit.

Table 1. Ingredient composition and analyzed chemical composition (dry matter basis) of TMR samples for NS (No Straw) and S (Straw) experimental diets.

	NS	S	SEM[1]
Ingredient, % of DM			
Conventional corn silage	39.72	39.73	
Haycrop silage	6.91	2.33	
Wheat straw, chopped	...	3.45	
Citrus pulp, dry	4.82	4.82	
Whole cottonseed, linted	3.45	3.45	
Soybean meal, 47.5% solvent	...	1.12	
Molasses	3.20	3.20	
Concentrate mix	41.89	41.88	
Chemical composition			
CP, % of DM	15.0	15.1	0.3
NDF, % of DM	30.8	30.1	0.4
Acid detergent lignin, % of DM	3.8	3.8	0.1
Starch, % of DM	25.0	25.5	0.5
Sugar, % of DM	7.4	8.1	0.4
Ether extract, % of DM	5.9	5.7	0.1
7-h starch digestibility, % of starch	73.3	74.3	0.9
Physically effective NDF $_{1.18\ mm,}$ % of DM[2]	23.9	25.9	0.7
30-h uNDFom, % of DM[3]	13.1	14.9	0.3
120-h uNDFom, % of DM[3]	9.0	10.2	0.2
240-h uNDFom, % of DM[3]	8.5	9.7	0.2

[1]Standard error of the means.
[2]peNDF determined with method described by Mertens (2002).
[3]undigested NDF determined with method described by Tilley and Terry (1963) with modifications (Goering and Van Soest, 1970).

Diets were similar except that the S diet had a portion of haycrop silage replaced with chopped wheat straw and soybean meal. Each diet was formulated to meet both ME and MP requirements. The TMR was mixed and delivered once daily at approximately 0600 h and pushed up approximately 6 times daily.

The diets were designed to differ meaningfully in physically effective NDF (peNDF) and undigested NDF (uNDF) measured at 30, 120, and 240 h of in vitro fermentation. Otherwise, the two diets were similar in analyzed chemical composition.

Twelve multiparous and 4 primiparous ruminally fistulated cows were used to form 4 focal groups for ruminal fermentation data. Each focal group was balanced for DIM, milk yield, and parity. Ruminal pH was measured using an indwelling ruminal pH measurement system (Penner et al., 2006; LRCpH; Dascor, Escondido, CA) at 1-min intervals for 72 h on days 12, 13, and 14 of each period. Daily ruminal pH measurements were averaged over 10-min intervals. Measurements were then averaged across days and among cows into a pen average for each period

Ruminal pH results are presented in Table 2. As expected, increasing the peNDF content of the diet reduced the time spent below pH 5.8 (P = 0.01) as well as decreasing the severity of sub-acute ruminal acidosis (SARA) as observed through a reduction in area under the curve below pH 5.8 (P = 0.03). Higher stocking density increased time spent below pH 5.8 (P < 0.01) and tended to increase the severity of SARA (P = 0.06).

Table 2. Ruminal pH responses to diets containing straw (S) or no straw (NS) fed at 100 or 142% stocking density (STKD).

	100%		142%			*P*-value		
Variable	NS	S	NS	S	SEM	STKD	Diet	STKD x Diet
Mean pH	6.17	6.13	6.09	6.10	0.03	0.07	0.62	0.39
Minimum pH	5.70	5.67	5.62	5.59	0.05	0.11	0.53	0.95
Maximum pH	6.63	6.58	6.56	6.53	0.04	0.07	0.22	0.68
Time pH < 5.8, h/d	2.29	1.90	4.12	2.77	0.41	<0.01	0.01	0.10
AUC < 5.8 pH, pH x unit[1]	0.38	0.19	0.58	0.34	0.10	0.06	0.03	0.75

[1]Area under the curve.

Furthermore, there was a trend for an interaction between stocking density and diet, indicating greater SARA when cows were housed at higher stocking density and fed the lower fiber diet. Importantly, greater stocking density had a larger effect on ruminal pH than changes to the diet, with a 1.4-h difference between 100 and 142% stocking density but only a 0.9-h difference between diets. Reductions in SARA through the addition of straw was observed at both stocking densities (0.4-h difference at 100% and 1.4-h difference at 142%), although there seemed to be greater benefit of boosting dietary peNDF or uNDF at the higher stocking density.

Cows were milked 3 times daily and milk yields were recorded electronically on d 8 to 14 of each period. Milk samples were collected across 6 consecutive milkings for each cow on d 13 and 14 of each period and analyzed for composition. Ingestive, rumination, and lying behavior as well as the location (feed bunk, stall, alley, standing or lying) of these performed behaviors were assessed on all cows using 72-h direct observation at 10-min intervals (Mitlöhner et al., 2001) on d 8, 9, and 10 of each period.

Table 3. Behavioral responses for cows fed diets containing straw (S) or no straw (NS) at 100 or 142% stocking density (STKD).

	100%		142%			*P*-value		
	NS	S	NS	S	SEM	STKD	Diet	STKD x Diet
Eating time, min/d	233	237	242	240	4	0.13	0.76	0.48
Eating time/kg NDF, min	31.0	28.7	34.1	30.0	1.3	0.04	0.01	0.35
Eating time/kg peNDF, min	37.8	35.1	41.3	36.4	1.7	0.11	0.03	0.44
Eating, bouts/d	6.8	6.7	7.0	6.9	0.1	0.60	0.11	0.64
Meal length, min/meal	34.8	36.4	35.6	37.0	0.9	0.43	0.11	0.90
Eating latency for fresh feed, min	20	28	39	40	4	0.02	0.35	0.46
Length of first meal, min	39	43	41	44	2	0.23	0.02	0.66
Rumination time, min/d	498	491	489	496	9.0	0.72	0.96	0.19
Rumination time/kg NDF, min	65.8	59.4	68.0	61.8	2.2	0.21	<0.01	0.95
Rumination time/kg peNDF, min	80.3	72.6	82.4	75.0	3.1	0.39	0.02	0.95
Rumination within stall, % of total	86.2	86.0	80.5	81.1	<0.1	<0.01	0.96	0.60
Lying time, min/d	832	827	779	797	11	<0.01	0.56	0.31
Lying time within stall, % of use	89.7	89.9	91.7	92.8	<0.01	0.01	0.39	0.50
Time spent in alley, min/d	121	125	192	181	9	<0.01	0.65	0.37

Eating time (238 min/d, SEM=4) and rumination time (493 min/d, SEM=9) did not differ among treatments ($P > 0.10$). However, rumination within a free-stall as a percent of total rumination decreased at higher stocking density. As resting and rumination are significant contributors to buffer production (Maekawa et al., 2002b), it is possible that this shift in the location of rumination may affect the volume or rate of buffer production, partially explaining the increased risk of SARA at higher stocking densities. Ruminal pH

differences between diets are likely explained by increased buffer volume produced during eating and rumination for the straw diets as evidenced by Maekawa et al. (2002a) where increases in the fiber-to-concentrate ratio resulted in increased total daily saliva production.

Higher stocking density increased the latency to consume fresh feed – i.e., it took cows longer to approach the bunk and initiate eating with higher stocking density. Additionally, higher stocking density reduced lying time, but boosted the time spent lying while in a stall indicating greater stall-use efficiency. Overall, time spent standing in alleys increased markedly with overstocking.

There were no differences in DM intake among treatments, although as expected the straw diet increased both peNDF and $uNDF_{om}240$ intake. Changes in milk production were small, which would be expected given the short periods (14-d) used in this study.

Table 4. Short term (14-d periods) feed intake and milk yield as influenced by stocking density (STKD) and diets containing straw (S) or no straw (NS).

	100%		142%			*P*-value		
	NS	S	NS	S	SEM	STKD	Diet	STKD x Diet
Intake responses								
DMI, kg/d	25.4	25.3	25.3	25.2	0.4	0.78	0.69	0.87
NDF intake, kg/d	7.5	8.3	7.2	8.0	0.3	0.23	<0.01	0.91
peNDF intake, kg/d	6.2	6.8	6.0	6.6	0.3	0.42	0.02	0.95
$uNDF_{om}240$, kg/d	2.2	2.5	2.1	2.5	0.1	0.50	<0.01	0.22
Lactational responses								
Milk, kg/d	41.2	40.4	40.7	40.0	0.7	0.21	0.06	0.79
SCM, kg/d	42.6	42.4	42.7	41.5	0.8	0.25	0.09	0.23

EXPERIMENT 2:
OVERSTOCKING AND REDUCED FEED ACCESS

Nutrition models calculate nutrient requirements assuming that cows have ad libitum access to feed and are not overstocked. The reality is that the majority of cows in the US are fed under overstocked conditions – and increasingly farmers are feeding for lower amounts of daily feed refusals in an effort to minimize wastage of expensive feed. Consequently, we need to understand the interaction of stocking density and feed availability on ruminal pH, behavior, and productive efficiency.

Forty-eight multiparous and 20 primiparous Holstein cows were assigned to 1 of 4 pens (n = 17 cows per pen). Pens were assigned to treatments in a 4 x 4 Latin square with 14-d periods using a 2 x 2 factorial arrangement. As in experiment 1, two stocking densities (STKD; 100 or 142%) were used. In experiment 2, we evaluated 2 levels of feed restriction (0-h or no restriction; NR) and 5-h of feed restriction (R) that resulted in

4 treatments (100NR, 100R, 142NR, and 142R). As in experiment 1, stocking density was achieved through denial of access to both headlocks and free-stalls (100%, 17 free-stalls and headlocks per pen; 142%, 12 free-stalls and headlocks per pen) and pen served as the experimental unit.

Feed access was achieved through pulling feed away from headlocks approximately 5 h before the next feeding. Previous research has shown that blocking access to the feed bunk for 5 to 6 h/d mimics so-called “clean bunk” management (French et al., 2005). Sixteen multiparous ruminally fistulated cows were used to form 4 focal groups for ruminal fermentation data. Each focal group was balanced for days in milk, milk yield, and parity.

The effect of stocking density and feed access on ruminal pH characteristics is shown in Table 5.

Table 5. Ruminal pH responses as influenced by stocking density (STKD) and feed restriction (FR; no restriction, NR; 5-h restriction, R).

	100%		142%			*P*-value		
Variable	NR	R	NR	R	SEM	STKD	FR	STKD x FR
Mean pH	5.96	6.03	5.98	5.89	0.06	0.14	0.80	0.08
Minimum pH	5.42	5.50	5.51	5.39	0.07	0.81	0.78	0.12
Maximum pH	6.49	6.61	6.48	6.53	0.04	0.25	0.06	0.29
Time pH < 5.8, h/d	6.62	5.23	6.78	8.77	1.27	0.02	0.49	0.02
AUC < 5.8 pH, pH x unit[1]	1.66	1.24	1.73	2.55	0.63	0.09	0.52	0.11

[1]Area under the curve.

Higher stocking density, as in experiment 1, increased risk for SARA with greater time spent below pH 5.8 (P = 0.02) and tended to increase severity (P = 0.09). While there were no differences in ruminal pH responses for the feed access treatment, there was a significant interaction between stocking density and feed access (P = 0.02), indicating an exacerbated risk for SARA when cows were housed at higher stocking density and had restricted access to feed. Compared to experiment 1, feed access when isolated did not have as great an impact on ruminal pH compared to differences in fiber levels of the diet. However, when combined with high stocking density, reduced feed access had a greater impact than the low fiber diets. The implications of these results on commercial dairy farms where overstocking and feeding to low levels of feed refusals is commonly practiced need to be better understood.

Further analyses for the experiment are currently underway and include behavioral responses, pen-level feed intake, and lactational performance.

CONCLUSIONS

Stocking density exhibited a consistent negative effect on ruminal pH and increased the risk for SARA. The presence of additional stressors in combination with stocking density exacerbated these negative effects on ruminal pH, although the magnitude varied depending on the type of stressor. However, manipulation of the feeding environment can help mitigate the negative effects of stocking density, such as increasing peNDF in the diet or reducing time without feed.

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NEW MILK ANALYSIS TECHNOLOGIES TO IMPROVE DAIRY CATTLE PERFORMANCE

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INTRODUCTION

Two years ago we introduced the application of new mid-infrared (**mid-IR**) for rapid milk fatty milk analysis (Barbano, et al., 2014) and reported positive correlations of bulk tank milk fat test with a higher proportion and concentration of de novo fatty acids in bulk tank milk. The form of the fatty acid data from the mid-IR was structured to provide information on the relative proportions of de novo (C4 to C14), mixed origin (C16:0, C16:1, C17:0), and preformed (C18:0 and longer) fatty acids in milk. We can also provide that information in units of grams per 100 grams of milk. Since that time, we have continued to collect data on milk fatty acid variation in bulk tank milk and it's relationship to feeding and farm management. A field study of 20 Holstein and 20 Jersey farms was completed in 2014 (Woolpert et al., 2016) and a follow up study of 40 Holstein farms was completed in 2015 (Woolpert, 2016) with the objective of determining farm feeding and management practices relate to milk fatty acid compostion and bulk tank milk fat and protein concentration. Starting in February of 2016, information on milk fatty acid 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.

In addition, in the last 2 years we have expanded our milk analysis research on fatty acid analysis to individual cow milk samples at Cornell and in collarboration with Miner Institute in Chazy, NY. Additional work is in progress in collaboration with Penn State and Michigan State Universities. Today, I will focus on the use of milk fatty acid (**FA**) information for feeding management of dairy cows at the bulk tank level and report the status of our work on individual cow data, particularly transition cows.

EXPERIMENTAL APPROACH

Prior to the current study a group of partiail least squares (**PLS**) chemometric prediction models were developed from mid-IR spectra. The spectra of modified milk calibration samples (Kalylegian et al., 2006a,b), bulk tank milks, and individual cow milks were used in combination with chemical reference chemistry for fat (AOAC, 2000; method 989.05; 33.2.26), total protein (AOAC, 2000; method 991.20; 33.2.11 and nonprotein nitrogen (AOAC, 2000; method 991.21; 33.2.12) with true protein calculated by difference, anhydrous lactose (Lynch et al., 2007) and gas liquid chromatography (Barbano and Sherbon, 1980; Lynch et al., 1992) for FA analysis using a Varian CP-SIL88 capillary column [(100m x 0.25 mm x 0.2 µm film thickness), ID code # CP7489; Varian, Inc., Lake Forest, CA], installed in a Hewlett Packard 6890 GC System

equipped with an automatic liquid sampler and a flame ionization detector (Hewlett Packard Co., Wilmington, DE). A more complete descripiton of the fatty acid analysis methods and PLS model for fatty acid prediction model development was reported by Wojciechowski and Barbano (2016).

A library of chemometric prediction models for the major components in milk and milk FA composition for use on a Lactoscope FTA and Lactoscope CombiScope FTIR 600/300 (Delta Instruments, Drachten, The Netherlands) has been developed. A variety of individual FA and groups of FA were measured. The following individual FA were measured by mid-IR: C16:0; C18:0; C18:1 *cis*9, *cis*12; C18:1 t*rans* 10; andC18:1 *trans* 11. The following groups of FA were measured: total FA; DeNovo (C4:0 to C14:0), mixed origin (C16:0, C16:1, C17:0), preformed (C18:0 and longer); total unsaturated FA, total cis FA; total trans FA; mono unsaturated FA; and poly unsaturated FA. All FA measures produce results from the IR in grams of FA per 100 grams of milk. Some researchers have used the grouping of FA as short, medium, and long chain FA but the exact definition of those groups varies among researchers. The group definitions of de novo, mixed origin, and preformed FA are much more clear and consistent because they are based on the biochemical pathways for FA synthesis and have better potential to be correlated with the biology, metabolism, and feeding of dairy cows.

In addition to the measures of FA concentrations, two fat concentration independent measures of FA structure were also done on each sample: mean FA chain length (expessed as mean carbon number per FA) and mean FA unsaturation (expressed as double bonds per FA). The measure of total FA (not fat) in g/ 100 g of milk is used as a new basis for a more accurate measurement of total fat content in the milk. This approach eliminates most of the weakness of traditional measurses of fat by IR using the Fat A (C=O stretch) and Fat B (C-H stretch) because it compenstates sample by sample for differences in FA composition when trying to estimate the total fat content of the milk in comparison to ether extraction (Kaylegian et al., 2009a,b). The relative proportion of the total FA in milk that are represented by an individual or group of FA can be expressed on a relative basis as a precent of total FA in the sample. Thus, it is possible to produce a simulated gas chromatograph FA analysis of milk fat directly from the same (IR spectra) of milk tested on the IR for fat, protein, and lactose concentration.

The calibration adjustment of the fat, true protein, anydrous lactose and all FA measures on the IR milk analyzer is done once per month using a set of 14 modified milks described by Kaylegian et al. (2006a,b) that has reference values in (g FA per 100 g of milk) for each of the individual or groups of FA measured. The set of calibration samples is produced monthly at Cornell and was used to check the calibrations during the month.

RESULTS

2014 Farm Study (Woolpert et al., 2016)

This study investigated the relationship of management practices, diet characteristics, milk composition, and lactation performance with de novo fatty acid (FA) concentration in bulk tank milk from commercial dairy farms with Holstein, Jersey, and mixed breed cows. It was hypothesized that farms with higher de novo milk FA concentrations would more commonly use management and nutrition practices known to optimize rumen conditions that enhance de novo synthesis of milk FA. Farms (n = 44) located in Vermont and northeastern New York were selected based on a history of high de novo (HDN; 26.18 ± 0.94 g/100g FA; mean ± SD) or low de novo (LDN; 24.19 ± 1.22 g/100g FA) FA in bulk tank milk. Management practices were assessed during one visit to each farm in March or April, 2014. Total mixed ration samples were collected and analyzed for chemical composition using near infrared spectroscopy. There were no differences in days in milk at the farm level.

Yield of milk fat, true protein, and de novo FA per cow per day were higher for HDN versus LDN farms. The HDN farms had lower freestall stocking density (cows/stall) than LDN farms. Additionally, tiestall 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. The difference in income per cow would depend on the actual milk price at any point in time. However, the average fat and protein price for the Federal Milk Order No. 1 for March and April 2014 was $4.62 and $10.17 per kg, respectively. Therefore, at 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 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 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. This research indicated that overcrowded freestalls, reduced feeding frequency, and greater dietary ether extract content are associated with lower de novo FA synthesis and reduced milk fat and true protein yields on commercial dairy farms.

2015 Farm Study (Woolpert, 2016)

The objective of this study was to evaluate the relationship of management practices and dietary factors with de novo fatty acid concentration in bulk tank milk from commercial dairy farms milking Holstein cows. Farms were selected based on de novo fatty acid concentration during the 6 mo previous to the farm visit and were categorized as high de novo (HDN; 24.61 ± 0.75 g/100 g of FA, mean ± standard deviation; n = 19) or low de novo (LDN; 23.10 ± 0.88 g/100 g of FA; n = 20). Farms were visited once in February, March, or April, 2015 and evaluated based on management and facility design known to affect cow behavior, physical and chemical characteristics of the diet,

and the ration formulation and forage analyses obtained from the farm's nutritionist. The mean milk composition for HDN and LDN farms is shown in Table 1.

No differences in milk, fat, and true protein yields were detected between HDN and LDN farms, but milk fat and true protein content were higher ($P < 0.01$) on HDN farms (Table 1). This positive relationship between de novo FA and milk fat and true protein percentage agrees with previous results of Barbano et al. (2014) who evaluated bulk tank milk composition on over 400 commercial dairy farms. De novo FA expressed as g/100 g of FA and as g/100 g milk were higher ($P < 0.01$) on HDN farms, and preformed FA expressed as g/100 g of FA and as g/100 g milk were lower ($P < 0.01$ and $P = 0.02$, respectively) on HDN farms. These results are consistent with previous research (Woolpert et al., 2016) that indicated that HDN farms have higher milk fat and true protein content in bulk tank milk. De novo FA yield, expressed as g/d, was higher ($P < 0.01$) for HDN farms with no difference detected in milk yield ($P = 0.91$) suggesting that cows on HDN farms synthesized more de novo FA. However, milk weights per cow were not measured directly, but were estimated indirectly based on the number of cows milking on the day of the farm visit and the average bulk tank milk shipped per day during the month of the farm visit. Thus, the uncertainty in milk weight data was higher than the uncertainty in milk composition data. Consequently, further research is needed under conditions where milk weight per cow per day can be accurately measured, along with milk composition, to determine whether greater de novo FA synthesis is always associated with greater milk fat and true protein yields.

There were no differences in farm size, time away from the pen for milking, days in milk, or body condition score for HDN versus LDN farms. No differences between HDN and LDN farms in milk, fat, or true protein yield were detected; however, milk fat and protein content and de novo fatty acid yield per day were higher for HDN farms, as was gross income per unit of milk sold.

The relationships between various milk fatty acid parameters across 40 farms and bulk tank milk fat test are shown in the Figures 1 thru 5 below.

Table 1. Least squares means of milk composition factors for high de novo (HDN) and low de novo (LDN) farms for the month of the farm visit.

Item	HDN	LDN	SEM	*P* value
Milk yield, kg/d	31.9	32.1	0.9	0.91
Fat, %	3.98	3.78	0.04	<0.01
Fat, kg/d	1.27	1.21	0.03	0.25
De novo fatty acids[1]				
g/100 g milk	0.99	0.86	0.01	<0.01
g/100 g FA	25.99	23.78	0.22	<0.01
g/d	315.6	276.2	9.5	<0.01
Mixed fatty acids[2]				
g/100 g milk	1.48	1.35	0.02	<0.01
g/100 g FA	38.86	37.36	0.37	<0.01
g/d	472.0	434.2	15.2	0.08
Preformed fatty acids[3]				
g/100 g milk	1.32	1.38	0.02	0.02
g/100 g FA	34.60	38.21	0.50	<0.01
g/d	419.0	439.3	10.4	0.17
True protein, %	3.19	3.08	0.02	<0.01
True protein yield, kg/d	1.02	0.99	0.03	0.44
MUN, mg/dL	12.1	12.9	0.5	0.25
Anhydrous lactose, %	4.65	4.66	0.02	0.66
Anhydrous lactose, kg/d	1.46	1.51	0.05	0.51

[1] C4 to C14.
[2] C16, C16:1, and C17.
[3] Greater than or equal to C18.

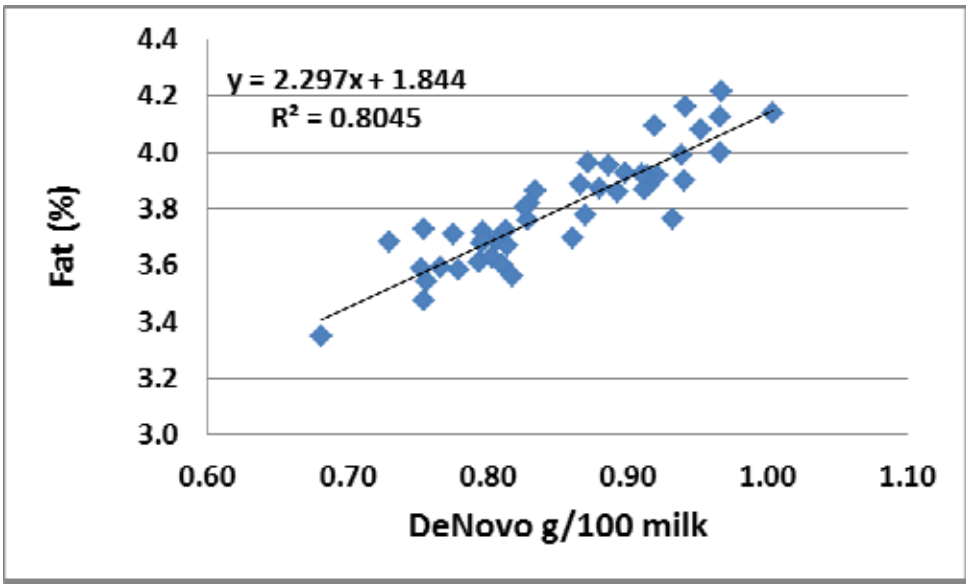

Figure 1. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of de novo fatty acids in milk. In general, a farm needs to have a concentration of de novo fatty acids higher than 0.85 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

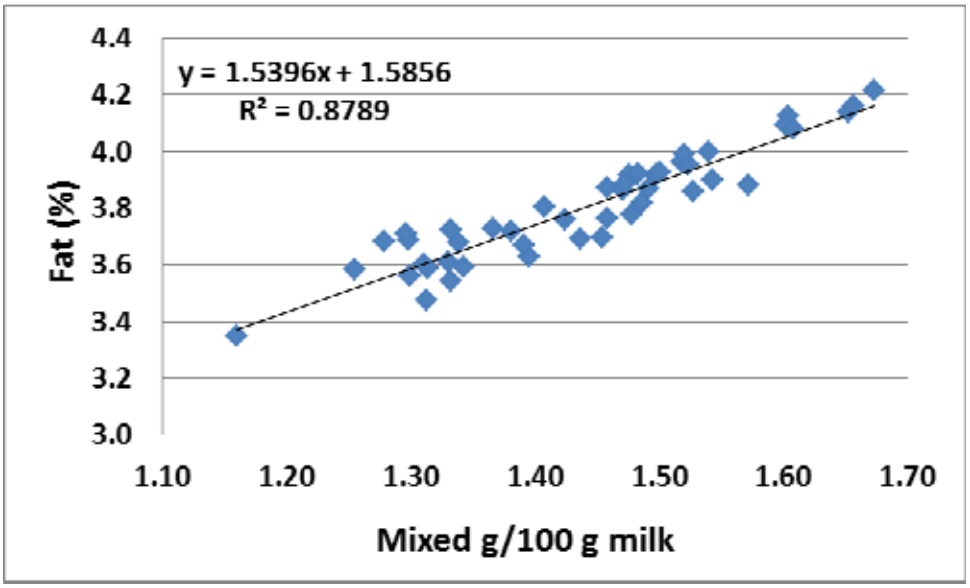

Figure 2. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of mixed origin fatty acids in milk. In general, a farm needs to have a concentration of de novo fatty acids higher than 1.40 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

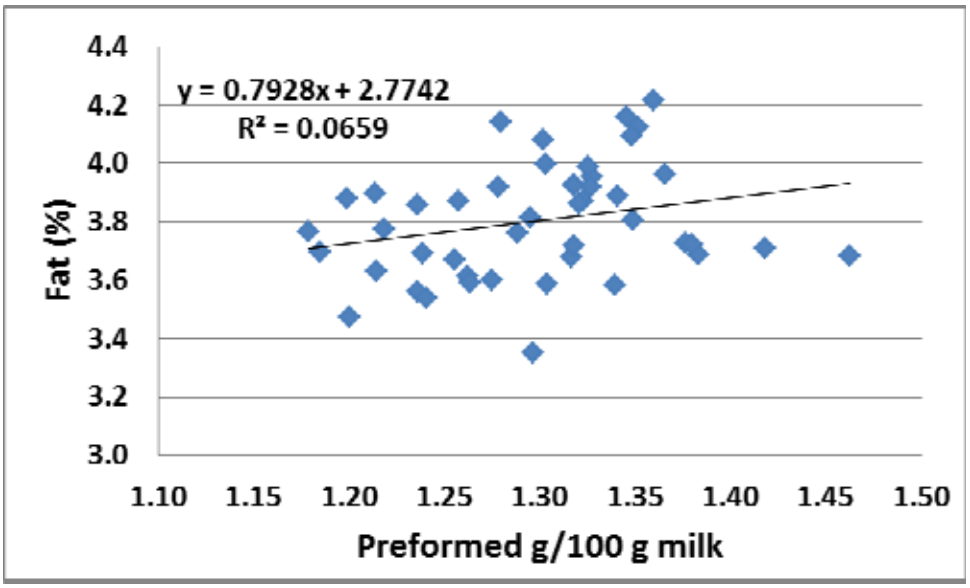

Figure 3. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of preformed fatty acids in milk. In general, the variation in preformed fatty acid concentration in Holstein herds is less than de novo and mixed origin fatty acids and is not well correlated with bulk tank milk fat test.

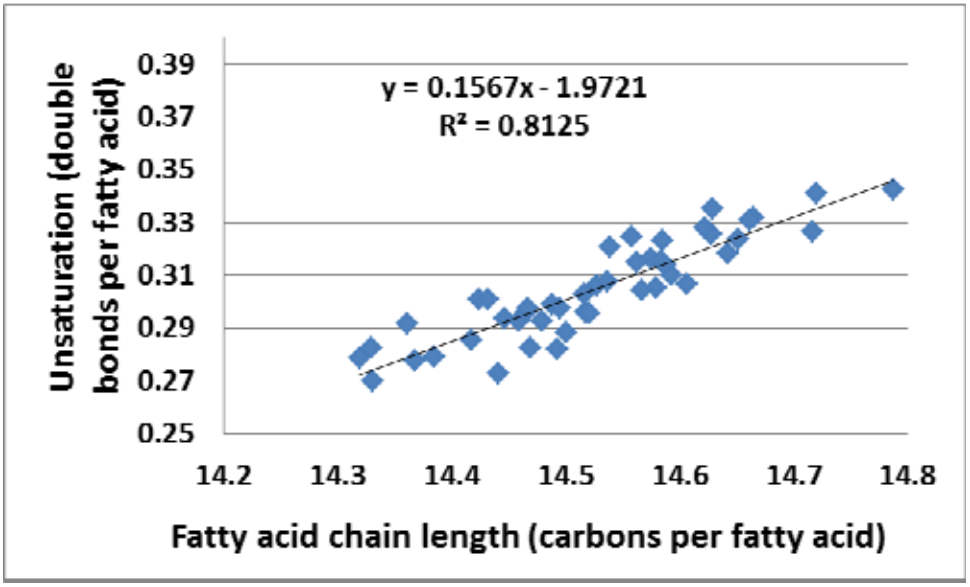

Figure 4. Relationship of bulk tank milk fat fatty acid unsaturation to fatty acid chain length. As fatty acid chain length fatty acid unsaturation increases and this appears to be due mostly to an increase in oleic acid (C18:1 cis 9).

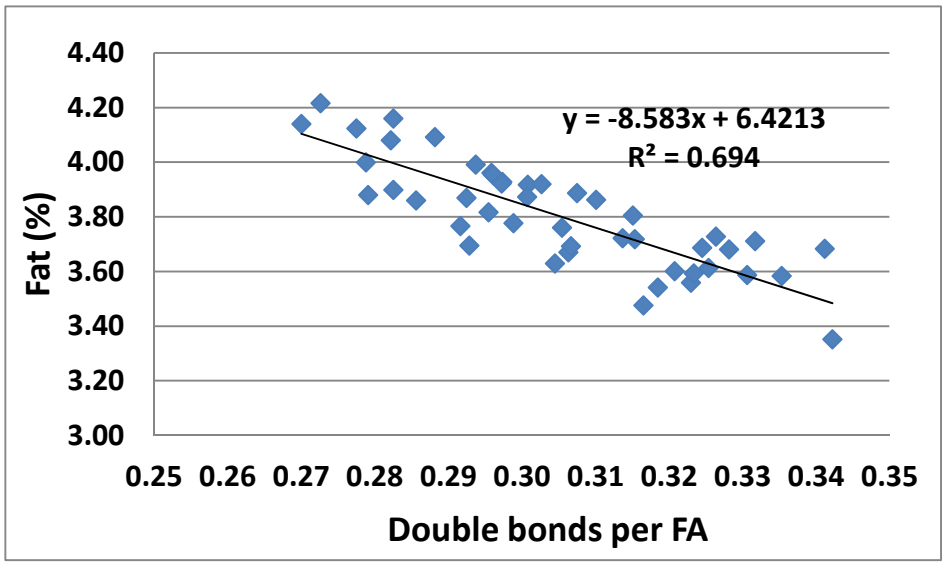

Figure 5. Relationship of bulk tank milk fat fatty acid unsaturation with bulk tank milk fat test. As double bonds per fatty acid increases the bulk tank milk fat test decreases. To achieve a 3.75% fat test a farm needs to have a double bond per fatty acid of less than 0.31. The double bonds per fatty acid may be an indirection of the rumen unsaturated fatty acid load (RUFAL) and the rate of unsaturated fat release from forage sources (e.g., corn silage, distiller grains, and oil seeds) in the rumen. The double bonds per fatty acid may be an index of the level of milk fat depression in a dairy herd.

The relationship between de novo milk fatty acid concentration across 40 farms and bulk tank milk protein test is shown in the figure below.

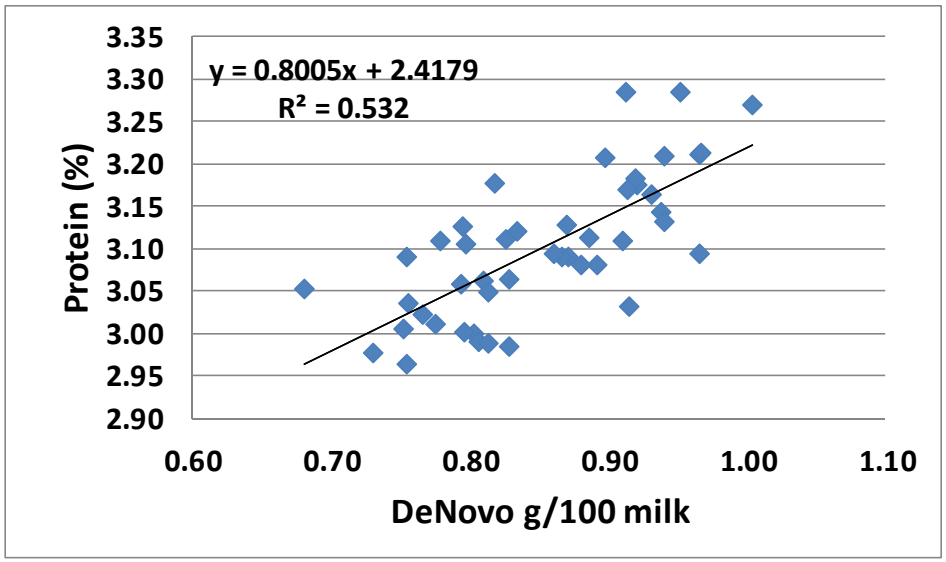

Figure 6. Relationship of bulk tank milk protein test to concentration (g/100 g milk) of de novo fatty acids in milk. In general, a farm needs to a concentration of de novo fatty acids > 0.85 g/100 g milk to achieve a bulk tank fat test higher than 3.10% true protein.

It is hypothesized that when de novo fatty acid production is high, the the biomass of rumen microflora is high and this provides a higher level of essential amino acids produced in the rumen. When double bonds per fatty acid increase bulk tank milk protein test decreases (data not shown).

The difference in income per cow between HDN and LDN herds would depend on the actual milk price at any point in time. However, the average fat and protein price for Federal Milk Order No. 1 for February through April, 2015 (US Department of Agriculture, 2015) was $4.19 and $5.74 per kg, respectively. Therefore, at 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 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 30 kg of milk would result in a gross income difference of $9,125 for fat and $6,935 for true protein per 100 milking cows per year. High de novo farms tended to be more likely to deliver fresh feed twice versus once per day, have a freestall stocking density less than or equal to 110%, and provide greater than or equal to 46 cm of feed bunk space per cow. There were no detectable differences in forage quality or ration dry matter, crude protein, or starch content. However, ether extract was lower and physically effective neutral detergent fiber was higher for HDN compared with LDN farms. The results of this study indicate that feeding management, stocking density, dietary ether extract content, and the physical characteristics of the diet are related to de novo fatty acid, fat, and protein concentration in bulk tank milk from high-producing Holstein dairy farms.

SUMMARY OF BULK TANK MILK TESTING

The key FA parameter that was positively correlated with bulk tank milk fat and true protein concentration was *DeNovo* FA (g/100 g milk). Structural parameters of FA chain length (carbon number) and total unsaturation (double bonds /FA) were negatively correlated with fat and protein (g/100 g milk). This was true for both Jersey and Holstein. In general, a Holstein farm needs to have a concentration of de novo fatty acids higher than 0.85 g/100 g milk and a concentration of mixed origin fatty acids higher than 1.35 g/100 g milk to achieve a bulk tank fat test higher than 3.75%. As double bonds per fatty acid increase both fat and protein will decrease. Double bonds per fatty acid may be an index of effective RUFAL level in diet. Keeping the milk double bonds per fatty acid at 0.3 or lower produce higher milk and protein. Over crowding of cows in pens was correlated with lower de novo and mixed origin fatty acids and lower milk fat and protein test. Generally, when de novo fatty acid production is higher milk production per cow will be equal to or higher than when de novo is lower, but both milk fat and protein test (g/100 g of milk) will be higher. This will increase the income per unit of milk produced.

Milk Testing for Individual Cows (Barbano et al., 2015)

As the milk production per cow has increased, there is more demand placed on the physical and metabolic system of each individual dairy cow. More attention through automated information collections systems to the metabolic and physical condition of each cow is needed to keep each cow healthy and productive. Because each cow makes an individual contribution to both farm costs and income, it becomes a management challenge particularly in large dairy herds, to make each cow a "cow-of-interest" and make correct decision about health and reproduction to achieve improved overall performance of the dairy herd.

To achieve a focus on individual cow status, measurement of de novo, mixed origin, and preformed fatty acids in milk is also useful for individual cow milk testing, particularly during the transition period. The changes in de novo fatty acids are a relative percentage of total fatty acids reflects the energy balance status of the cow. Recently, we have developed a new milk mid-IR test that produces an estimate of blood NEFA level by testing the milk. This testing would be done on the same milk sample at the same time as the fat, protein, lactose, solids, MUN and fatty acid analysis using the mid-IR milk analyzer.

High blood NEFA indicates that a cow is mobilizing body fat and increases the risk of metabolic disorders. Milk and blood samples were collected from 60 lactating Holsteins once per week for the first 3 weeks of lactation. Cows were milked 3 times per day. Within + or – one milking of the time of blood collection, a milk sample was analyzed using a Delta Instruments (model FTA) mid-IR milk analyzer. A Wako NEFA HR test kit was used as an *in vitro* enzymatic colorimetric method for the quantitation of NEFA in blood serum and these values were used as reference values for development PLS regression model to predict blood NEFA from the mid-IR milk spectra. There are no NEFA in milk, so a model to predict blood NEFA from a milk sample uses differences in the milk spectra from sample to sample that are correlated with changes in blood NEFA. The final PLS model had 9 factors, used wavelengths in the following ranges (3000 to 2800, 1800 to 1700, 1585 to 1000 cm^{-1}) with a standard error of cross validation of 172 µEq/L. Validation milk and blood sample pairs (n = 53) were collected from Holstein cows from a different herd. The mean value for the blood reference test was 713 µEq/L of serum and the mean value for the milk based blood NEFA prediction was 703 µEq/L of serum with a standard deviation of the difference (SDD) of 218 µEq/L for the 53 validation samples. Blood NEFA measured on blood is a snapshot of the NEFA concentration at an instant in time, while blood NEFA predicted from milk analysis represents a time average for the total time between milkings. The FTIR milk analysis to estimate blood NEFA is rapid (about 10 seconds), done simultaneously with all other milk component and fatty acid measures, and uses no reagents. This approach could be useful for rapid evaluation of risks of ketosis, displaced abomasum and possibly reproductive disorders. The relationship between the milk estimated blood NEFA level and the change in de novo milk fatty acids may have predictive to power to provide an advanced warning that a cow is going to have a displaced abomasum.

Concepts for integration of mid-IR milk analysis directly into the milking systems on large farms are being considered. The combination of milk weight and the component concentrations (i.e., fat, protein, lactose, and milk NPN/Urea content) will allow calculation of energy output in the milk and in combination with feed input data will allow an estimate of energy and protein balance of individuals or groups of cows within the herd.

Some other measures that we have developed for use in individual cow milk testing are predicted blood NEFA for ketosis prediction, in addition to milk BHB and acetone concentrations. We are developing a milk estimated blood BHB method currently. The measurement and rate of change of blood NEFA estimated by milk analysis during the early transition period will provide a view of the metabolic status combined with energy balance estimates. Indirect measurement of rumen pH through milk analysis is in development and might provide insight into how a cow is interacting the complex mixture of nutrients in the rumen, as that impacts the chemistry of the milk.

Combinations of individual parameters that provide more predictive indices of feed efficency, ketosis, and probability of successful breeding may be derived from the current PLS models for milk analysis. In the future, development of models to determine pregnancy status and loss of pregnancy will bring further benefit in the applications of mid-IR milk testing for real-time farm management milk testing.

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AFLATOXIN CONCENTRATIONS IN MILK FROM HIGH-PRODUCING US HOLSTEINS FED NATURALLY-INFECTED MAIZE AND MILKED 3X PER DAY

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INTRODUCTION

Aflatoxin belongs to a group of fungal toxins known as mycotoxins; these are highly oxygenated, heterocyclic, difuranocoumarin compounds produced by *Aspergillus flavus and Aspergillus parasiticus* (Diaz, Calabrese, & Blain, 2008). Aflatoxins are hepatotoxic and carcinogenic secondary metabolic products from these fungal species. Once aflatoxins are produced by the fungi, they are heat, cold, and light stable. They persist to some extent in food even after the inactivation of the fungi by food processing methods, such as ultra-high temperature products, due to their significant chemical stability. Aflatoxins are colorless, odorless, and tasteless. Because even low concentrations can be important, and with the uneven distribution in commodities, aflatoxins are difficult to detect accurately (Peraica, Radić, Lucić, & Pavlović, 1999).

Although *Aspergillus flavus* and *A. parasiticus* grow well in tropical and subtropical climates, they can also be found and produce aflatoxins in more temperate areas (Binder, Tan, Chin, Handl, & Richard, 2007). While fungi are a normal part of the microflora of standing crops and stored feeds, the production of the secondary metabolites, such as aflatoxin B1, are promoted by certain physical and biological factors at points during harvesting, handling, and storage (W. L. Bryden, 2007). Physical factors include moisture, humidity, temperature and mechanical damage of the crops. Biological factors include plant variety, stress from pre-harvest drought, insect damage and spore load.

HEALTH EFFECTS

More than 20 aflatoxin-like secondary metabolites have been identified. Aflatoxin B1 (AFB1) was shown to possess the most toxic and carcinogenic properties to humans and animals (Binder et al., 2007). Along with carcinogenic properties, AFB1 can reduce feed consumption and reduce milk yield in the short term and result in chronic immune suppression and reduced reproductive performance. These chronic effects may be of more economic importance than the acute effects (Bodine and Mertens, 1983). In both humans and animals, AFB1 is metabolized by the liver, creating a hydroxylated metabolite called aflatoxin M1 (AFM1). Both AFB1 and AFM1 are considered group 1a carcinogens (IARC 2007). Aflatoxin M1 is of particular importance to the dairy industry as it is the major aflatoxin metabolite present in mammalian milk.

REGULATIONS

There is current legislation in almost 100 countries regulating the maximum allowable AFB1, total aflatoxin, and AFM1 levels in human food, animal feeds and milk (Berg, 2003). In the US, the FDA creates and enforces action levels for AFB1 and AFM1 in feed, food and milk. These regulatory levels take into account the advisory regulations put forth by the Joint FAO/WHO Expert Committee of Food Additives (JEFCA). In the EU, the European Commission takes into account the advice of the European Food Safety Authority (EFSA) on regulatory levels for AFB1 and AFM1 in feed, food and milk. Both advisory committees work with the Codex Alimentarius Commission of the World Trade Organization to develop harmonized international food standards, guidelines, and codes of practice. The FDA dictates a maximum allowable concentration of 20 µg/kg total aflatoxin in food and feed intended for dairy consumption, and 0.5 µg/kg AFM1 in milk and milk products. These regulations are based mainly on risk analysis of aflatoxins. The European Commission dictates a maximum allowable concentration of 4 µg/kg total aflatoxin in food and feed intended for dairy consumption, and 0.05 µg/kg AFM1 in milk and milk products. These regulations are based on 7 years of occurrence data, risk analysis, and dose-response modeling from animal and epidemiological data. Regulations specific to AFM1 contamination have also influenced regulatory limits in feed for dairy animals. In order to ensure compliance with the maximum levels in milk, stringent maximum levels in dairy feedstuffs are also necessary (European Food Safety, 2004).

AFLATOXIN M1 AND MODERN DAIRY PRODUCTION

A factor that is considered to be important for influencing regulatory limits of both total aflatoxin and AFM1 is the rate at which AFB1 is converted and excreted as AFM1 into the milk of dairy cows (Masoero, Gallo, Moschini, Piva, & Diaz, 2007). The ability of cattle to transform AFB1 in the feed to AFM1 in the milk has been examined in many studies, which demonstrated that such carry-over in dairy cows milked 2 times daily was usually 1% to 2% of the ingested AFB1 for low-yielding cows (< 30 kg milk yield/day) and up to ~6% for high-yielding cows (> 30 kg milk yield/day). Most previous studies on the carry-over of aflatoxins from feed to milk were in what would be considered today as low-yielding dairy cows (Britzi et al., 2013).

Due to specialized breeding programs, technological innovations and other structural changes in milk production in the US, total milk production increased by 45% between 1975 and 2000. There has also been a huge shift from pasture-based dairy operations to confinement feeding operations which has increased the proportion of concentrates such as cornmeal in the feed, and the higher milk yield has increased the overall consumption of feed as well (Blayney, 2002). In order to ensure continued adherence to regulations for AFM1 concentrations in milk, it is now necessary to re-examine the actual transfer of toxicity as aflatoxin in the feed to AFM1 in the milk, and suggest new safety thresholds for feed aflatoxins.

PRELIMINARY WORK

Local survey

We surveyed 38 local farms in upstate NY, taking feed and milk samples to establish a general occurrence level of mycotoxins and formulate a field data carry-over rate for aflatoxin from feed to AFM1 in milk. When possible, we took small samples of TMR from all the bunkers from which the animals on each diet were fed. We then collected milk samples from groups or individual cows from each of the feeding groups. It was often logistically difficult to obtain a good milk sample from just one milking group and on farms without TMR were more difficult to sample cows' actual intakes. It is always difficult to get a representative sample for mycotoxins in feeds because of their heterogeneous distribution (Wayne L. Bryden, 2012). Also, according to other studies, because of the quick excretion rate of aflatoxins in milk, it is hard to collect exactly what feed became part of which milk sample (Decastelli et al., 2007). While we expected farms with unregulated homegrown feed sources to have high milk mycotoxin levels, that was not the case. However we did find that over 14% of the milk samples collected would violate EU regulations for AFM1. We put together a field carry-over graph using 120 points of aflatoxin and AFM1 measurement data (Figure 1). While there was an apparent relationship between feed and milk aflatoxin levels, the specific feed sampled in the bunkers and troughs were assumed to be similar to the feed the cows had consumed to make the milk sampled, but had it usually been fed more recently.

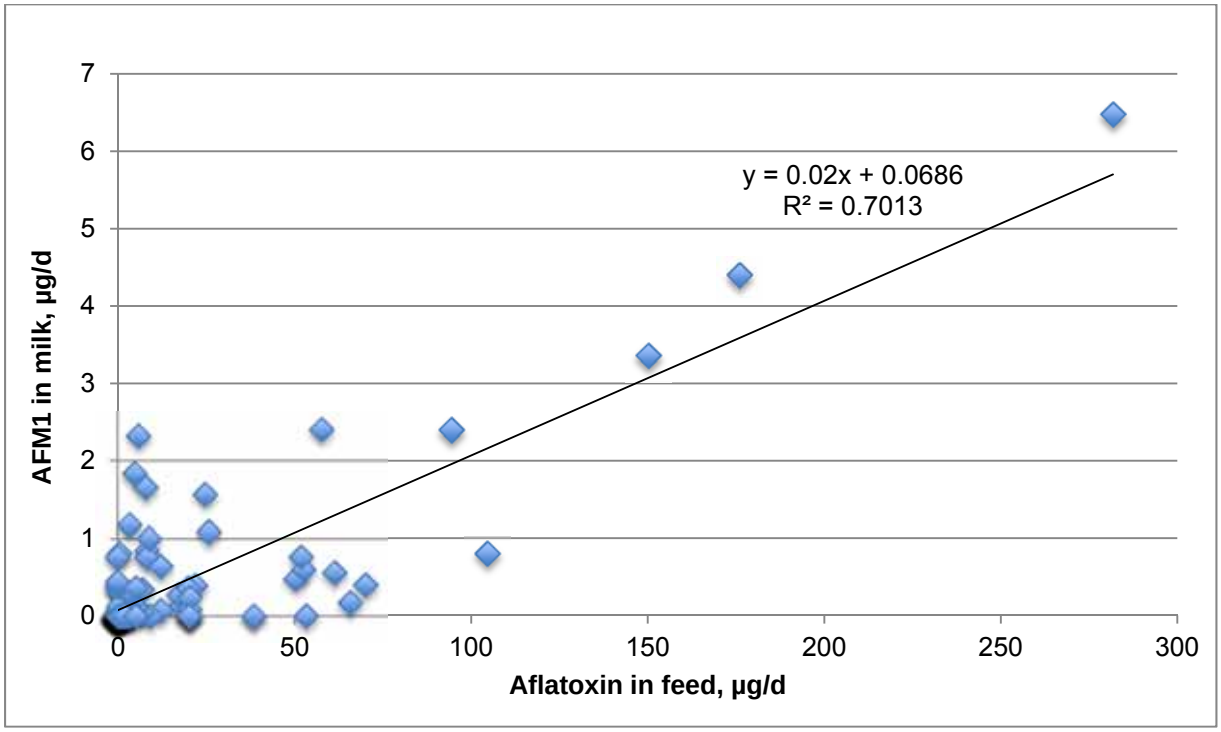

Figure 1. Local field survey data for mass of aflatoxin M1 in milk vs mass of aflatoxin in feed.

Meta-analysis for previous results

We collected individual aflatoxin data and specific methods information from 13 studies from 1967 to 2014 (Figure 2). Some aflatoxin intake data were inferred from methods and other information provided in the papers. Consolidation of these historical works provides an overall picture based on the limited number and scope of past studies. These data are presented together with our field carry-over data in Figure 2 with trend lines added to show approximate carry-over percentages.

Many of the studies represented in Figure 2 used cows with relatively low milk production. The apparent carry-over percentage of 1.2% for these studies is in line with the understanding that lower-producing cows excrete less AFM1 into the milk as a percentage of AFB1 consumed. The relatively low r^2 value for these previous studies may be due to a lack of individual cow data available within each study.

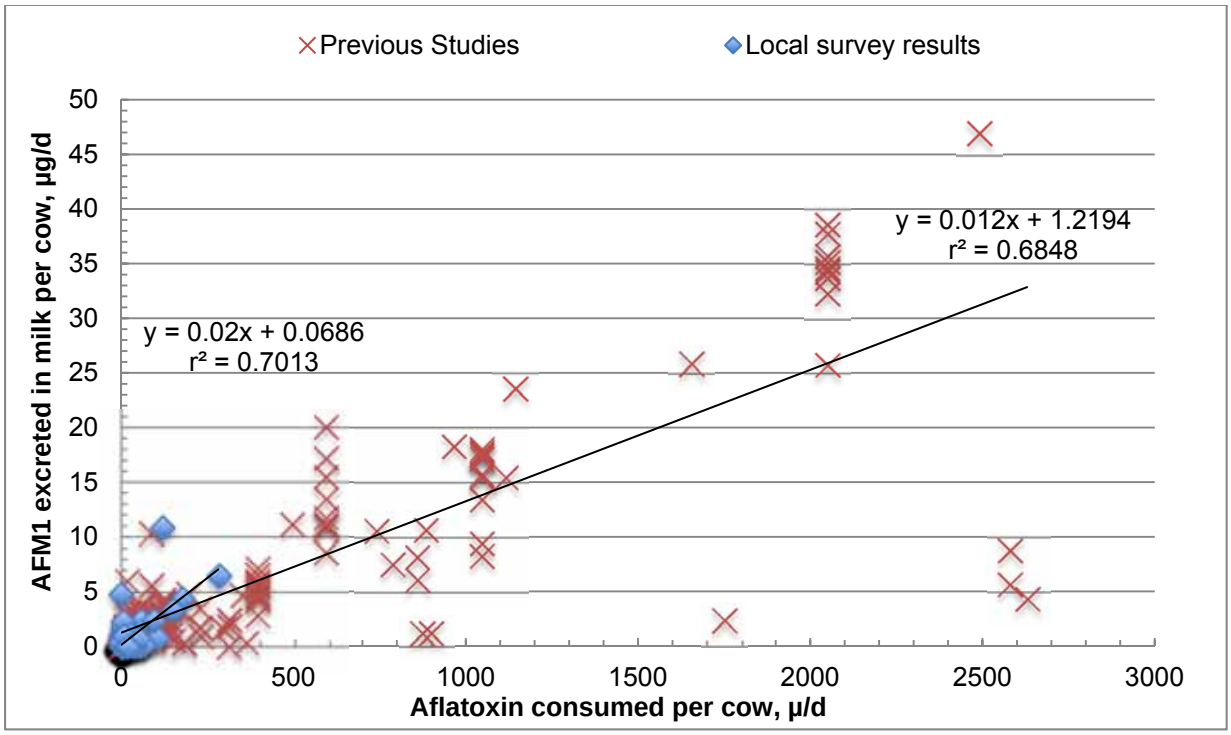

Figure 2. Average mass of AFM1 excreted in milk vs average mass of aflatoxin consumed from field survey data and data collected from previous carry-over experiments (Allcroft, Roberts, & Lloyd, 1968; Applebaum, Brackett, Wiseman, & Marth, 1982; Britzi et al., 2013; Chopra et al., 1999; Frobish, Bradley, Wagner, Long-Bradley, & Hairston, 1986; Lafont, Lafont, Mousset, & Frayssinet, 1980; Masoero et al., 2007; Munksgaard, Larsen, Werner, Andersen, & Viuf, 1987; Patterson, Glancy, & Roberts, 1980; Price, Paulson, Lough, Gingg, & Kurtz, 1985; Veldman, Meijs, Borggreve, & Heeres-van der Tol, 1992).

CONTROLLED FEEDING STUDY

Regulations for maximum limits for aflatoxins in human and animal foods are influenced by experiments to calculate the carry-over of aflatoxins into dairy milk and, in the US, the limit of detection for aflatoxins from 1969 (Binder et al., 2007). In Europe, the expected carry-over rate for aflatoxins into milk is used as part of their worst case scenario calculations for establishing safe maximum limits for aflatoxins in food and milk (Zain, 2011). It is believed that chronic, low level exposure of aflatoxins on high-producing dairy cows will provide a better picture for the current dairy industry and current regulation and safety recommendations (Wayne L. Bryden, 2012). These findings could influence scientific research groups responsible for advising committees in charge of food safety regulations.

Our study used cows with a high milk production level for a more realistic picture of high-producing, intensive dairy operations in the US. We included cows with milk production levels reported as high in only one previous study on mycotoxins (Britzi et al., 2013). While that other study used feed spiked with pure AFB1, we fed subclinical levels of total aflatoxin through the use of naturally contaminated cornmeal imported from the southeastern US.

Methods

We did three rounds of trials each using 12 high-producing dairy cows in early- to mid-lactation, feeding them naturally contaminated corn meal top-dressed on their daily TMR. One trial lasted 2.5 weeks with a week-long adjustment period to the stalls and the clean TMR, 2 days of infected cornmeal feeding, and milk sampling at all three daily milkings done before, during and after aflatoxin exposure until AFM1 levels in milk returned to 0 µg/kg. The other two trials were similar but infected cornmeal was fed for 7 days and milk was sampled every other day at all three milkings until AFM1 levels in the milk returned to 0 µg/kg.

Each trial had 4 cows each fed 1 of 3 diets with: 1) control (0 µg/d), 2) low (300 µg/d), or 3) high (600 µg/d) levels of aflatoxin in the cornmeal. Refusals of the previous day's TMR for each cow were sampled before removing them from the feed buckets. At feeding time each morning, after taking a representative sample of the fresh TMR offered, 1 kg of contaminated cornmeal was top-dressed on the fresh feed for each cow. Samples from each cornmeal bag were collected for each day. Feed samples were taken, refrigerated, and tested in our lab using Aflatest columns and the related published procedure on page 30 of the Aflatest manual from VICAM. Milk samples were taken, refrigerated, and immediately tested in our lab using Afla M1 FI^{+} columns and the related published procedure on page 11 of the Afla M1 FI^{+} manual from VICAM. Ingested aflatoxin levels were calculated by adding the aflatoxin in the cornmeal top-dress with the aflatoxin (if any) found in the base TMR and then subtracting any aflatoxin found in the refusals for each cow. Concentrations (µg/kg) of aflatoxin in milk and feed samples were converted to µg of aflatoxin using measured milk yield and DMI.

Results and discussion

Average levels of aflatoxin ingested for each feeding group in each trial are presented in Table 1 as well as average peak levels of AFM1 recorded in the milk for each group in each trial.

Table 1. Average dietary aflatoxin concentration for each feeding group for each trial and average maximum excretion concentration of AFM1 in milk for each feeding group for each trial. Numbers in italics violate the US action levels for aflatoxin and AFM1.

Diet	Trial 1 (7d)	Trial 2 (7d)	Trial 3 (2d)
Aflatoxin ingested (µg/kg feed DM)			
Control	0	0	0
Low	5.2	12.3	9.4
High	*21.7*	*21.9*	16.0
AFM1 excreted (µg/kg milk)			
Control	0	0	0
Low	0.278	*0.543*	0.174
High	*1.010*	*0.966*	*0.504*

Total aflatoxin ingested and total AFM1 excreted over the trial period were calculated using measured AFM1 levels in the milk, measured aflatoxin levels in ingested feed, DMI and milk yield at each milking. Using the total aflatoxin ingested and the total AFM1 excreted instead of a daily average of each provides a better overall picture of the carry-over effect without omitting or averaging data during the time to steady-state conditions and the time to 0 µg/kg AFM1 in the milk following the cessation of dosing. Linear regression was used to calculate the direct carry-over into milk as 6.5 µg/100 µg consumed (Figure 3).

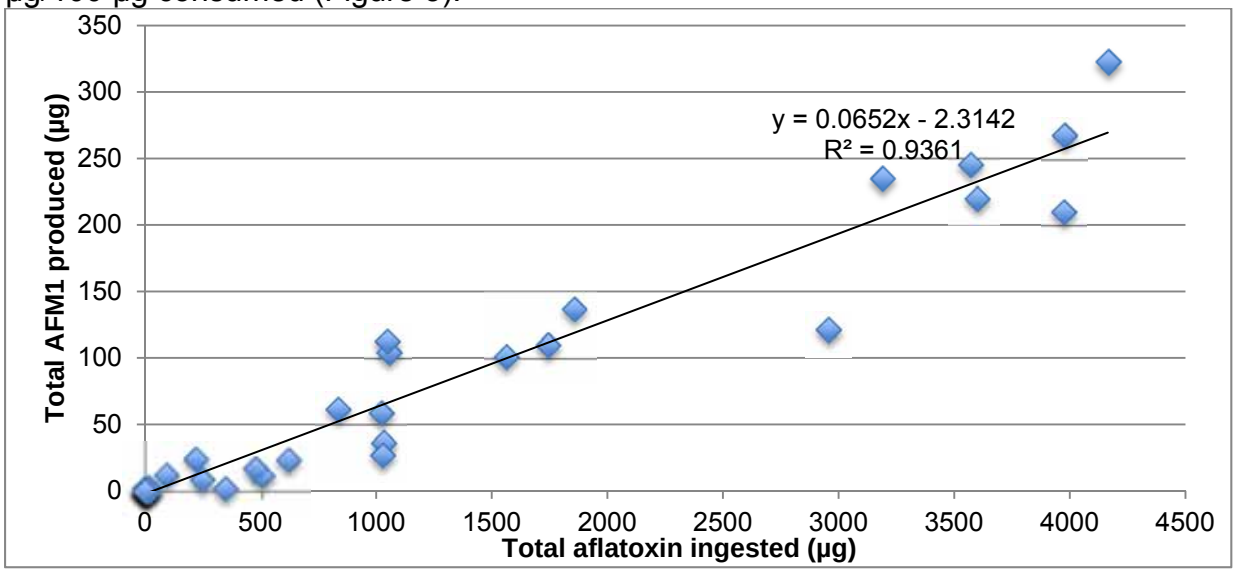

Figure 3. Individual cow data for total mass of AFM1 excreted in milk vs mass of aflatoxin ingested from the feed over the trial period.

Average concentrations of ingested aflatoxin in feed and average concentrations of excreted AFM1 in milk were calculated for each cow in the low and high groups for the experimental feeding period (Figure 4). The vertical and horizontal red lines mark the US regulatory limits for total aflatoxin in feed and aflatoxin M1 in milk respectively. Linear regression was used to calculate the relationship between ingested and excreted concentrations of aflatoxin and AFM1. The linear regression line crosses the line marking the US regulatory limit for AFM1 in milk at an aflatoxin level of 15 µg/kg (ppb) in the feed suggesting that this level of aflatoxin in the feed is the maximum likely to produce milk below the US regulatory limits.

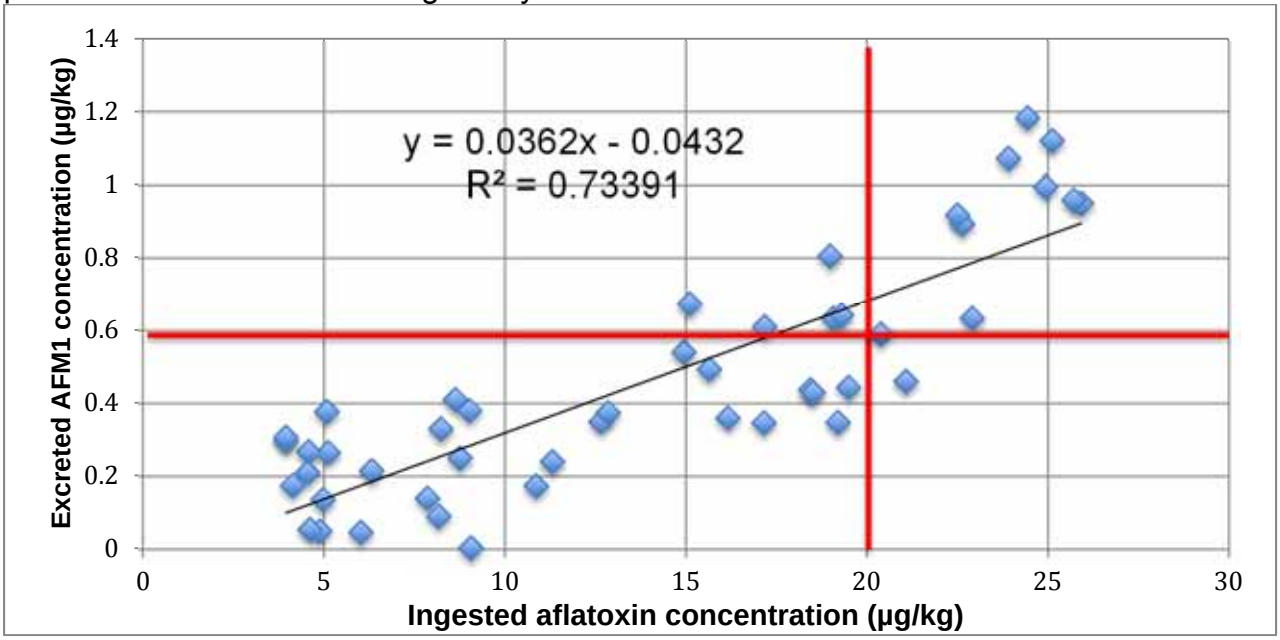

Figure 4. Average concentration (µg/kg) of excreted AFM1 for each cow over the experimental feeding period vs average concentration (µg/kg) of ingested aflatoxin for each cow.

FUTURE IMPLICATIONS AND RECOMMENDATIONS

While commercial feed and some milk production operations screen for a range of mycotoxins, only aflatoxin is subject to action levels by the FDA. In the US, milk is not regularly screened for mycotoxins on a commercial or small farm level. While we did not observe a concerning number of feed or milk samples taken from small dairy farms in upstate NY that violated US regulatory limits, our trial put to the test the safety of feeding high-producing dairy cows feed with the legal limit of aflatoxin present. Our data suggest very strongly that the current “safe” limits of aflatoxin allowable in feed for dairy cows do not protect against violating the current regulations for AFM1 residue in the resulting milk. The carry-over percentage of 6.5% found from our study shows that high-producing dairy cows will have a higher carry-over percentage than the 1 to 2% that has been suggested by previous studies using low-producing cows.

These findings suggest that the current regulations of 20 µg/kg total aflatoxin levels allowable in dairy cow feed are not protective to avoid violation of the 0.5 µg/kg

AFM1 regulatory levels for milk in high-producing cows. Farmers should be vigilant about proper harvesting, storing, and regular testing of feedstuffs for dairy cows to ensure the safety of the animals and the humans consuming their products.

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NUTRITION FOR SKELETAL MUSCLE HEALTH WITH AGING
THE ROLE OF DAIRY PROTEIN

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Sarcopenia and the Graying of society

In the United States, the population over age 65 is increasing, parallel with an increasing human life expectancy (cdc.gov). Although people are living longer, quality of life is not necessarily improving; the number of years of impaired living may actually be increasing due to metabolic disease and physical impairments associated with obesity. Based on a publication in *Nature* in 2012 (Scully, 2012) as we age, the prevalence in impaired activities (≥ 1 activities) of daily living (e.g., getting dressed, bathing, transferring laundry, cooking, etc.) increases, suggesting increased physical dysfunction with aging and a larger window of diminished quality of life for older adults. This is in contrast to the decline in prevalence of metabolically related disease (e.g. cancer and diabetes) with increasing age. Overall, older individuals are faced with reduced ability to perform activities of daily living and loss of independence.

Skeletal muscle plays an important and necessary role in overall human health and independence. Skeletal muscle is essential for physical activity and exercise, skeleton support, metabolic processes and endocrine functions. The human body of a young adult is comprised of approximately 35-45%. However, as we age, skeletal muscle mass declines to approximately 25-30% of body weight by the age of 70 years. Loss of skeletal muscle mass begins as early as the third decade of life (Lexell J, 1988) and continues until death. Intriguingly, men lose skeletal muscle mass and strength and gain fat mass at a greater rate than women (Goodpaster et al., 2006; Janssen et al., 2000). Age-related changes in skeletal muscle, such as the involuntary loss of muscle mass (sarcopenia) and associated decrease in strength, increase the risk for falls and fractures as well as loss of mobility and independence (Bales and Ritchie, 2002; Doherty, 2003) and have been linked to physical disabilities in older adults (Rantanen et al., 1999). Concurrent with sarcopenia, fat and fibrotic tissue accumulation within and around skeletal muscle increase with age which combined likely increases one's risk for functional disability and metabolic dysregulation (Zoico et al., 2004).

There are many endogenous (e.g., muscle denervation, changes in autocrine, paracrine, and endocrine factors, inflammation, oxidative stress, mitochondrial dysfunction, and changes in protein metabolism) and exogenous factors (e.g. decreased physical activity and changes in dietary intake) that influence skeletal muscle deterioration. These effectors instigate muscle atrophy, functional decline, and metabolic dysfunction, which ultimately promotes weakness, loss of mobility, and metabolic disease, culminating in loss of independence.

Optimal therapies to maximize muscle health for older adults are essential. Unfortunately, optimal therapies for preventing or augmenting muscle deterioration have yet to be clearly defined. Dietary protein and branched chain amino acids, particularly leucine, are among the recommended therapies to attenuate muscle deterioration.

Dietary protein and amino acid requirements

Protein balance is important for maintaining muscle mass. Imbalanced protein metabolism from inadequate protein intake generally results in clinical manifestations such as muscle atrophy, impaired muscle (re)growth, and functional decline. Adequate dietary protein is essential for overall human health with recommendations differing throughout the human lifespan. As there are no true body stores for protein, insufficient protein intake to satisfy body requirements leads to a negative protein balance (i.e. protein synthesis lower than breakdown). The Recommended Dietary Allowance (RDA) for dietary protein is 0.8 g · kg body weight^{-1} · day^{-1}. However, in individuals with increased stress (e.g., infection, trauma, etc.), the dietary protein needs increase to minimize skeletal muscle atrophy from rapid degradation of skeletal muscle proteins. There is some consensus that older adults may also need a higher level of dietary protein intake.

Amino acids are the basic building blocks of endogenous proteins. Amino acids are provided from exogenous dietary protein and amino acid sources and from endogenous protein breakdown. On a daily basis, 300 g of endogenous protein are synthesized and degraded; approximately 3-4 % of our total body protein is turned over daily. The overall goal of daily protein consumption is positive protein balance. Protein balance is the balance of protein synthesis and protein breakdown. Amino acids are used for multiple purposes in the body including protein synthesis, energy production, and synthesis of glucose, non-protein derivatives, and non-peptide hormones. During the fed state, amino acids are provided through dietary sources. However, during the fasting state, skeletal muscle protein breakdown provides the amino acids necessary to complete body needs and functions.

Impaired skeletal muscle protein synthesis in old adults

Older adults have a blunted anabolic response to ingestion of dietary protein and leucine, a key essential amino acid for stimulating skeletal muscle protein anabolism. In relatively healthy, older adults, acute studies demonstrate that old (vs. young) adult skeletal muscle has an impaired protein synthesis response to dietary protein intake less than 20 g, but a comparable anabolic response is observed between younger and older adults following 25-30 g of protein in a single meal (Paddon-Jones and Rasmussen, 2009). Additionally, when compared to young adults, old adults have impaired or blunted anabolic responses to amino acid ingestion (7-10 g of EAA (Cuthbertson et al., 2005; Katsanos et al., 2005)). Additionally, research from our laboratory demonstrated that the skeletal muscle gene expression profile reflects an accommodative response to dietary protein in older males, such that older adults given the a diet containing dietary protein intake at and above the RDA had lower expression

of genes related to protein synthesis and modification, while consumption of a diet containing dietary protein at or below the RDA the older skeletal muscle had higher expression of genes related to protein catabolism (i.e. degradation) (Thalacker-Mercer et al., 2010).

Paddon-Jones et al., using the NHANES dataset demonstrated that the U.S. population is inclined to consume protein in a skewed dietary pattern, with more protein consumed at dinner and the least amount consumed at breakfast (Paddon-Jones et al., 2015). Mamerow et al. (Mamerow et al., 2014) demonstrated that consumption of dietary protein in the skewed pattern led to lower 24 h mixed muscle fractional synthesis rate compared to a diet containing dietary protein consumed in an even distribution (i.e. equal amount of dietary protein consumed at each meal throughout the day). Based on these and other studies, investigators have proposed a new pattern of dietary protein intake to maximally stimulate skeletal muscle protein synthesis in older adults, with the overarching goal of maximizing the anabolic response to dietary protein and/ or leucine intake. Researchers proposed older adults consume 30 g of dietary protein at breakfast, lunch, and dinner (90 g of protein consumed daily). The proposed 30:30:30 g protein diet for older adults has raised several concerns by clinicians, including how to get older adults to consume dietary protein at such a high amount, three times a day, when clinicians have a challenging time getting older adults to consume dietary protein.

Dairy protein for maximizing muscle protein balance in older adults

Although relatively few studies have investigated the long-term use of dairy products for skeletal muscle health, acute and short-term studies have demonstrated that dairy protein may provide the necessary substrate for maximal gains in skeletal muscle protein synthesis and muscle mass compared to other protein sources. Studies have demonstrated that milk and whey protein (vs. soy protein) yield better gains in lean body mass (a measurement of body composition containing skeletal muscle mass) when coupled with resistance exercise. Gains in lean body mass among younger adults are potentially due to greater stimulation of muscle protein synthesis and greater net protein balance following resistance exercise coupled with milk consumption (500 mL) compared to soy protein that is isonitrogenous, isoenergetic, and macronutrient matched (Wilkinson et al., 2007). Further, maximal stimulation of protein synthesis, in younger adults, could be attributed to the whey protein in milk products; whey protein stimulates greater protein synthesis and net protein balance after resistance exercise compared to casein and soy (Tang et al., 2009). Similarly, among older adults, whey protein stimulates muscle protein accretion more effectively than casein or casein hydrolysate (Pennings et al., 2011). In a review of studies examining the effects of protein source on changes in lean body mass, Phillips et al. (Phillips et al., 2009) demonstrated that milk and the milk protein whey resulted in greater gains in lean mass with resistance exercise training in both younger and older adults. The research is further supported by Radavelli-Bagatini et al. (Radavelli-Bagatini et al., 2014) who identified that older age women consuming ≥ 2.2 servings per day of dairy (1.5 servings per day of milk) had greater appendicular skeletal muscle mass than older women consuming ≤ 1.5 servings of dairy per day (0.8 servings per day of milk). While these

studies support the promising benefits of dairy products, particularly whey protein, for skeletal muscle protein metabolism and potentially mass, it is important to note that the leucine content of milk and whey protein is 77 and 108 mg leucine / g, respectively, compared to 62 mg leucine / g protein in soy protein; therefore, the beneficial effects of milk and whey proteins could come from greater leucine content in the milk and whey proteins. Never-the-less, research supports that dairy proteins could be beneficial for maintaining skeletal muscle mass in older adults.

Obesity in older adults—challenges faced and the role of dairy protein

Thirty-35% of older adults (65-85 years of age) are obese. Obesity and sarcopenia are both associated with increased risk for falls and immobility and sarcopenic obese older adults have decreased walking speeds and hand-grip strength compared to obese older adults without sarcopenia. Caloric restriction-induced weight loss is controversial for older adults as it initiates loss of lean body mass in conjunction with loss of fat mass. Therefore, there is the need for therapies to reduce fat mass among older adults while maintaining skeletal muscle mass (Smith and Mittendorfer, 2015). High calcium diets have been shown to promote adipocyte apoptosis. Additionally, high branched chain amino acid content in milk protein is an important factor in repartitioning dietary energy from adipose tissue to skeletal muscle. The high calcium and branched chain amino acid content of milk supports a potential dietary therapy that would minimize adiposity and maximize lean body mass (Zemel, 2004; Zemel and Miller, 2004; Zemel et al., 2004).

Milk and other dairy proteins are a promising protein product for older adults

In addition to adverse changes in skeletal muscle protein metabolism and body composition, older adults are challenged by a number of biological and social changes that affect their ability obtain and consume the necessary dietary protein to meet their needs. Challenges include but are not limited to changes in economic status; adverse changes in dentation (loss of teeth and sensitivity to foods); the inability or loss of desire to prepare food; and single serving food preparation.

Table 1. Protein content of common dairy products*

	Protein (g)	Lactose (g)
Greek yogurt, plain, nonfat, 150-170 g	15-17	4-7
Cottage cheese, 0.5 cup	15	3
Milk, 8 oz glass		
Whole (3.25% milk fat)	7.7	12-13
2% milk fat	8.1	12-13
1% milk fat	8.2	
Skim (nonfat)	8.3	12-13

*protein and lactose contents are approximations and will vary based on product and manufacturer.

Dairy products are a high quality protein source (Table 1) and address the observed dietary challenges faced by older adults, including affordability. Dairy protein comes in single servings that require no to minimal preparation and can be consumed despite dentation challenges faced by older adults due to the liquid or soft texture. With ongoing research regarding the balanced 30:30:30 g dietary pattern for protein intake, particularly among older adults, the common goal is to maximize skeletal muscle protein synthesis throughout the day with high quality protein. In general, commonly consumed dairy products offer a great food source of dietary protein for older adults given the dietary considerations faced by older adults.

There is some concern regarding the use of dairy products and higher protein in the diets of older adults. The common challenges and/ or concerns with consuming diets high in protein and/ or dairy products include lactose intolerance and chronic kidney disease. Lactose intolerance occurs as a result of fewer lactase enzymes, the enzymes required to digest lactose. There is a reported higher prevalence of lactose intolerance among African- and Native-Americans (~75 %) as well as Asian-Americans (~90%). However, the lactose content of dairy products varies (Table 1); dairy proteins can be chosen based on their lactose content or lactose-free products are also available. In addition to lactose, there is a general concern regarding the consumption of too much protein. Diets high in protein can lead to kidney stones and/or impaired function of the kidney due to extra metabolic stress. Patients with chronic kidney disease need to limit their protein intake. (http://www2.kidney.org/professionals/KDOQI/guidelines_ckd/p6_comp_g9.htm).

FUTURE DIRECTIONS

Studies have demonstrated dairy products are associated with greater skeletal muscle gains and/ or maintenance of skeletal muscle among older adults suggesting dairy enriched diets may be optimal for muscle health. However, there is insufficient evidence to determine whether gains in muscle anabolism and mass, due to a diet rich in dairy products, are associated with gains in skeletal muscle quality and function. Additionally, research up to this point has focused primarily on the impact of specific proteins or amino acids (i.e. leucine) on skeletal muscle protein synthesis. However, it is likely other bioactive compounds found in dietary protein sources are important for promoting skeletal muscle health and metabolism. Preliminary data from our laboratory suggest that other nutrients, aside from leucine, are essential for skeletal muscle health in older adults. Recognizing dairy products provide a unique nutrient profile associated with skeletal muscle health in older adults is essential for establishing a dairy-enriched diet as an optimal therapy for attenuating sarcopenia.

SUMMARY

In summary, the population over the age of 65 years is a growing demographic in the United States and globally. Older adults are challenged by impairments in activities of daily living and functional decline with advancing age; adverse physical challenges

related to skeletal muscle health. Appropriate therapies to attenuate skeletal muscle deterioration are necessary but have yet to be clearly defined. Dairy proteins offer a promising therapy for maximizing skeletal muscle protein metabolism and gains and potentially preserving muscle during energy restricted weight loss among obese older adults. The translation of acute studies to long-term improvements in skeletal muscle and overall metabolic health of older adults has yet to be addressed. Despite the need for additional research regarding dairy proteins for long-term muscle health, dairy products are an ideal source of dietary protein and other nutrients for older adults, particularly for overcoming physiological and sociological challenges faced with advancing age.

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NEW PERSPECTIVE OF NUTRIENT DIGESTIBILITY AND RETENTION IN DIETS CONTAINING DEFATTED MICROALGAE

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INTRODUCTION

The world population has already reached 7.4 billion and the number is still growing. Total food production has increased every year to meet the massive demand for this rapidly increasing global population. In 2015, the U.S. poultry industry raised 9 billion broiler chickens and served more than 40 billion pounds of chicken products on ready-to-cook basis (Broiler Chicken Council, 2016). Total poultry production has increased from 1,500 million pounds of meat in 1950 to 40,000 million pounds in 2016. Approximately 17 million and 13.6 million metric tons of corn and soybean meal, respectively, are used as animal feed annually. This use directly competes against the need of corn and soybean as a staple for human consumption. Thus, it is necessary to find alternative sources of feed for maintaining sustainable animal production.

Recently, microalgae have gained a great deal of interest for their potential as the third generation of feedstock for biofuel production and the high contents of protein and other nutrients in the defatted biomass as a new source of animal feed. Previous research in our laboratory has shown that broiler chicks, laying hens, and weanling pigs are able to utilize 7.5% to 10% defatted microalgae in their diets without decreases in growth or egg production performance (Ekmay et al., 2014, 2015; Gatrell et al., 2014; Leng et al., 2014; Kim et al., 2016). However, the exact nutrient digestibility or retention of these diets containing the defatted microalgae remains unclear.

NUTRITIONAL APPLICATION OF MICROALGAE AND DEFATTED MICROALGAE

Microalgae are aquatic photoautotrophic single cellular organisms that have the potential to convert carbon dioxide to biofuels, foods, and feeds [Chisti, 2007; Brune et al., 2009]. The biofuel industry has recently developed oil extraction techniques from microalgae that are more efficient than from the biomass of conventional crops [Chisti, 2007]. In addition to high levels of protein and amino acids, the defatted microalgal biomass contains beneficial components including n-3 fatty acids and bioactive compounds [Spolaore et al., 2006]. Both microalgae and their defatted biomass are used as a viable feed protein source for aquatic as well as terrestrial animals [Becker, 2007; Kiron et al., 2012]. In an early study (Combs, 1952), dried Chlorella was used in the broiler diet to replace 10% soybean meal that resulted in improved body weight gain and feed efficiency. Thereafter, a variety of microalgae including *Chlorella sp*., *Spriulina sp*., *Coelastrum* and *Scenedsmus* have been used as animal feed ingredients. During the past seven years, our laboratory has evaluated nutrient composition and feeding values of three types of defatted microalgae including *Staurospira sp*., *Desmodesmus sp*., and

Nannochloropsis Oceanica in the diets for weanling pigs, broiler chicks, and laying hens [Austic, 2013; Ekmay, 2014; Leng et al., 2014]. The defatted microalgae was effective in enriching n-3 fatty acids in the liver and muscle (breast and thigh) of broiler chicks (Gatrell et al., 2015) and eggs (Kim et al., 2016).

NUTRIENT DIGESTIBLITY AND RETENTION OF MICROALGAE

Few studies in the past have determined nutrient digestibility and retention of microalgae. The digestibility of crude protein in *Chlorella*, *Spriulina* and *Coelastrum* were reported to be 89.3, 89.2, and 88.6% respectively, when being used as the sole source of protein (10% of the diet) in rats [Saleh et al., 1985]. Meanwhile, the digestibility of crude protein in *Sprirulina maxima* was found to be between 75.5 to 76.7% at 15% level in the rat diet [Clement et al., 1967]. Although these digestibility values derived from feeding microalgae as the sole source of dietary protein were encouraging, their nutritional relevance was problematic. This was because feeding microalgae over 20% caused adverse growth performance in chicks [Combs, 1952], let alone feeding them with microalgae as the only protein source [Grau and Klein, 1957]. Likewise, adverse performance was also observed from feeding chicks with high levels of Diatom microalgae due to relative deficiency in methionine and cysteine [Austic et al., 2013]. Therefore, the nutrient digestibility or retention of microalgae may be estimated by feeding testing animals graded doses of the biomass and then developing a linear regression equation between the dietary concentration of the biomass and the respective diet digestibility [Kies et al., 2006]. Nutrient digestibility and retention can be determined by direct method (total collection of excreta) or indirect method (using indigestible marker such as chromic oxide). However, indirect determination of mineral digestibility using chromic oxide might not be always reliable because chromic oxide recovery rate varies with different conditions. Direct method provides more accurate estimates, but requires more efforts.

DEFATTED MICROALGAE ON NUTRIENT DIGESTIBILITY AND RETENTION

We determined impacts of supplemental 10% defatted microalgae (*Nannochloropsis oceanica*, 45% crude protein and 3.8% ether extract) from biofuel production in a corn-soybean meal basal diet (BD) on nutrient digestibility and retention in broiler chicks. Day-old hatchling Cornish Giant cockerels were divided into two groups (5 cages/group, 4-5 chicks/cage) and fed the BD or the microalgae diet for 6 week. Starting week 3, chicks were fed diets containing 0.2% chromic oxide as an indigestible marker. Total excreta of individual cages was collected daily for consecutive 5 and 3 d during weeks 5 and 6, respectively. At the end of week 6, chicks were euthanized to collect ileal digesta from 1 chick/cage. Apparent nutrient retention was calculated based on total excreta collection and chromic oxide as an indigestible marker. The latter was also used to estimate apparent ileal digestibility of nutrients. Chicks fed the two diets had similar average daily feed intake and gain/feed ratio. Feeding the microalgae diet enhanced ($P < 0.05$) and decreased ($P < 0.05$) apparent retention and digestibility of dry matter by 3.3 and by 1.8%, respectively. Feeding the microalgae diet elevated (1.6 to 3.8%, $P < 0.05$) apparent retention of ether extract determined by the indirect method,

but not by the direct method. Supplemental defatted microalgae did not affect apparent retention of crude protein determined by both methods at either time-point except for a 17.8% decrease ($P < 0.01$) by the 5-day total collection. Feeding the microalgae diet decreased ($P < 0.05$) apparent ileal digestibility of 8 essential amino acids, and 6 non-essential amino acids, ranging from 32% for isoleucine to 7% for glutamic acid. Feeding that diet also decreased ($P < 0.05$) apparent retention of 6 essential amino acids and 5 non-essential amino acids, ranging from 16% for threonine to 0.6% for leucine. In conclusion, supplementing 10% of defatted microalgae in the corn-soybean meal diet did not show consistent effect on apparent retention or ileal digestibility of dry matter, ether extract, or crude protein determined by the two methods at the two time-points, but the diet decreased apparent retention or ileal digestibility of a number of amino acids.

SUMMARY

Defatted microalgae from the biofuel production have the potential to spare corn and soybean meal as conventional ingredients for feeding food-producing animals. This will help improve fuel, food, and environmental sustainability. For the efficient nutritional application, accurate and systematic determination of nutrient digestibility and retention in the defatted microalgal biomass and diets containing the biomass warrants future research.

ACKNOWLEDGEMENT

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LOOKING AHEAD – DAIRYING IN NEW YORK AND THE USA IN 2066

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Capacity of USA's agricultural land to feed the greatest population is fulfilled best by a diet that includes dairy products (Peters et al., 2016). Dairy provides required nutrients and dairy-based diets rank above vegan diets. This bodes well for the future of dairying. Mankind has been harvesting milk from cows for 200 centuries--that makes it less likely that dairying will not continue.

This dairy futuring exercise was developed initially in response to Michigan State University's invitation to the author to present the 2016 Tucker Endowed Lecture. The author sought and acknowledges inputs from Drs. Mike Hutjens, Chad Dechow, Jeff Stevenson, Pam Ruegg and Gordie Jones of the USA; Drs. Hillary Dobson and Martin Sheldon of the United Kingdom and Dr. Patrice Humblot of Sweden.

OUR WORLD IN 2066

Population of the earth is forecast to be 10.4 billion in 2066 – about 3 billion greater than today (Figure 1). About 80% of the planet's inhabitants will live in Asia or Africa. Population of the USA will be 410 million, a lower global proportion than today. Worldwide, personal income will have increased and increased demand for high-quality dietary proteins will require increased output, efficiency and sustainability.

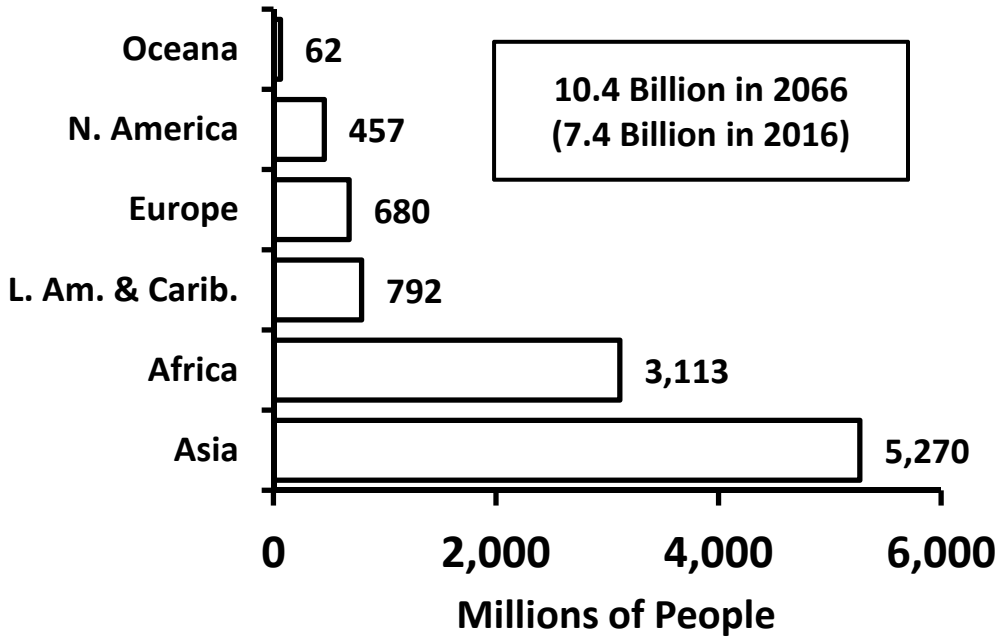

Figure 1. Estimated world population in 2066 (De Wulf, 2016).

CHANGING CLIMATE AND AGRICULTURE

Climate will change by 2066. This will drive shifts in where food is produced. Irrigation water will be rate-limiting, particularly for producing commodity crops, forages and grasslands. High-value crops will be produced with desalinated water. Crops will be genetically altered to use less water and water with higher salinity. Agriculture in the USA will remain highly-dependent on rainfall.

Currently 42% of milk in the USA is produced in states that will have shortages of water in 2066 (Figure 2). Dairy farming in the USA will relocate to regions with more water, particularly the upper Midwest and Great Lakes (Figure 2). In 2066, these regions will have growing seasons extended by 6 to 8 weeks in their most northern latitudes. Annual precipitation will be near current levels. A warmer climate, longer growing seasons and availability of water from precipitation makes these areas attractive for growth in dairy farming.

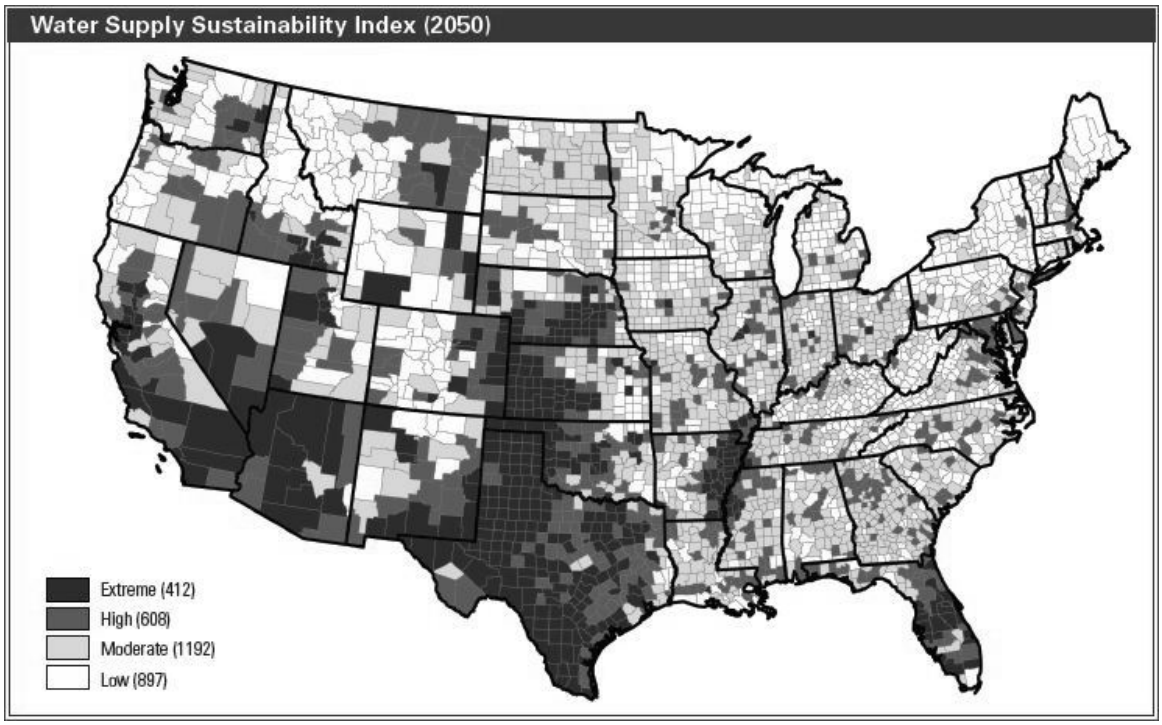

Figure 2. Forecast of water supply sustainability in 2050–darker areas less sustainable.

DAIRY CATTLE GENETICS AND MILK PRODUCTION IN 2066

Commercial dairy cattle in 2066 will comprise genes from multiple cattle breeds, but also from other species and even artificial genes (Figure 3). It is already possible through gene editing to change a single gene and therefore to “crossbreed” at the single gene level. In the future, genetic edits may be done in single embryos, essentially crossbreeding in the laboratory. Genetic companies of the future will market embryos as their primary product for commercial herds.

In 2066, farms will have on-farm systems that separate milk into components or classes, so milk will be sold on the basis of these characteristics. Quantity will still be important because total yield of components is correlated highly with total amount produced.

Annual yield will more than double in 2066 (Figure 4). Linear extrapolation reaches 36,000 lbs of milk per cow and exponential extrapolation reaches 72,000. Forecasters thought 57,500 lbs was on target. Individual cows in the USA first surpassed this level about 40 years ago, and recently a cow produced almost 75,000 lbs a year on 2X milking. The average cow in the USA today produces 2.65-times the amount that the average cow produced 50 years ago. If we multiply today's average by 2.65, it equals 59,341 lbs per year--forecasters feel comfortable with their estimate.

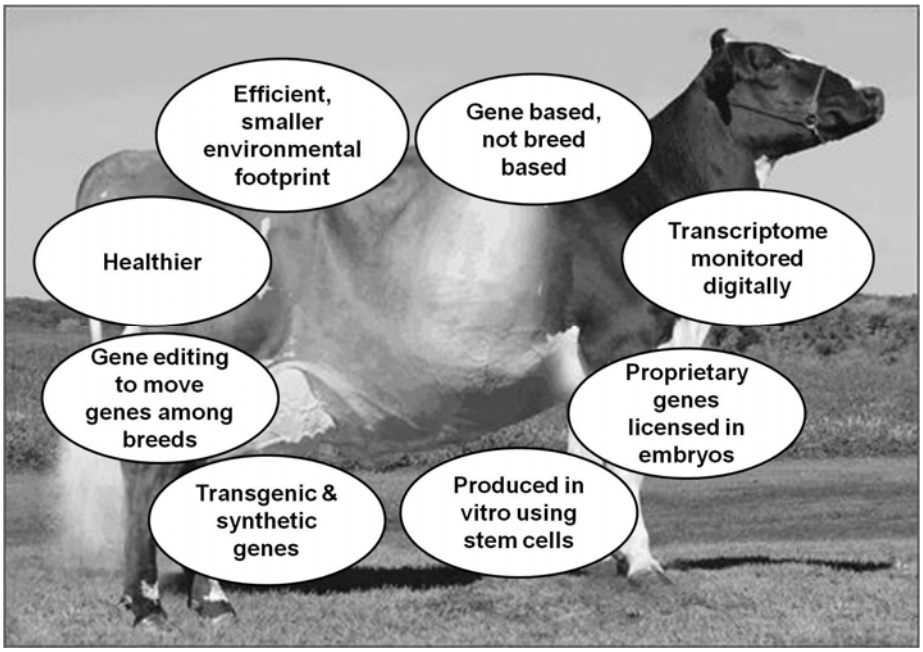

Figure 3. Examples of genetic changes in cows in 2066.

Are yields of milk and crops pushing their biological limits? The author assessed average and top yields in 2014 for three farm products (Table 1). Record yield was 7 to 12 standard deviation units (SDs) above average. Biological capacity to produce is far beyond average output in the USA.

Table 1. Record and average yields in USA.

	Corn (bu/acre)	Soybeans (bu/acre)	Milk (lbs/cow)
Record	504	161	74,650
Average	171	48	22,498
Std. Dev.	47.9	13.0	4,500
R-A(SDs)	7.0	8.7	11.6

Increase in yield per cow will drive down number of cows needed to meet the nation's demand for dairy products. The exact number will depend upon average yield and average consumption of fluid milk equivalents. The author assumed that consumption in fluid milk equivalents will be near today's level of 600 lbs per capita, which has been fairly constant over decades. If average yield reaches 57,500 lbs per cow, then we would need fewer than 5 million cows to supply this demand. These cows will be distributed in 500 to 600 herds nationwide.

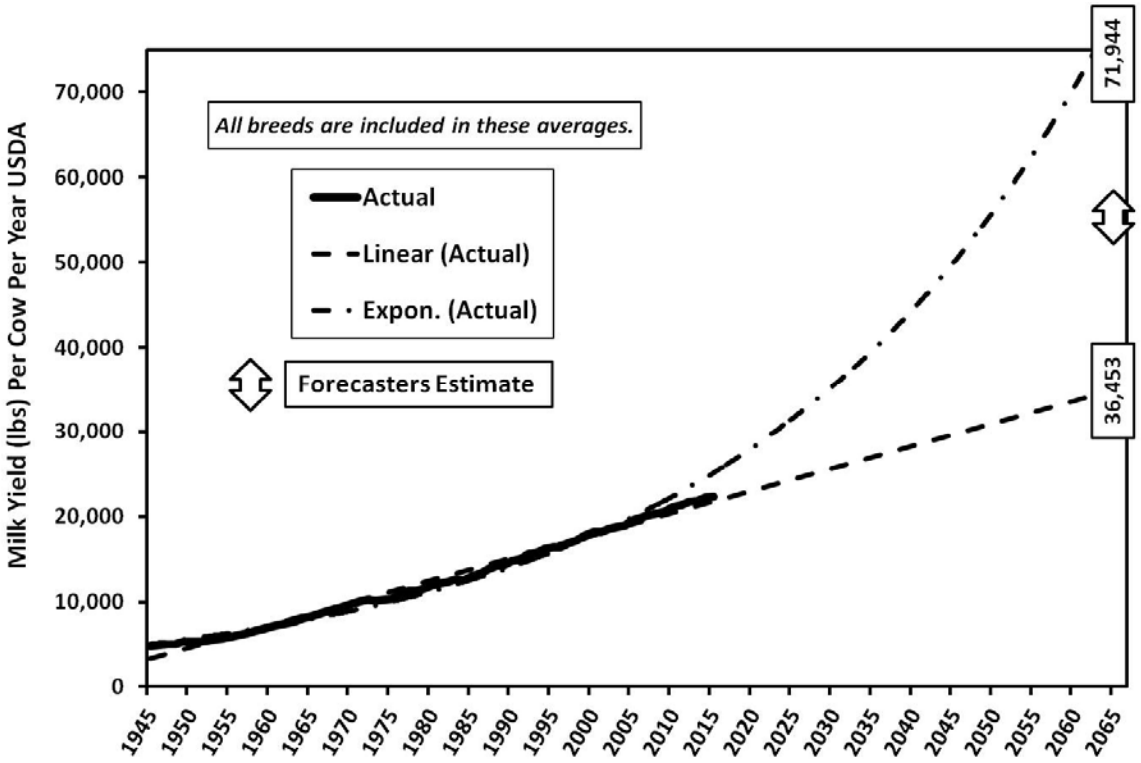

Figure 4. Estimated annual yield of milk in 2066.

DAIRY ENTERPRISES IN 2066

A dairy enterprise in 2066 will have about 10,000 cows in a cluster arrangement (Figure 5) that includes three milking herds supplied through shared heifer and dry cow units. A herd will comprise cows milked in the same parlor. Herds in the USA will be larger than those in Europe, but other countries may have herds of the sizes of the USA. Some general operating characteristics of herds are illustrated in Figure 5. Milk will be separated into market-based components at the farm level. Animal welfare plans will be required and will be inspected regularly. Forage crops will be more digestible and may include energy grasses that have been modified to be highly palatable and digestible.

Agro-ecology practices that ensure that herds operate sustainably within their ecosystems will be required. Nutrient management plans as we know them today may be relegated to history by 2066, because most valuable nutrients will be extracted from waste and used as fertilizer and marketed as co-products. Energy will be produced from

fermentation of residual waste materials and downstream residuals will be used as soil amendments.

Concrete will not be a contact surface for cows' hooves in 2066, but it may provide a subsurface substrate. Carbon-based materials that have high strength along with cushioning properties will provide comfortable surfaces for cows.

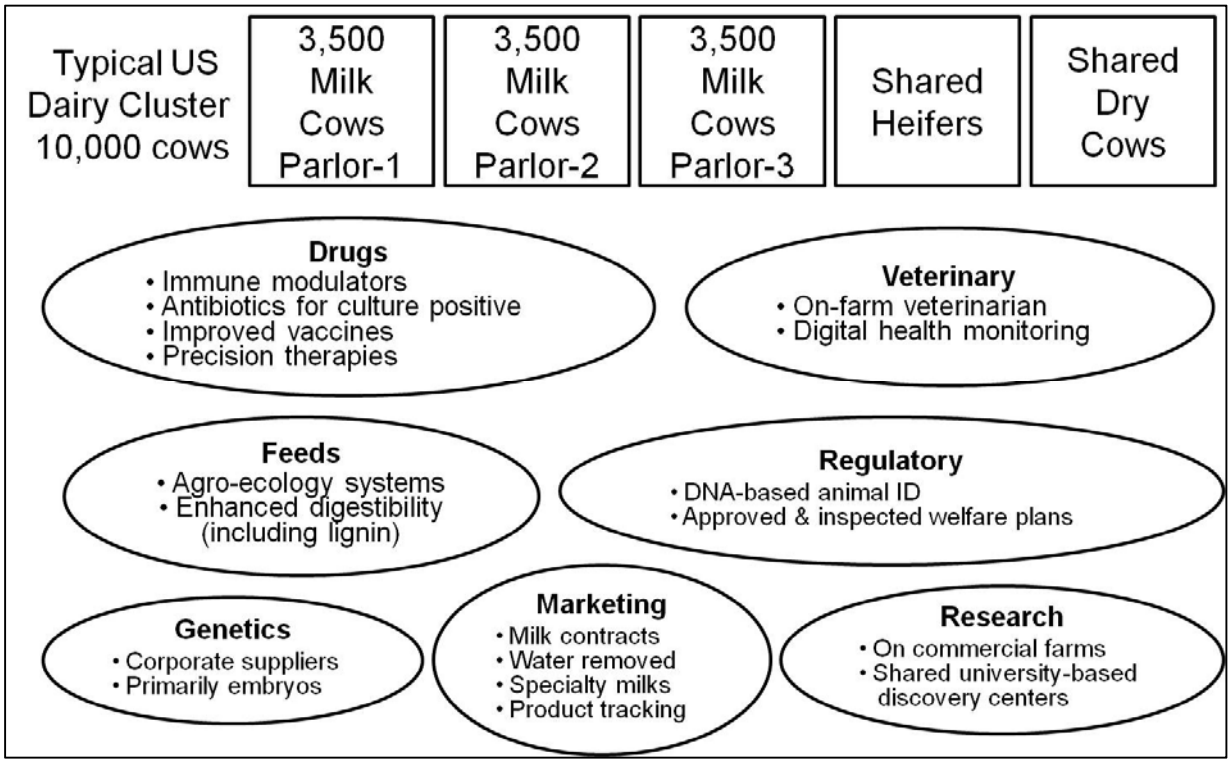

Figure 5. Characteristics of herds in 2066 including milking herd clusters and practices implemented on dairy farms.

DAIRY FARM TECHNOLOGIES IN 2066

Dairy farms in 2066 will be larger and feature robotics, sensors, automation, integrated systems, and natural-based methods for managing health, productivity and sustainability. Herds will be viewed as functioning like independent superorganisms.

Robotics, Sensors, Automation and Integrated Systems

Sensors will be employed across farmsteads to monitor soils, ground water and wells, waterways, roads, natural areas, waste storage systems, bedding and stalls, alleys, feed bunks, silos, commodity sheds, air, odors, particulates, pests, and safety (Figure 6). Some of these will provide data necessary for regulatory actions, but most will be part of enhanced focus on agro-ecology in farming operations worldwide.

Sensors will monitor activities of individual cows, including biodegradable sensors that will monitor gene transcription in the udder and liver. Health, activity and welfare will be monitored through sensors that measure immune status, infection or disease status, rumen function and several other biological traits. Data will be integrated and interpreted by the computer system and cows will have a “green, yellow or red” status for daily management.

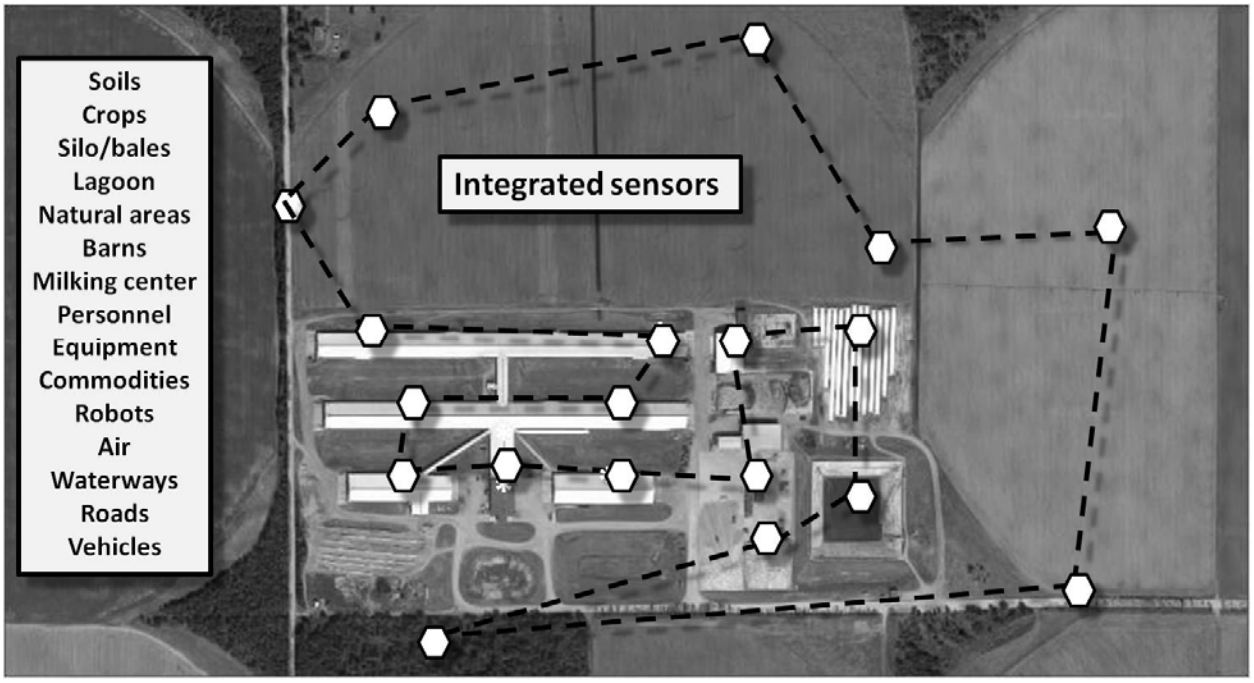

Figure 6. Sensors will be employed extensively on farms to monitor and manage resources and to comply with regulatory requirements.

Robotics and automation will largely replace manual labor on dairy farms and this will improve consistency in feeding, milking and animal health and welfare. Driverless equipment and robots will handle and mix feed, manage stalls and bedded areas, train heifers for entering the milking herd, clean alleys, aisles and milking areas, administer vaccinations, microbiomes, supplements and other products and complete other procedures that are part of the daily routine. In situations where manual labor is necessary, workers will be monitored electronically to ensure and improve compliance with standard operating procedures. Procedures will be improved consistently through feedback by the integrated data system.

Epigenetic Management

On average, about 20% of variation in dairy traits is attributable to DNA sequence or genome. Most variation is attributable to environmental effects according to the classical equation: Phenotype = Genetics + Environment. Environmental effects can be mediated through epigenetic processes in which the DNA sequence is not changed, but the expression of genes is altered. DNA bases can be methylated, histone

proteins can be acetylated and small RNAs can be transcribed to control gene expression, all without a change in the DNA sequence. Manipulation or management of the epigenome in food animals will develop extensively over the next few decades (Sinclair et al., 2016)

The Britt Hypothesis (Britt, 1992; Carvalho et al, 2014) illustrates an example of an apparent detrimental epigenetic effect. In this case, significant negative energy balance during 3- to 5 weeks postpartum results in ovulation of defective oocytes 2-3 months later. Oocytes are affected initially within ovarian follicles during earliest stages of development and are ovulated about 100 days after the follicles are activated. After ovulation and fertilization, oocytes die at a rate much higher rate during 7 days after fertilization. This latent effect is an example of how epigenetics works.

Understanding of epigenetics is reaching a point where epigenetic management is beginning to be deployed in the dairy industry. For example, increasing milk intake and weight gain in heifers before weaning results in increased milk yields 2 years later. This type of delayed effect is classical for epigenetic processes and probably is mediated through an effect on mitotically-active cells like mammary epithelium.

By 2066, there will be many management procedures targeted toward manipulating the epigenome. These will include precisely-timed feeding, supplementing, regulating and controlling the ambient environment or specific processes to alter biological systems. These procedures will enhance reproduction, health, immune status, energetic efficiency, milk quality and yield and other important bioprocesses.

Managing the Microbiome

Animals, plants, soils, stored feeds, manure and the general environment of dairy farms comprise an “unseen” population of beneficial and symbiotic microorganisms (microbiome). Exploitation of this microbiome to enhance health and well-being of cattle, yield of crops, fertility of soils, quality of feeds and sustainability of environmental systems is emerging.

For several decades we have used antibiotics, insecticides, fungicides, sterilants, disinfectants and other anti-microbial materials to control infections, pests and crop diseases without giving appropriate attention to their effect on the beneficial microbiome. This will change quickly as we learn how to preserve and enhance the beneficial microbiome and apply it to benefit biological and environmental systems (Deusch et al., 2015). Examples of how managing the microbiome will be used in dairy herds in 2066 are illustrated in Table 2.

Table 2. Examples of microbiomic management that will be used in dairy farm production systems in 2066.

Agronomic	Environmental	Cow Specific	Therapeutic
Seeds Soils Crops Silages Forages Feeds	Drinking water Waste water Irrigation water Manure Bedding Facilities	Delivered by robotic systems	Sterile packs Intrauterine Intramammary Neonatal Some Rx

Microbiome profiles differ among herds and locations, so there will be no universal product that will work everywhere. Farms will purchase custom microbiome products from suppliers that will provide mixtures for their herd and location. Some products will be proprietary and will demand a premium price. Some products may require a prescription, depending on their activity and their classification by regulatory agencies, especially those that are genetically modified.

Farms will also manage their inherent microbiome profiles by altering practices that are detrimental to beneficial microorganisms. Microbiome scouts, much like crop scouts, will visit farms regularly to sample various microenvironments and provide advice and direction on the best microbiome products to utilize in that herd and location. Testing services will utilize bulk milk, feed, manure and waste water samples to monitor microbiome profiles of farms, much as feed testing is done today.

Feeding the Herd

Feed sources for dairy cattle will differ significantly by 2066. Research on recalcitrance of bioenergy crops will pay off for dairy farmers because high-yielding perennial crops with high levels of starch and high digestibility will displace corn as a feedstuff. In theory, perennial sugar cane has a capacity to yield about 90 tons of dry matter per acre per year with high starch levels (Moore 2009). Cold-tolerant lines of sugar cane are being developed and it will be grown at latitudes extending into the upper Midwest and Great Lakes regions as the climate warms. Other perennial forage grasses that need little nitrogen fertilizer to produce at high levels will also be arising from research that is ongoing today.

Herd as a Superorganism

The commercial dairy production unit is the herd, not the cow, and we have an inadequate understanding of how and why herds differ in health, productivity and sustainability. Generally it is said differences are due to management, but there is ambiguity about what that really means.

Scientists that study colonies of animals such as bees, termites and flocks of starlings refer to these groups as superorganisms. My interest in this was stimulated by reading *Honeybee Democracy* by Dr. Thomas Seeley, a Cornell neuroscientist (Seeley,

2010). Individual bees and terminates in hives or colonies are subservient to the reigning queen, so that makes them somewhat different than individual cows. Nevertheless, superoganisms comprise animals that live in the same environment, consume the same sources of food, are exposed to the same diseases and pests and may be under care of mankind. Does this sound familiar—same environment, same feed, same diseases and same management?

In contrast to those that study hives and colonies, most dairy scientists study individual animals or their systems (digestive, reproductive, mammary), organs, cells or genes. Little of the findings from such studies tell us much about what makes herds different. Should we devote more effort to understanding herds? If so, the herd becomes the experimental unit, not the individual cow. As we consider this, we might start by studying herds located within defined geographic areas that share similar weather, soils, types of crops and markets (Figure 7).

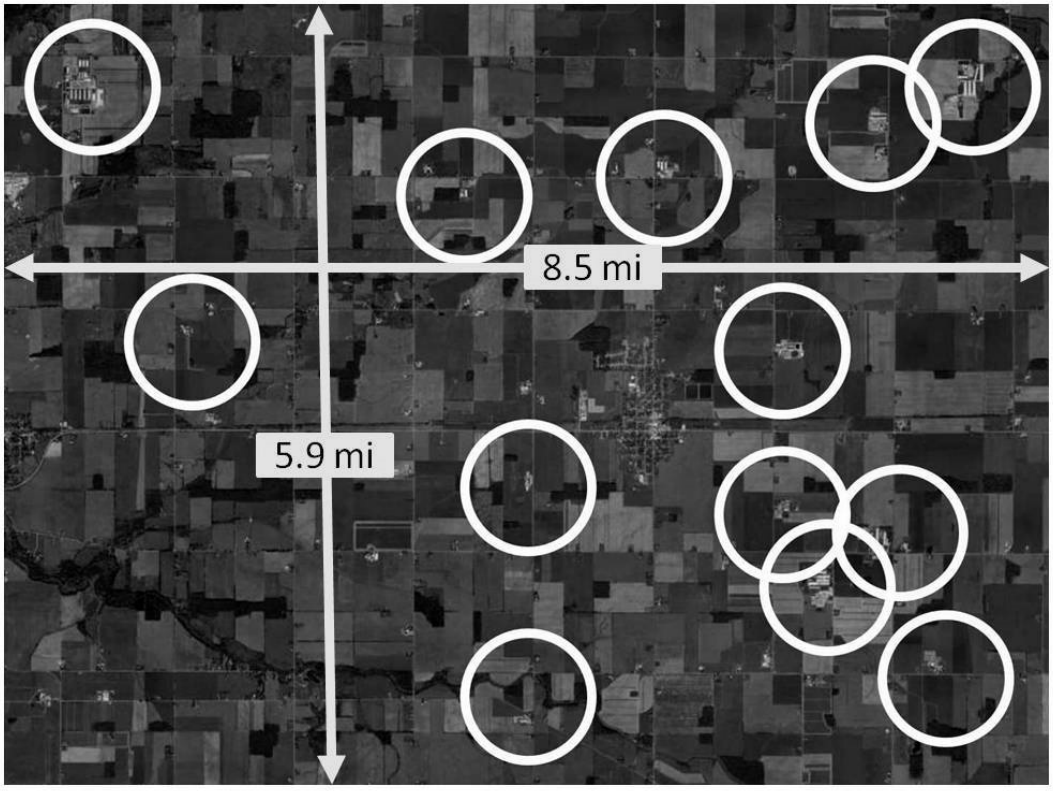

Figure 7. Example of 13 dairy herds in close proximity in a Midwestern area that shares common weather and growing seasons. Herds range from approximately100 to 1500 cows (created with Google maps).

How do goals of owners or managers differ? How do their practices differ? Where does feed quality rank among overall impact factors? Is biosecurity important? What role does compliance play? How do cows within these herds communicate with each other? The list of questions could be very long and there may be some important questions that are not asked because of our preordained opinions.

Why is it important to undertake this effort? By 2066 it will be imperative that food production be as efficient and sustainable as possible. We need answers to questions about productivity and sustainability of herds and the lands and waters that support herds in order to achieve these goals Furthermore, many of the findings would be useful in principal for dairy farmers worldwide, regardless of the sizes of their herds.

An undertaking to study herds should include university specialists, industry suppliers and customers, governmental agency representatives, farmers and farm organizations and consumers. It would be important to engage industrial engineers, data miners, systems specialists and other experts in such a study. Herd owners and managers that participate in such a study should receive periodic feedback that would be beneficial to them in the long run. There would not be typical “control” and “treatment” groups, so the types of statistical analyses might differ greatly from our traditional methodology. This is where outside experts would be extremely helpful.

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EVOLUTION OF THE CNCPS - DEVELOPMENT OF V7

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INTRODUCTION

The CNCPS was first described in a conference proceedings addressing protein requirements for cattle in the 1980's (Fox et al., 1982; Van Soest et al., 1982). Ten years later, a series of manuscripts were published outlining the CNCPS in more detail including carbohydrate and protein digestion (Sniffen et al., 1992), microbial growth (Russell et al., 1992), amino acid supply (O'Connor et al., 1993) and animal requirements (Fox et al., 1992). These publications laid the foundation for a substantial R&D effort over ~30 years that has seen numerous model updates published culminating in summary papers describing new versions of the CNCPS (Fox et al., 2004; Tylutki et al., 2008). The effort continues today with the most recent updates describing v6.5 of the CNCPS (Higgs et al., 2015; Van Amburgh et al., 2015). A unique aspect of the CNCPS, which sets it apart from other modeling efforts, is its widespread commercial use and global reach. Estimates have various versions of the CNCPS being used by thousands of people in dozens of countries to formulate the rations of millions of cattle around the world every day.

The initial intention in developing v7 was to improve predictions of AA supply. Our strategy was to include estimations of protozoal and endogenous N flows from the rumen as part of v6.5. However, given the extensive cycling of N within the rumen, to the liver, and among the entire GIT, it became apparent that to adequately capture all these dynamics, a more holistic approach would be required. The entire GIT of the CNCPS has been rebuilt in v7 along with a new system to estimate post absorptive components of N metabolism such as urea recycling and AA supply. Development of v7 has changed the CNCPS from a static to a more dynamic and mechanistic model. New capability has also been included based on work from (Ross, 2013) and (Raffrenato, 2011). This provides new capability to understand variation in nutrient supply and can help refine ration formulation. A complete model description is provided by Higgs (2014). This paper will focus on aspects of the model that impact N metabolism and its subsequent flow out of the rumen.

AN OVERVIEW OF V7

Model structure

Since inception, the CNCPS has been developed for field application with care taken to ensure model inputs are routinely available on most farms (Fox et al., 2004). Practical application has remained a core philosophy in model development and v7 adheres to the same fundamental principles. While new capability is available within the model, ensuring the model would be field usable was a priority.

Version 7 is constructed in the system dynamics modeling software Vensim (2010). Vensim uses a diagrammatic interface with embedded mathematical statements and calculates iteratively over time. The time unit used in the development of this model is hour, and the model simulates for 300 h with integration every 6 minutes. The simulation time used was the shortest period needed for the model to reach dynamic equilibrium or 'steady state' (Sterman, 2000) across a range of diets. The diagrammatic interface of Vensim is convenient and allows for visual critique of the model which aids interpretation, particularly when considering biological processes. The visual nature also means the model can be more easily understood and interrogated by current and future contributors to the R&D effort.

Diets are generally balanced for the average cow in a group on a per day basis. Although v7 calculates continuously over time, and the unit used within the model is hour, the output from the model is expressed on a per day basis. To do this, the model is sampled for 24 h after simulating for 276 h (once it has reached steady state). Therefore, the format of the outputs generated are similar to those from v6.5.

Several aspects of the model have been completely rebuilt using entirely new approaches. Protein digestion is one example which is now calculated on an N basis and is reconciled by compartment to ensure N balance through the model is correct. This facilitated the construction of a mechanistic system to estimate urea recycling and simplified the estimation of AA flows through the GIT.

Intake and nutrient digestion

Digestion of nutrients in the original CNCPS (Sniffen et al., 1992) followed the system proposed by Waldo et al. (1972) where the kinetics of digestion and passage are integrated to predict substrate digestion. Assuming a single potentially digestible pool, the system can be described by the following equation:

$$\frac{dA}{dt} = -k_1 A - k_2 A$$

where,
A = the amount of potentially digestible substrate in the rumen,
k_1 = the digestion rate,
k_2 = the rate of passage,
t = time in hours.

The derivative of the previous equation gives:

$$R = Ae^{-(k1+k2)t}$$

where, assuming a single feeding,
R = the remaining potentially available substrate present in the rumen after *t* hours,
A = the amount of substrate fed.

Using this system, the ratio of $k_1/(k_1 + k_2)$ gives the fraction of substrate digested in the rumen from a single feeding and has been used to statically capture the dynamics of rumen digestion in both the CNCPS and the protein sub-model of the NRC (2001).

The new rumen sub-model follows the same general system previously used, but because the model is dynamic, rather than static, and calculates continuously, an intake term can and must be added to the model which allows the estimation of substrate pool size at steady state. The general form of the system is shown in Figure 1 and is represented by the equation:

$$\frac{dA}{dt} = k_1 A - k_2 A - k_3 A$$

where,
A = the amount of potentially digestible substrate in the rumen,
k_1 = the rate of substrate intake,
k_2 = the digestion rate,
k_3 = the rate of passage,
t = time in hours.

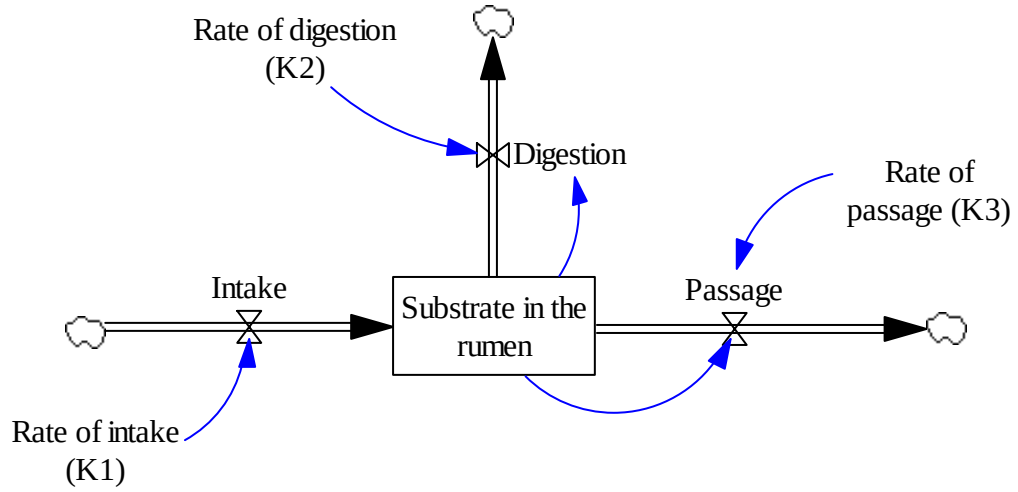

Figure 1. Diagram representing the dynamics of a single pool of substrate digestion in rumen

The pattern of intake affects many aspects of the model including, but not limited to, microbial growth, rumen N supply and rumen pool sizes. Changing the intake pattern from a constant influx to pulses that represent meals creates variation in the predicted rumen pools sizes. More frequent smaller meals result in less variation than larger, less frequent meals. Meal duration is also important with longer slower meals resulting in less variation than the same meal size over a shorter period of time. The model could also accommodate unequal meal sizes allowing for the comparison of different systems (tie-stalls, free-stalls or grazing) and different management scenarios (over-crowding, slug feeding, etc.).

An important factor that intake pattern strongly influences is rumen NH_3-N, which in turn, impacts microbial growth. A comparison of predicted NH_3-N using continuous intake, 4 meals/d and 8 meals/d is found in Figure 2. Microbial growth in the model becomes limited when rumen NH_3-N falls below 5.0 mg/dl (Satter and Roffler, 1975). This interaction causes the behavior observed in Figure 2 when NH_3-N falls below 5.0 mg/dl when the meal pattern is 4 meal/d. The effect of N recycling within the model is evident as rumen NH_3-N slowly increases until the next meal is consumed. The same general pattern is presented by Schwab et al. (2005) using in-vivo data. With continuous feeding and with 8 meal/d rumen NH_3-N remains above 5.0 mg/dl demonstrating the importance of feeding pattern on rumen N supply.

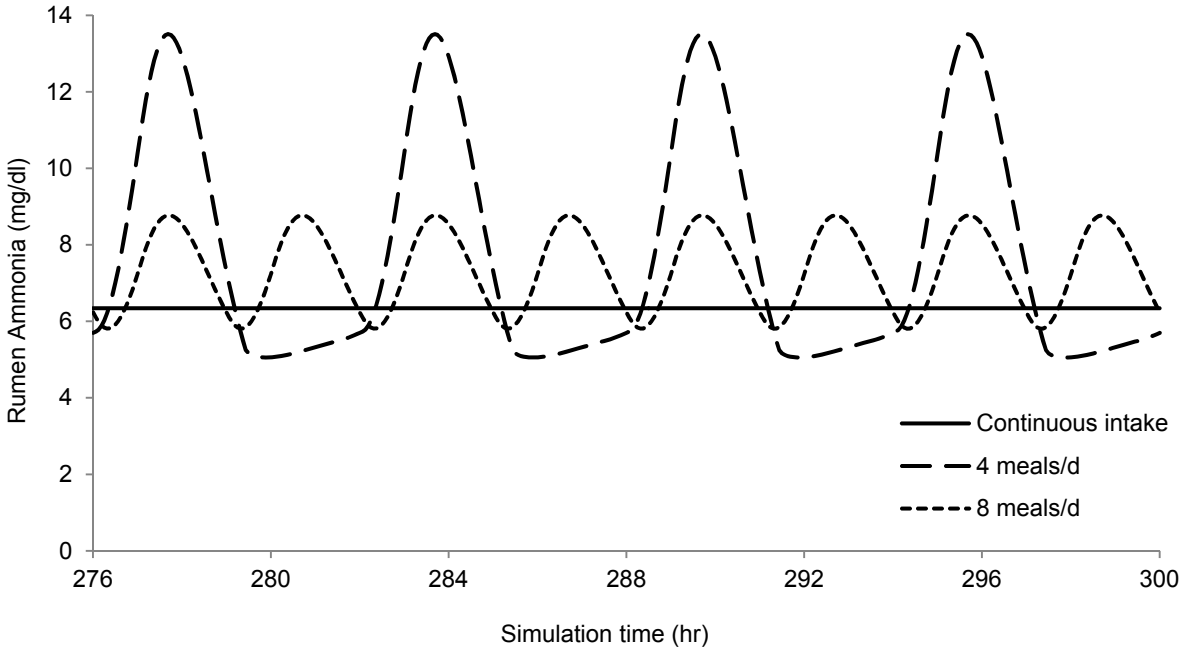

Figure 2. Variation in rumen NH_3-N (mg/dl) among three different meal distributions represented by continuous intake, four meals per day and eight meals per day. The ammonia concentration does not decrease below 5 mg/dl in this example because below that value, microbial yield is decreased thus offsetting the demand.

Protozoa

Protozoa have been accommodated in previous versions of the CNCPS by reducing the theoretical maximum growth yield of bacteria from 0.5 to 0.4 g cells per g CHO fermented (Russell et al., 1992) but do not contribute to digestion or microbial protein production. Protozoa have important effects not only on bacterial yield, but also nutrient digestion and cycling within the rumen (Firkins et al., 2007; Hristov and Jouany, 2005) and can make up 40% to 50% of the total microbial biomass (Hristov and Jouany, 2005). Further, protozoa can contribute 5-10% of the microbial flow in high producing dairy cows, and their AA profile differs to that of bacteria, particularly in Lysine content where it is greater. To capture these effects, aspects of protozoal growth and metabolism

were constructed in v7. To appropriately account for microbial growth, the bacterial growth yield was set at the maximum amount (0.5 g cells per g CHO fermented; Isaacson et al., 1975) to then allow for protozoal predation of bacteria.

An example of how predictions of microbial growth behave, with and without protozoa, are presented in Figure 3. Dietary comparisons include high and low levels of forage at high or low levels of intake. Diets were formulated to provide a 600 kg animal with enough energy and protein to support 45 kg milk at the high level of intake and 20 kg milk/d at the low level of intake. Simulations are run for 300 h which is the time required for all microbial functions to reach steady state within the rumen sub-model.

Using this example, predicted rumen pools of fiber bacteria (FB-N) and non-fiber bacteria (NFB-N) are reduced by protozoal growth. This occurs due to predation and also competition for substrate. Non-fiber bacteria are most affected as they exist in the fluid phase and are more accessible for protozoa to engulf (Dijkstra et al., 1998). Fiber bacteria are also engulfed as a collateral effect of fiber engulfment (Dijkstra et al., 1998). Protozoal pool sizes when intake was high were 4.2% and 9.2% of the microbial N for the low and high forage diets, respectively, and are within the range and follow the same trend reported by Sylvester et al. (2005). Pool sizes on the lower intake diets are higher which is due to lower predicted passage. A positive feedback exists within the model where, as the protozoal cell mass increases, more substrate can be engulfed. This is controlled by lysis, passage and also the ability of protozoa to digest engulfed material.

Protozoa make a significant contribution to microbial protein turnover in the rumen which increases peptides, free AA and NH_3-N (Walker et al., 2005). In situations where rumen N is deficient, the effect of protozoa in the model stimulates bacterial growth and CHO digestion through increasing the rumen N supply, although net microbial flow out of the rumen is still reduced through predation. Predicted microbial turnover ranged from ~10% to 40% which is lower than what is typically reported (Hristov and Jouany, 2005), but this might be expected in high producing animals (Firkins et al., 2007). Overall efficiencies of microbial growth in the faunated simulations ranged from 17.4 to 28.5 g microbial N kg^{-1} RD OM which is similar to the finding of Broderick et al. (2010). Values in the defaunated simulations were higher than what might be expected and demonstrates the importance of including protozoa in the model.

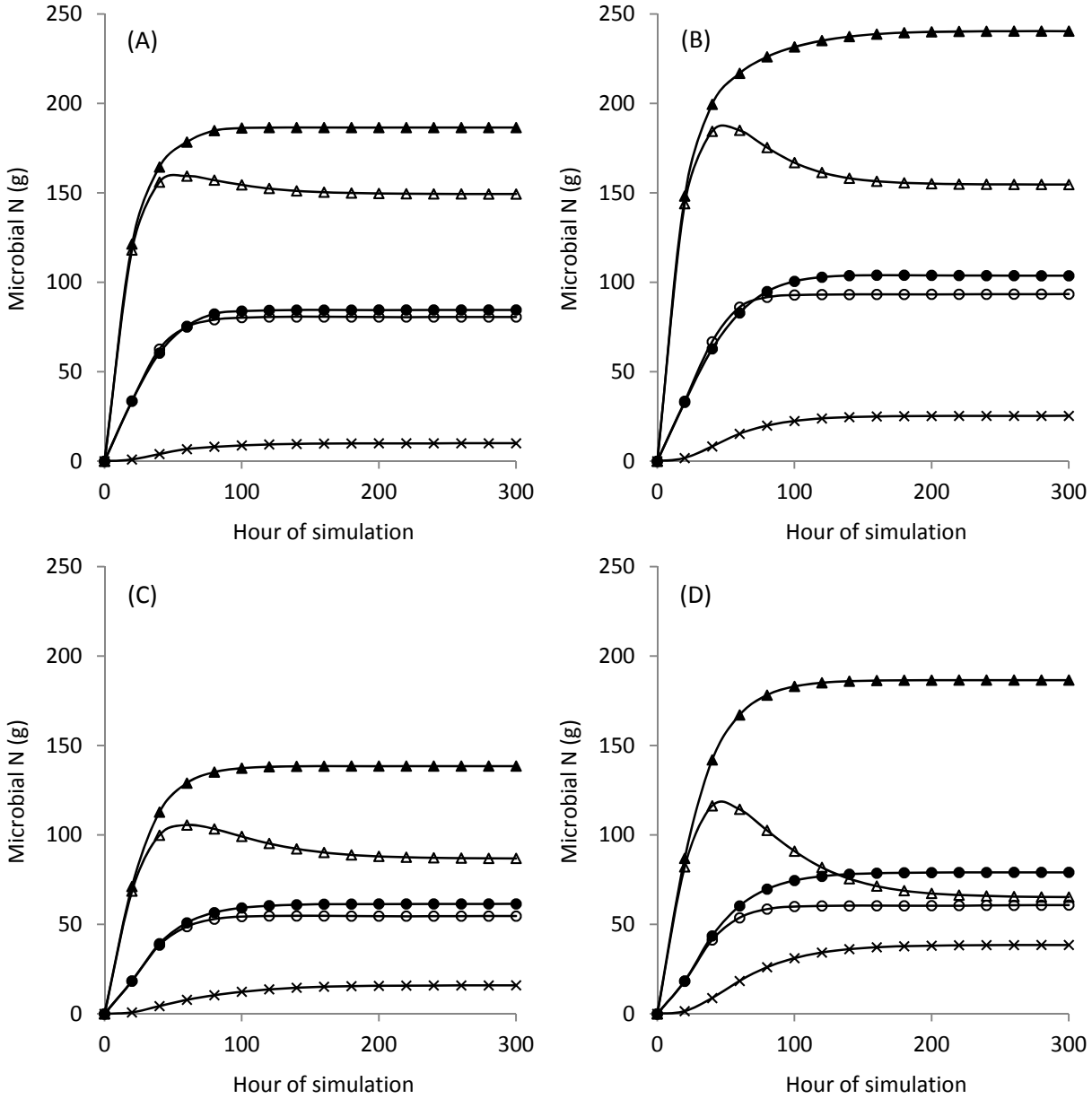

Figure 3. Rumen microbial N pools in diet simulations at high intakes with low (A) or high (B) levels of forage or low intakes with low (C) or high (D) levels of forage where the rumen was either faunated or defaunated. Microbial populations in the faunated rumen include: Non-fiber bacteria (∆), fiber bacteria (○) and protozoa (×). Microbial populations in the defaunated rumen include: Non-fiber bacteria (▲) and fiber bacteria (●).

Intestinal digestion

In previous versions of the CNCPS, material that escapes rumen digestion and arrives in the lower GIT can either be digested or passed out in the feces (Sniffen et al., 1992). This is calculated using an intestinal digestibility coefficient that represents the entire lower GIT. In reality, digestion in the small intestine and large intestine occur by

different processes with the small intestine being enzymatic and the large intestine fermentative (Van Soest, 1994). In the current model, digestion in these two compartments has been separated with digestion in the small intestine modeled using a single digestion coefficient, while the large intestine utilizes a mechanistic structure, similar to the rumen model.

Urea recycling

Ruminants have a remarkable ability to recycle N back to the GIT on order to sustain favorable conditions for microbial protein synthesis and in general, this recycling appears to be an obligate function with a low energy requirement (Reynolds, 1992). While previous versions of the model have accounted for N recycling (Fox et al., 2004), the dynamics are difficult to capture in a static model. A great deal of work has taken place to try and understand the exact mechanisms and quantitative aspects of N recycling, and while the exact mechanisms remain evasive, quantitative aspects of N fluxes are reasonably well understood and described (Lapierre and Lobley, 2001; Marini et al., 2008; Marini and Van Amburgh, 2003; Recktenwald et al., 2014).

The proportion of urea returned to the GIT relative to urea production is remarkably uniform among experiments when animals are fed diets at, or in moderate excess of MP requirements (Lapierre et al., 2004, Ouellet et al., 2004, Recktenwald, 2007, Valkeners et al., 2007). However, recycling increases when N supply is limited (Reynolds and Kristensen, 2008, Valkeners et al., 2007) and decreases when N supply is greatly in excess (Lapierre et al., 2004, Reynolds and Kristensen, 2008). To estimate the proportion of urea returned to the GIT in v7, the equations presented in Recktenwald et al. (2014) and Reynolds and Kristensen (2008) were used in combination. Recktenwald et al. (2014) showed a linear relationship between urea production and urea recycling in high producing cows fed diets ranging from 15% - 17% CP, while, Reynolds and Kristensen (2008) showed an increase in the proportion of urea recycled at very low N intakes. Therefore, using the equations in combination allowed for a wider range in dietary conditions to be represented. Recycled urea is distributed to either the rumen, large intestine or small intestine and continues to cycle through the system at steady state. To integrate these transactions, a new system of N recycling was constructed in v7.

A summary of the pools and flows of N in v7 once absorbed from the GIT is found in Figure 4. The model assumes non-ammonia N absorbed in the small intestine (NAN Ab to PDV) has two general fates: 1) it is utilized for a function of maintenance or production (Liver-NAN Utilized) or, 2) it is converted to urea in the liver (Liver-NAN to Urea). Nitrogen requirements for maintenance or production include milk, growth, reserves, fetal growth, scurf, metabolic urinary losses and gut secretions. Absorbed NH_3-N from either the rumen (R-NH3 N to PDV), large intestine (LI-NH3 N to PDV) or small intestine (SI-NH3 N to PDV) is assumed to be completely converted to urea in the liver (PDV-NH3 to Urea). Nitrogen converted to urea can either be returned to the GIT (Urea-N Liver Recycled to the GIT), or excreted in the urine (Urea-N Liver Irreversible Loss).

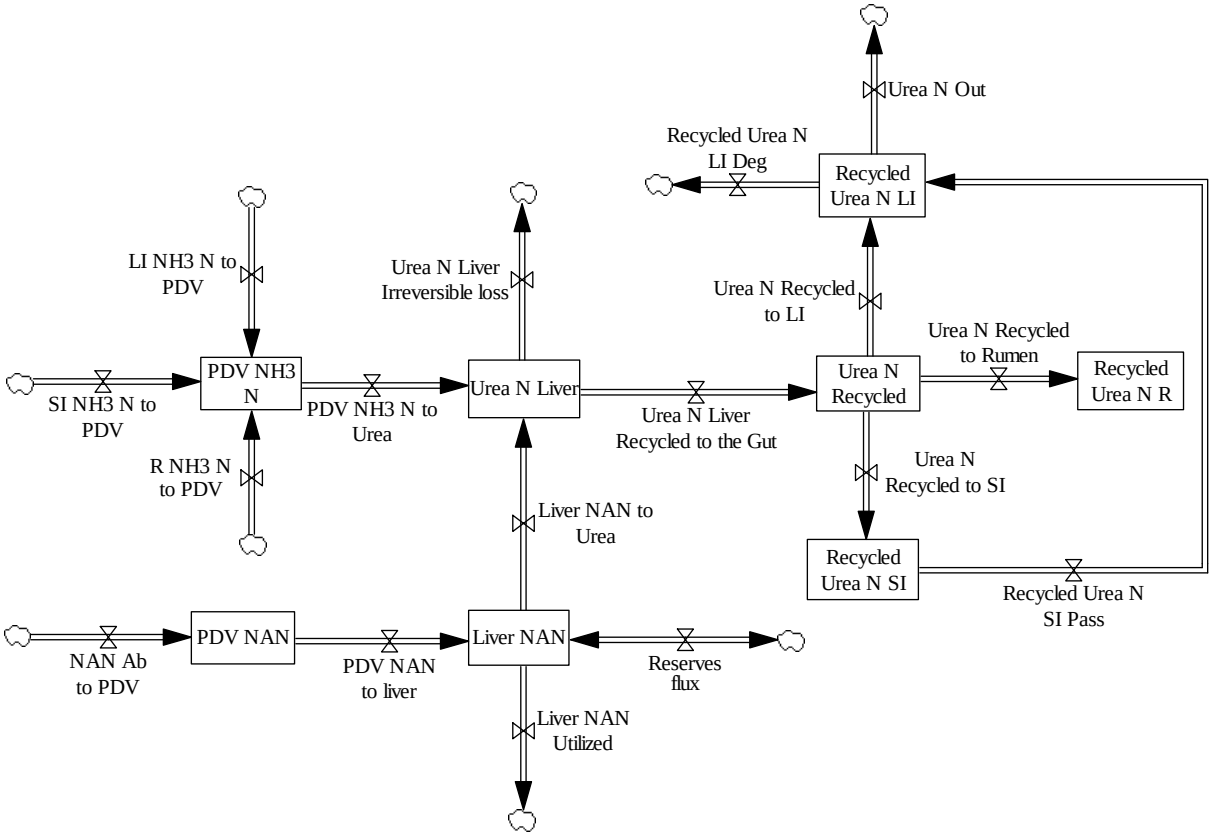

Figure 4. Post absorptive N transactions in the model. Boxes represent pools and arrows represent flows.

Endogenous nitrogen

The contribution of endogenous AA to total AA flows were recognized by O'Connor et al. (1993) when the original CNCPS was being described, but at the time, it was deemed there was not enough quantitative information available to include them in the model. A great deal of work has been conducted since the 90's which has improved the quantitative understanding of endogenous N transactions along the GIT (Marini et al., 2008; Ouellet et al., 2010; Ouellet et al., 2004; Ouellet et al., 2002). Using these data, endogenous N (EN) losses into the GIT were modeled mechanistically in v7 to capture the various transactions along the GIT and between microbial pools.

Endogenous secretions occur at various places along the GIT. Important sources include saliva, gastric juices, bile, pancreatic secretions, sloughed epithelial cells and mucin (Tamminga et al., 1995). Gross EN to the forestomach and intestines were estimated according to Ouellet et al. (2002) and Ouellet et al. (2010) which were subsequently partitioned into individual components (Table 1) using estimates reported in Egan et al. (1984). Endogenous contributions are reasonably consistent among diets when expressed relative to DMI or OMI (Marini et al., 2008; Ouellet et al., 2010; Ouellet et al., 2002; Tamminga et al., 1995). Thus, the model expresses each component as g

EN per kg DMI. A summary of the EN contributions to various points in the GIT are in Table 1.

Table 1. Endogenous contributions used to predict endogenous AA requirements and supply in v7 of the CNCPS.

Endogenous component	Secretion (g N/kg DMI)
Saliva	0.9
Rumen sloughed cells	4.3
Omasum/abomasum sloughed cells	0.3
Omasum/abomasum secretions	0.2
Pancreatic secretions	0.4
Bile	0.1
Small intestine sloughed cells[1]	0.7
Small intestine secretions[1]	0.7
Large intestine sloughed cells	0.3

[1] Includes secretions past the pancreatic and bile duct and prior to the terminal ileum

The mechanistic framework of v7 enabled EN to be modeled in all parts of the GIT including the microbial transactions in the rumen and large intestine. Endogenous N transactions through each compartment in the model are summarized in Figure 5 using the 'Hay' treatment in the study of Ouellet et al. (2010) as an example. Endogenous N in the rumen has three potential fates: 1) It is degraded to ammonia; 2) escapes the rumen and can be digested in the SI 3) or is incorporated into microbial protein. In the example used, total EN secretions into the GIT were 135.4 g/d of which 46.4 g/d was recovered as either free EN in the duodenum or incorporated in microbial protein. The balance (89.0 g/d) was considered lost by the animal and part of the maintenance requirements for protein. Of the 89.0 g/d lost, 31.8 g/d appeared in the feces and 57.2 g/d was degraded in the GIT to NH_3. The total estimated requirement (89.0 g/d) when expressed relative to DMI is 5.1 g EN/ kg DMI which, interestingly, is similar to estimates of metabolic fecal N for the same diet (5.0 g MFN/kg DMI) in v6.5. Metabolic fecal N is the major MP requirement for maintenance in v6.5. Therefore, although v7 estimates these losses in an entirely different way and accounts for their reutilization (microbial incorporation), the maintenance requirements for protein are similar to v6.5.

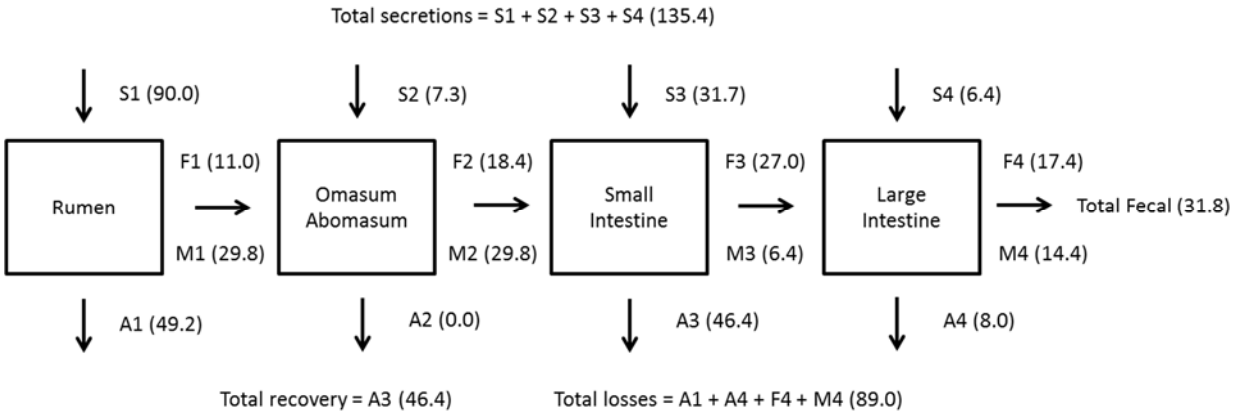

Figure 5. Model predicted endogenous transactions (g endogenous N/d) by compartment for the hay treatment presented in Ouellet et al. (2010). S1-S4 are the endogenous secretions into the gut; F1-F4 are the flows of free endogenous N; M1-M4 are the flow of endogenous N in bacteria; A1-A4 is the endogenous N absorption at different sites. Recovery is only possible in the small intestine (A3) where the N can be absorbed as AA.

AMINO ACID REQUIREMENTS

Requirements for each individual EAA in the CNCPS are predicted for processes that are quantified by the model (maintenance, lactation, pregnancy, growth) and subsequently divided by the efficiency of transfer to that process to give the total AA requirement (O'Connor et al., 1993; Fox et al., 2004). The efficiency of transfer could also be thought of as the additional requirement for each AA relative to the requirements quantified by the model. Such processes include oxidation across the gut or in other tissues, anaplerotic requirements, synthesis of non-essential AA, gluconeogenesis etc. (Lapierre et al., 2005; Lapierre et al., 2006; Lemosquet et al., 2010; Lobley, 2007). The apparent efficiency of AA use for any given diet can be calculated by dividing model predicted amino acid requirement (AAR) by amino acid supply (AAS), which can be variable, and typically decreases as AAS increases relative to AAR and also energy (Hanigan et al., 1998). This decrease in apparent efficiency of AA use represents AA being increasingly used for purposes other than those quantified or described by the model. If the utilization of each AA for every process in metabolism could be adequately quantified, the term ‘efficiency of use’ would become obsolete as it would be 100% (there would be no additional requirement above model predictions). The ability of cows to direct AA to other uses demonstrates the interactions among different nutrients and is an example of the metabolic flexibility that allows productivity to be maintained across a wide range of nutrient inputs and supply (Lobley, 2007). The pertinent question for ration balancing is: what level of additional AA supply is required above the predicted requirements for milk protein synthesis and body protein requirements to maximize productivity and minimize AA wastage? The answer to this question is going to differ among models as supply and requirements are calculated in different ways.

The optimum supply of EAA in v7 was estimated similarly to Doepel et al. (2004) using a dataset of studies that infused AA into the abomasum, duodenum, or

intravenously and fitted a logistic curve (Higgs, 2014). The optimum supply of each EAA was defined as the point in which a logistic curve was approaching plateau most rapidly (Lysine example; Figure 6). This point is similar to the break-point in the segmented linear model used in the NRC (2001). The optimum ratio of model predicted AAR to AAS (efficiency of use) for each AA in v7 are in Table 2. The impact of energy supply on the utilization of AA was also investigated by regressing the ratio of AAR and AAS against AA supply relative to total ME (Lysine example; Figure 7). Interestingly, the optimum supply of Met and Lys estimated using this approach was 15.1% and 5.7% of EAA, respectively, which is similar to results found in other studies that used different approaches (Rulquin et al., 1993; Schwab, 1996; Schwab et al., 1992). However, under these circumstances, no relationship was observed between the 'efficiency' of AA use when AA supply was expressed relative to MP supply but a strong relationship was observed when AA were expressed relative to ME supply which is in agreement the findings of Van Straalen et al. (1994). These data suggest when balancing rations it might be more appropriate to consider AA supply relative to ME which is the approach used in swine (NRC, 2012). Establishing requirements for monogastrics is less complicated than in ruminants as the true AA supply is more easily determined (Lapierre et al., 2006). And to extend the comparison, the predicted Lys requirement for a lactating sow in the NRC (2012) model is 2.72 g Lys/Mcal ME which is similar to the 3.03 g Lys/Mcal ME calculated in this study for dairy cows. Likewise, the recommended ratios for each EAA and Lys are similar in the dairy cow and sow with the exception of Met and His (Table 2). These data suggest, as improvements are made to the predictions of true AA supply in dairy cows, consideration of the approach used to balance AA in other species where AA supply is more easily determined could provide opportunities to improve productivity and the efficiency of nutrient use. The data in Table 2 do not represent recommendations for v6.5 of the CNCPS. Those recommendations are provided by Van Amburgh et al. (2015).

Table 2. Efficiency of use and optimum supply of each EAA relative to total EAA, ME and Lys.

AA	Efficiency of use	% EAA	g AA/ Mcal ME	Lys:AA Dairy[1]	Lys:AA Swine[2]
Arg	0.55	10.2%	2.04	1.49	1.85
His	0.70	4.5%	0.91	3.33	2.50
Ile	0.61	10.8%	2.16	1.40	1.78
Leu	0.67	17.1%	3.42	0.89	0.89
Lys	0.62	15.1%	3.03	1.00	1.00
Met	0.53	5.7%	1.14	2.66	3.71
Phe	0.53	10.7%	2.15	1.40	1.82
Thr	0.53	10.7%	2.14	1.41	1.49
Trp	0.58	2.9%	0.59	5.16	5.33
Val	0.62	12.4%	2.48	1.22	1.15

[1] Optimum Lys:EAA ratio for the data set used

[2] Optimum Lys:EAA ratio for a lactating sow (NRC, 2012)

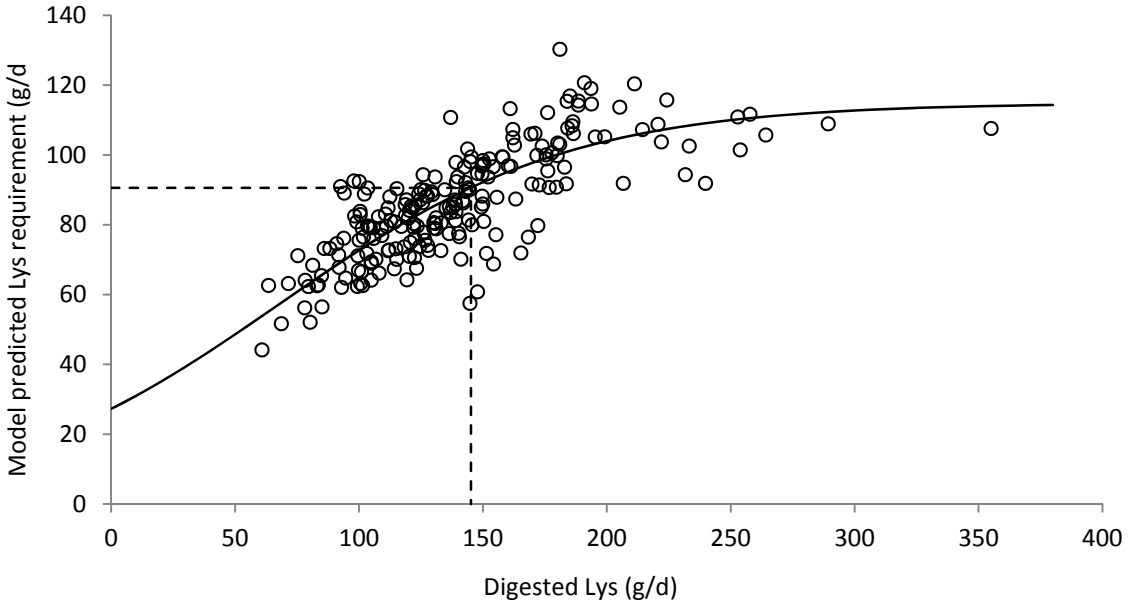

Figure 6. Logistic fit of model predicted Lys requirement and Lys supply. The dashed line represents the optimum ratio of Lys requirement and Lys supply

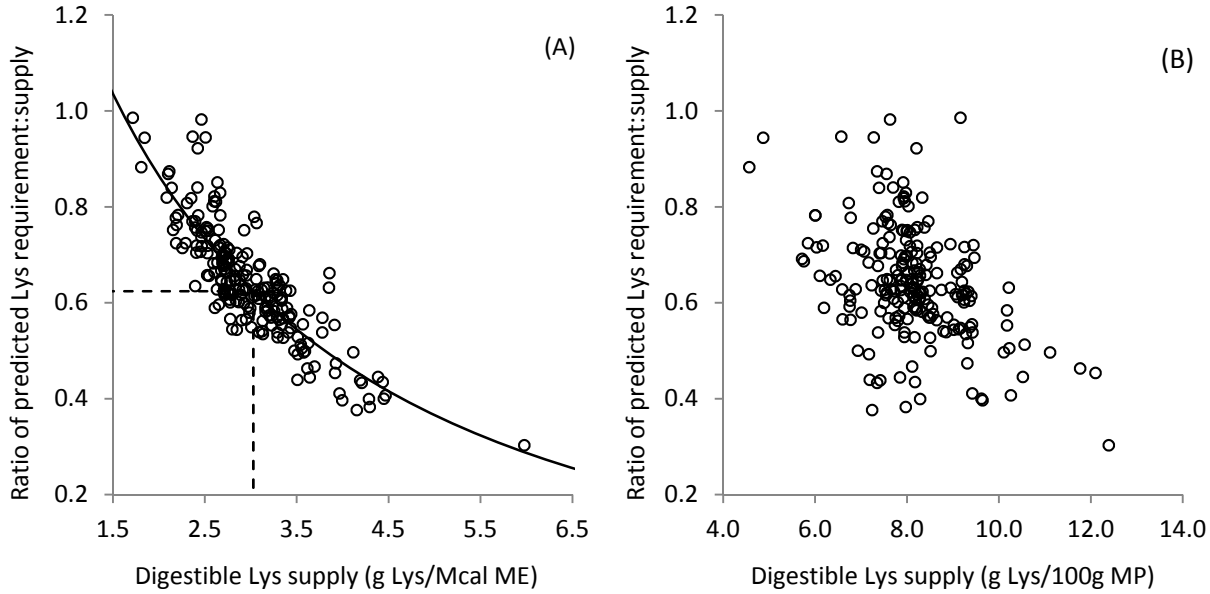

Figure 7. Relationship between model predicted Lys requirement:supply and Lys supply relative to ME (A) or MP (B). The dashed line in (A) represents the Lys supply at the optimum ratio of model predicted Lys requirement and supply. No significant relationship was determined in (B).

MODEL PERFORMANCE

The sections of v7 described here, and in Higgs (2014), represent an implementation of advancements that have been made in the understanding of N availability to the animal, including improvements in the characterization of feed chemistry (Higgs et al., 2015), multiple pool NDF digestion (Raffrenato, 2011), quantification of endogenous N flows (Ouellet et al., 2010; Ouellet et al., 2002), estimates of N availability in the small intestine (Ross, 2013) and changes to estimates of microbial growth to include protozoa. The broad goal of these updates has been to improve the ability of the CNCPS to predict N flows out of the rumen, to the small intestine, and the availability of AA to the animal. Validating the changes to the model against animal data is an important step in establishing the efficacy of the model updates. The data used to evaluate the N flows from the rumen were sourced from studies that measured microbial N (MN), rumen undegraded feed N (which would include endogenous N; RUN) and total non-ammonia N (NAN) flows at the omasum (16 publications; 61 treatment means). This dataset has previously been used by Van Amburgh et al. (2015) to evaluate v6.5.

Incorporation of protozoa into the dynamic structure of v7 represents a considerable change in the system used to estimate microbial growth in the CNCPS. Compared to omasal sampling data, predictions of microbial N flows were more accurate and had less bias than v6.5, particularly when intake was high (Figure 8; Van Amburgh et al., 2015).

Prediction of RUN was more variable than MN and tended to be over-predicted when RUN flows were high (Figure 9). What is generally reported in the literature as feed N will typically also include endogenous secretions as feed N is calculated as the difference between total NAN and MN (Broderick et al., 2010). Any error in the prediction of MN or NAN will be pooled in the estimates of RUN and, therefore, more variability might be expected. Also, the predictions of RUN rely on library values to estimate the rate of N digestion of the various N fractions which can vary within and among feeds (Broderick, 1987; NRC, 2001). Estimating digestion rates of feed N *in vitro* is challenging due to contamination with microbial protein (Broderick, 1987). However, relying on library values is no doubt one of the major limitations to improving predictions of AA supply in ration formulation models. Although some bias was observed in this version of the CNCPS, the slope and intercept were closer to unity than observed for the NRC (2001) by Broderick et al. (2010) and v6.5 by Van Amburgh et al. (2015).

Total NAN was predicted accurately, precisely and with little bias (Figure 10). The relationship was similar to v6.5 (Van Amburgh et al., 2015), however, earlier versions of the CNCPS do not include direct predictions of endogenous N or protozoa. Therefore, the apparent accuracy of v6.5 suggests an over-prediction of undegraded dietary protein flow out of the rumen. Given that endogenous N, feed N, bacterial N and protozoal N have different AA concentrations, profiles and intestinal digestibility, an important first step in improving predictions of AA supply is to accurately estimate the source of N. Version 7 accounts for each N component individually and appears to predict total N flows more accurately than previous version of the model. The subsequent prediction of individual

AA flows is beyond the scope of this paper, but has also been improved. The evaluation can be found in Higgs (2014).

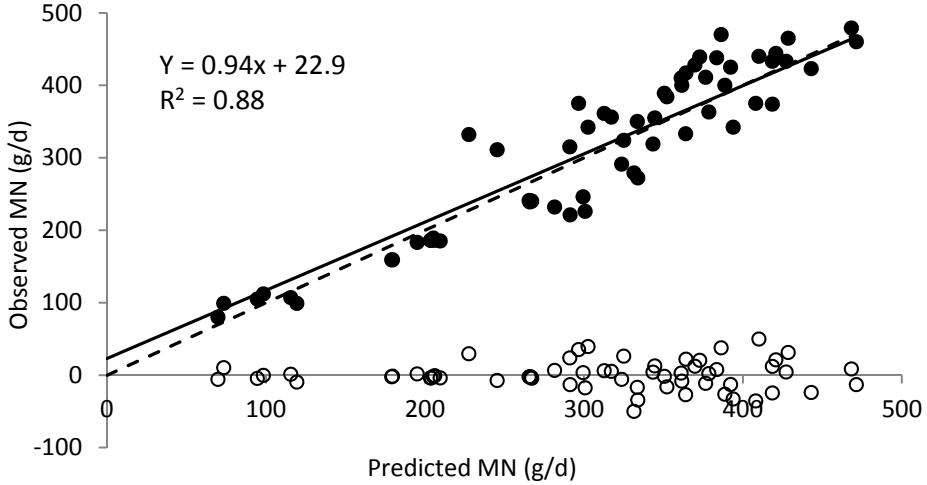

Figure 8. Predicted and observed microbial N (MN) flows at the omasum (●) and residual error (○) from the mixed model regression analysis. The solid line (—) represents the linear regression and the dashed line (- - -) is the unity line.

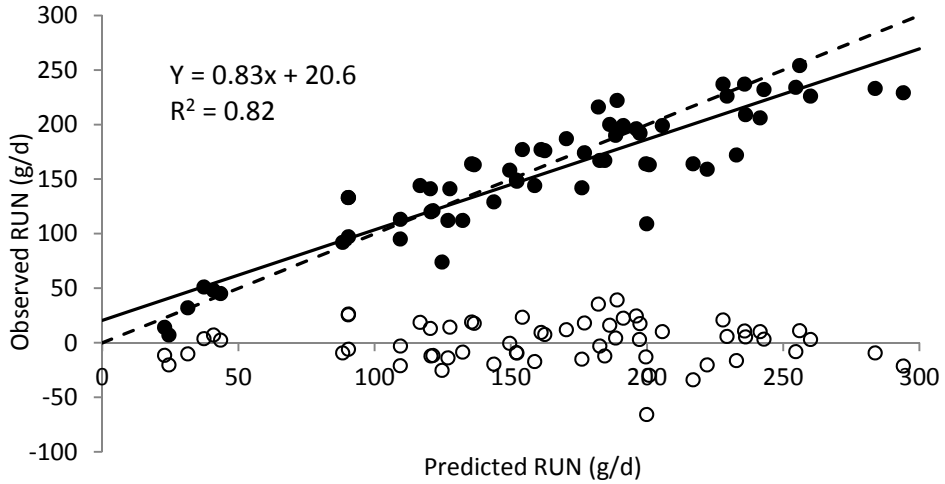

Figure 9. Predicted and observed rumen un-degraded and endogenous N flows (RUN) at the omasum (●) and residual error (○) from the mixed model regression analysis. The solid line (—) represents the linear regression and the dashed line (- - -) is the unity line.

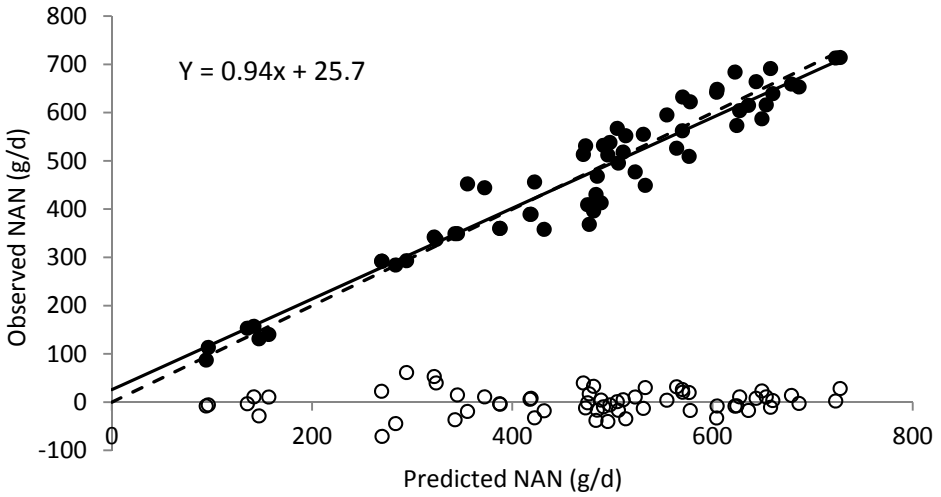

Figure 10. Predicted and observed non-ammonia N (NAN) flows at the omasum (●) and residual error (○) from the mixed model regression analysis. The solid line (—) represents the linear regression and the dashed line (- - -) is the unity line.

SUMMARY

The development of v7 has seen a shift from the original structure of the model that calculates statically, to a dynamic structure that calculates over time. A summary of the major updates to the CNCPS since version 6.0 (Tylutki et al., 2008) that have resulted in v6.1, v6.5 and v7.0 is found in Table 3. Updates that have resulted in v7.0 are described in detail by Higgs (2014).

FUTURE DIRECTION

The development of v7 of the CNCPS represents a progression from a static factorial model to a more dynamic mechanistic system. This has been possible through advancements in computing power, software availability, new laboratory techniques and a better understanding of the biological systems. For example, it is noteworthy to recognize the reason non-essential AA were not included in the original version of the CNCPS was not because the data weren't available, rather because there were not enough columns available in spreadsheets at the time (Sniffen, pers. comm.). However, the singularity effect being observed with the advancement of computing power and technology suggests that these factors are unlikely to constrain future progress, rather, biological understanding and our ability to keep pace will be first limiting.

Practical areas to target for advancement that have high value involve improving model inputs. Examples of recent advancements include the work of Ross (2013) and Raffrenato (2011). Areas of particular future importance include:

- Assays to predict rates of protein degradation.
- Simple ways to quantitatively estimate tissue accretion or mobilization.

- Further refinement of lab assays to reduce variation, particularly *in vitro* assays.

Target areas for new capability within the model include:

- Predicting the size and pattern of meals.
- Further developing the rumen model to estimate VFA production and pH.
- Rebuilding and updating the fatty acid sub-model of Moate et al. (2004) in the v7 framework which would allow for the prediction of milk components as well as yield.
- Behavioral models that better capture activity and general aspects of the farm system that effect maintenance and possibly revisiting metabolic body size related to energy partitioning.

Other, more advanced areas to target could involve optimization and automation techniques that allow rations to be formulated that satisfy a multitude of economic, farm system, compliance, and animal based constraints simultaneously and instantly. Linking this model to other models that describe other farm resource allocations such as manure to fields to minimize fertilizer use while optimizing forage yields and then allocating forages based on digestibility and nutrient profile to the appropriate groups would provide new insights into nutrient utilization related to both economics and environmental sustainability. The computational power and optimization methods already exist to achieve this.

With new model developments, the fundamental principles of the CNCPS need to stay intact and field usability needs to remain the priority. Complexity might be required in the background to improve capability or to acquire the needed inputs, but this should be carefully scrutinized, and a clean simple user experience prioritized at all times.

Table 3. Major developments in the CNCPS after the description of version 6.0 by Tylutki et al. (2008) resulting in v6.1 (Van Amburgh et al., 2007), v6.5 (Van Amburgh et al., 2015) and v7.0 (Higgs, 2014).

v6.1 (Van Amburgh et al., 2007)	v6.5 (Van Amburgh et al., 2015)	v7.0 (Higgs, 2014)
• Re-organization of passage rate assignments so soluble protein fractions flow with the liquid passage rate (Van Amburgh et al., 2007) • Reduction the digestion rates of A and B1 protein fractions to be more consistent with literature reports (Van Amburgh et al., 2007) • Reduction in the digestion rates of sugars to better reflect gas	• Updated feed chemistry in the feed library. • Updated pool structure for the protein fractions in the model where the A pool, previously defined as non-protein N, was changed to ammonia and is now defined as the A1 pool. • Updated AA profiles of feeds in the feed library. • Combined efficiency of AA use for milk production and	• New dynamic structure for the entire gastro-intestinal model. • Expansion of the post-rumen model to include a separate large and small intestine. • Development of a mechanistic large intestine. • Inclusion of protozoa in the microbial sub-model. • New system to mechanistically estimate N recycling.

	production data (Van Amburgh et al., 2007)	maintenance (Lapierre et al., 2007) • Capability to use $uNDF_{240}$ rather than lignin × 2.4 to characterize unavailable fiber (Raffrenato, 2011)	• Capability to model different meal patterns. • Capability to estimate N digestibility using an in vitro estimate of indigestible N (Ross, 2013) • Inclusion of endogenous N transactions along the gastro-intestine tract. • Revised efficiencies of AA use. • Expansion of potentially digestible NDF from 1 to 2 pools (Raffrenato, 2011) and the implementation of new passage rates for NDF from (NorFor, 2011)

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DOUBLE CROP ROTATIONS WITH WINTER CEREALS AND CORN SILAGE OR FORAGE SORGHUM

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Winter cereals, such as cereal rye (*Secale cereal* L.) and triticale (x *Triticosecale* Wittm.), grown as double crops in corn (*Zea mays* L.) silage rotations in the Northeast United States, have the potential to increase on-farm forage production as well as provide many environmental, economic and nutritional benefits to dairy farms. The past five years have shown that in some years this double crop rotation can be very successful, while in others late harvesting of the winter cereals in the spring can inhibit timely planting of corn silage while late corn harvest combined with early onset of winter can make planting of the winter cereals impossible. Forage sorghum (*Sorghum bicolor* L.) has shown comparable yields and quality to corn silage but its growing season is shorter and as a result, forage sorghum is now being looking into as a potential fit with winter cereals as well.

In this paper we summarize results of a statewide project that includes 59 on-farm winter cereal and the results of nine sorghum nitrogen (N) rate trials that evaluated (1) yield potential and forage quality of the winter cereals and sorghum, (2) economic viability of double cropping corn silage with winter cereals, and (3) the agronomic performance of forage sorghum as an alternative crop to corn silage in rotation.

WHAT IS DOUBLE CROPPING?

Double cropping is the practice of sequentially growing two harvested crops within one growing season. Double crops serve many of the same purposes as cover crops, such as erosion control, uptake and carryover of end-of-season nutrients, and addition of organic biomass, but in this context double crops are harvested in the spring for forage as well. Forage production on dairy farms in New York primarily consists of 3-4 years of corn silage rotated with 3-4 years of alfalfa (*Medicago sativa* L.) and/or grass hay. The development of high-yielding, short-season forage varieties has lead to increased opportunities for cover cropping, even with short growing seasons found in the Northeast. Cover cropping is a better alternative to leaving the ground bare following corn silage harvest. The latter could result in soil and nutrient loss to the environment. Double cropping (as cover cropping) covers the ground, reducing the risk of erosion and nutrient loss.

Double cropping can also help to reduce crop production risk. If a single crop is produced and production is compromised due to adverse weather or

other constraints, the producer must purchase outsourced forage to make up for the losses. Double crops provide an additional source of nutritious feed and provide environmental benefits to the rotation, in addition to serving as emergency feed in the case of yield deficits. Furthermore, increasing forage production on farmer-owned and operated acreage can reduce whole farm nutrient balances and greatly help farms become more environmentally sustainable.

Cereal rye and triticale are viable options for double crops in the Northeast due to their winter survivability and spring yields. Ideal planting dates for these winter cereals range from mid September and early October, and harvest at flag leaf typically occurs in mid to late May. Because the planting and harvest windows can overlap with the warm-season crop, such as corn silage, studies are ongoing to determine ideal planting and harvest times for optimal yield and quality of the winter cereals and to determine alternative main crops such as forage sorghum.

YIELD EXPECTATIONS FOR WINTER CEREALS

Earlier work in New York showed average yields of 1.6 tons dry matter (DM)/acre for cereal rye and 2.2 tons DM/acre for triticale when harvested at flag leaf stage in the spring (Ketterings et al., 2015). In the N-rate trials conducted in 2013-2016, yields at the most economic rate of N (MERN) averaged 1.5 tons DM/acre for cereal rye and 1.9 tons DM/acre for triticale (Table 1). These studies did not include a direct comparison of species on the same location so we cannot conclude from these data that cereal rye yields less than triticale (Table 1). Most cereal rye trials (70%) yielded between 1.0 and 2.0 tons DM/acre, while most triticale trials (76%) yielded between 1.0 and 2.5 tons DM/acre. According to soil test data, two fields that were very low yielding did not respond to N, likely due to deficiencies in P and K. Soil test data from these sites revealed one site was low in P and the other was low in P and very low in K, emphasizing the importance of soil testing for management of winter cereals.

The MERNs averaged 58 and 52 lb N/acre for cereal rye and triticale, respectively (Table 1). However, 34% of the trials show no yield response to N addition (i.e. MERN = 0 lbs N/acre), indicating that at some locations the soil supplied sufficient N. This is likely due to a variety of soil fertility parameters and management practices. Research is ongoing to determine how to predict yield levels and MERN values for specific sites using soil fertility indicators and field histories.

Table 1. Yield ranges, most economic rate of nitrogen (MERN), and yield at MERN for winter cereals harvested at flag-leaf stage in double cropping rotations.

Species*	Locations	Yield (ton DM/acre)			Avg. MERN	Avg. Yield at MERN
		Min.	Max.	Avg.	(lb N/acre)	(ton DM/acre)
Cereal rye	21	0.25	2.88	1.45	58	1.6
Triticale	38	0.27	5.07	1.86	52	2.0

*Winter cereal species were grown on different farms and different fields and thus should not be directly compared.

QUALITY PARAMETERS OF WINTER CEREALS AS DOUBLE CROPS

Quality parameters at the MERN averaged over all rye and triticale sites and N rates were similar between the species (Table 2). Crude protein ranged from 6.6-28.1% DM depending on N rate. Neutral detergent fiber (NDF) ranged from 40.0-64.3% DM, acid detergent fiber (ADF) from 20.0-38.0% DM, in vitro true digestibility (IVTD) from 77.5-94.0% DM, and neutral detergent fiber digestibility (48 hour fermentation; $NDFD_{48}$) from 61.0-86.2% DM. Crude protein typically increased with N rate while for other quality parameters, N application did not have an impact.

Table 2. Quality parameters at the MERN of winter cereals grown as double crops.

Species*	CP	NDF	ADF	IVTD	$NDFD_{48}$
	% of dry matter				
Cereal rye	16.7	52.3	28.1	87.9	77.1
Triticale	15.5	51.8	27.8	88.0	77.2

*Winter cereal species were grown on different farms and different fields and thus should not be directly compared.

THE ECONOMICS OF DOUBLE CROPPING WITH CORN SILAGE

An economic evaluation of double cropping with winter cereals was documented by Hanshar et al. (2015). For this study, the results of a survey of New York farm managers with double cropping experience (Ketterings et al. 2015) were used to determine costs of production, expected changes in profit, and desirable yields for winter cereals in rotation. Break-even winter cereal yields were calculated based on corn silage yield impact as well as fertilizer costs (Table 3).

Table 3. Break-even yields of winter cereals seeded after corn silage harvest and harvested before corn silage planting under four potential scenarios (Hanchar et al., 2015). Data are averaged across five case studies varying in location, species and tillage practices. These include conventional tillage (triticale in Northern NY and cereal rye in Central NY), reduced tillage with wide swath and merge harvest (triticale in Northern NY), no-till (cereal rye in Northern NY), and no-till with merge harvest (triticale in Western NY).

	No impact on corn silage yields	1 ton/acre reduction in corn silage yield
	ton DM/acre	
No additional N fertilizer needed	0.7	1.7
75 lb N/acre needed at green-up	1.0	2.0

This assessment showed that a minimum of 0.8 ton DM/acre was needed if the winter cereal did not need extra N and corn yield was not impacted. In the worst-case scenario, where 75 lb N/acre was needed and corn silage yields were 1.0 ton DM/acre lower than could have been obtained with a long-season variety, a minimum yield of 2.0 tons DM/acre for the winter cereal was needed. The dataset showed that for 24% of cereal rye sites and 53% of triticale sites yields exceeded 2.0 tons DM/acre. For cereal rye and triticale 95, 90, and 52% (cereal rye) and 97, 97, and 66% (triticale) of sites exceeded 0.8, 1.0 and 1.7 tons DM/acre, respectively.

FORAGE SORGHUM: A VIABLE ALTERNATIVE TO CORN SILAGE?

Sorghum has the potential to fit into double cropping rotations well due to a shorter growing season, resistance to extreme weather such as drought (Rosenow et al., 1983), yields competitive with corn silage, and high nutritional value for livestock (Oliver et al., 2004). The latter is especially true to brown mid-rib (BMR) varieties. Trials were implemented 2013-2016 utilizing a dwarf brachytic (branching) variety of BMR forage sorghum, which has greater resistance to lodging as well as increased forage quality as compared to older varieties. The 2016 trials are being harvested now. A preliminary summary of the previous three years showed yields at the MERN averaged 8.5 tons DM/acre, ranging from 5.8 to 12.6 tons DM/acre, with MERNs ranging from 0 to >300 lbs N/acre depending on the year and location (Table 4). CP at the MERN ranged from 5.5-8.9% DM across all sites. Additional quality parameters were analyzed for the 2013 and 2015 trials. For these years, neutral detergent fiber (NDF) values averaged 48.1-51.8% of DM, while acid detergent fiber (ADF) averaged 28.1-30.6% of DM, and total digestible nutrients (TDN) was 67.9-69.9% of DM. In 2015, $NDFD_{30}$ (30-hour fermentation) averaged 29.1% of DM. Additional trials are currently ongoing.

Table 4. Yield at the MERN and quality for BMR brachytic dwarf forage sorghum harvested at soft dough stage. Quality parameters include crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrients (TDN), and neutral detergent fiber digestibility (30 hour fermentation, $NDFD_{30}$). CP was found at the MERN due to differences between N rates; all other parameters are averaged across N rates.

Year	Yield at the MERN			DM	CP at MERN	NDF	ADF	TDN	$NDFD_{30}$
	----- ton DM/acre ------			%	---------- % of dry matter ----------				
	Min.	Max.	Avg.	Avg.					
2013	8.3	12.6	10.4	31	7.9	51.8	30.6	67.9	-
2014	6.2	9.3	7.8	27	6.5	-	-	-	-
2015	5.8	9.2	7.5	30	6.6	48.1	28.1	69.9	29.1

Harvesting sorghum silage early in the fall may be necessary to meet the ideal winter cereal planting date of September 15 in the Northeast. In 2015, sorghum was harvested every week for nine weeks to determine the tradeoffs between yield and quality if harvested before or after soft dough, the current harvest time recommendation for sorghum silage. Initial trends reveal that earlier harvest times result in reduced yield but increased CP, NDF, ADF and $NDFD_{30}$ (Figure 1).

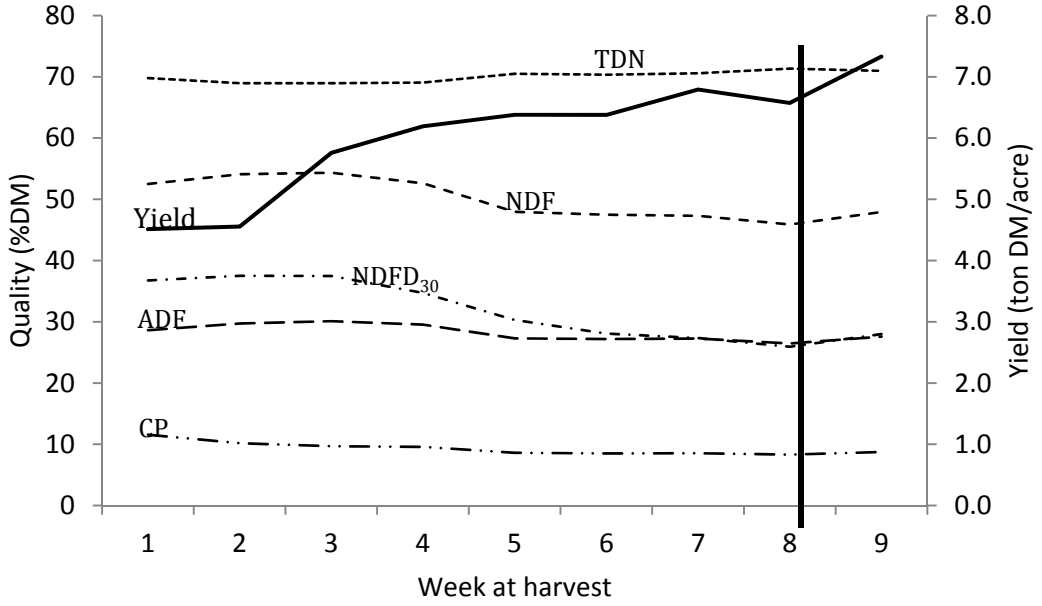

Figure 1. Trends in yield and quality of forage sorghum harvested at different growth stages for one site in 2015. Week 8 is when sorghum was at soft dough stage, and week 9 is following a frost. TDN = total digestible nutrients, NDF = neutral detergent fiber, $NDFD_{30}$ = neutral detergent fiber digestibility at 30 hour fermentation, ADF = acid detergent fiber, and CP = crude protein.

DOUBLE CROPPING POTENTIAL IN THE NORTHEAST

Double cropping in the Northeast has the potential to be an economically and environmentally favorable practice for dairy farmers. Preliminary results show that winter cereals can provide a significant amount of additional, nutritious forage without greatly interfering with corn silage production. However, alternative warm-season crops to corn silage could be viable options. Forage sorghum can be a nutritious silage crop with competitive yields. It fares well in years with extreme weather, such as in 2016 when a severe drought impacted corn silage throughout New York. Work is ongoing to determine specific planting dates, harvest times, and fertilizer recommendations for these crops to ensure successful implementation of these rotations.

CONTACT INFORMATION

To get more information about double cropping and participating in the New York On-Farm Research Partnership including these projects, contact Quirine M. Ketterings, Nutrient Management Spear Program, Cornell University, Department of Animal Science, 323 Morrison Hall, Ithaca NY 14850. Access: http://nmsp.cals.cornell.edu/NYOnFarmResearchPartnership/DoubleCrops.html and http://nmsp.cals.cornell.edu/NYOnFarmResearchPartnership/ForageSorghum.html for protocols and other materials on the projects.

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CHARACTERIZATION OF NON-NUTRITIVE FACTORS OF FEEDS FOR MODEL DEVELOPMENT

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INTRODUCTION

Protein is one of the most expensive macronutrients in dairy cattle rations, and overfeeding degradable protein relative to supply results in excessive N losses to the environment (Huhtanen and Hristov, 2009). Efficient use of feed N can be achieved by first meeting the requirements of the rumen microbial population, followed by balancing diets to meet the amino acid requirements of the cow. To decrease competition for quality protein that could otherwise be fed to humans, dairy cattle can be fed byproducts of human food production, thereby converting waste product streams into highly valuable milk protein. One such byproduct of commercial amino acid production is Fermenten (Arm and Hammer Animal Nutrition, Princeton, NJ). Commercial AA production is performed using bacterial cultures, resulting in a waste stream with high amounts of soluble nitrogenous compounds. A meta-analysis of in-vitro data from continuous culture fermenters using these fermentation byproducts demonstrated an almost 16% increase in microbial nitrogen output vs. a control with no fermentation byproduct addition (Lean et al., 2005). The response in that paper was attributed to a stimulation of microbial protein synthesis by AA and peptides contained in the fermentation byproduct (Cotta and Russell, 1982). However, in vivo results have been more varied, with some studies showing limited effect on rumen metabolism and cattle performance (Broderick et al., 2000), or effects mediated by other dietary components, such as sugar (Penner et al., 2009).

Lack of agreement between in vitro fermentation responses and in vivo metabolism and performance responses is not a new issue. Many compounds have been tested in vitro and found to have potent selective antimicrobial effects, however when moved to the cow, the effects disappear. This is likely due to differences in the environment, especially the concentrations of microbes to substrate and closed nature of the system. Even products that are known to have lasting effects on rumen fermentation do not always demonstrate the same mode of action in vitro as is observed in vivo, as was discussed by Recktenwald et al. (2014). In many diet formulation programs, in vitro results can be used in conjunction with performance studies to create surface level, semi-empirical response profiles, however more detailed in vivo studies are necessary to model the effects in a more mechanistic manner.

Mathematical models such as the Cornell Net Carbohydrate and Protein System (CNCPS) (Higgs et al., 2015; Van Amburgh et al., 2015) have been successfully used to quantify rumen microbial output and meet animal nutrient requirements while reducing N losses to the environment (Tylutki et al., 2008). The mechanistic elements of the

rumen sub-model in the CNCPS require appropriate experimental data to evaluate and develop equations to predict metabolizable AA outflows from the rumen. A new, dynamic version of the CNCPS (v. 7) was recently developed (Higgs, 2014; outlined in these proceedings) that describes rumen degradation of substrates with mechanistic representations of growth of bacteria and protozoa and includes interactions among protozoa and bacteria such as predation and intra-ruminal microbial N turnover. Evaluations of this model indicated a strong ability to predict the partitioning between microbial and non-microbial nitrogen flows; however the partitioning between protozoa and bacteria along with individual AA predictions might require some refinement (Fessenden, 2016). Further, the dynamic nature of v. 7 might allow the non-nutritive elements of some feeds to be described more completely compared with previous versions of the CNCPS. As with most model development, evaluations of the rumen sub-model with independent data can be helpful for determining areas for improvement.

Considering these factors, we identified the need to perform more quantitative studies investigating the non-nutritive aspects of some feedstuffs to better understand how to best characterize the differences between a nutrient driven effect on microbial behavior compared to a non-nutritive outcome. Given the importance of AA to the cow, feedstuffs with possible effects on rumen protein synthesis and flows were determined to be prime candidates for study. To maximize the value of the data generated during an intensive study, several quantitative techniques were combined to provide insight into rumen function. Omasal sampling and rumen evacuations were used to estimate pools and flows for kinetic digestion parameters, improved protozoa isolation techniques allowed for investigation of microbial metabolism, and more thorough AA analysis were used to more accurately quantify AA flows and improve model predictions when compared against a larger literature dataset.

QUANTITATIVE METHODOLOGY

To isolate the non-nutritive aspects of the fermentation byproduct, careful consideration was needed when designing treatments. Our goal in formulation of the control diet, which contained no fermentation byproduct, was to simulate as closely as possible the nutrient composition of the feedstuff. To achieve this, a control protein premix containing wheat midds and urea was used, which allowed for diet formulation to be iso-nitrogenous, iso-soluble protein, iso-NDF, and iso-energetic. Other minor differences included some shifts in mineral sources to account for the high sulfur content of the fermentation byproduct. This allowed for two treatments diets: one with the fermentation byproduct at 3% inclusion rate (EXP), and a control consisting of wheat-midds and urea (CON). Beyond the feedstuffs mentioned above, the rest of the diet was identical between the treatments. Diets differed only in the protein pools, with the EXP diet containing 18 g more non-ammonia soluble N than the EXP diet. This shift was intentional and given the feed chemistry of fermentation byproduct, the additional N was assumed to be in the form of soluble AA and peptides. The differences represented ~ 3.3 % of total N intake.

Eight ruminally cannulated multiparous Holstein cows averaging (mean ± SD) 60 ± 10 d in milk and 637 ± 38 kg of body weight were stratified by pre-trial milk production and randomly assigned to one of two treatment sequences in a switchback trial with three 28 d periods. In this design, each cow was fed each diet at least 1 time, allowing the variation associated with each cow to be controlled. Each period provided 21 d for diet adaptation and 7 d of data and sample collection.

Omasal Sampling

During the sample collection period, the omasal sampling technique was used to quantify post-rumen flows. Sampling though omasal cannulas has been performed since the 1960's (Oyaert and Bouckaert, 1961), however routine sampling was improved by Huhtanen et al. (1997) using a device that, once inserted into the omasum through a rumen cannula, would allow for repeated sampling over a longer time period without the need for more intensive omasal cannulation. This method was adapted by the University of Wisconsin researchers for a series of studies on omasal flows of nutrients (Reynal and Broderick, 2005). The technique has been validated against duodenal sampling (Ahvenjärvi et al., 2000; Ipharraguerre et al., 2007) and these evaluations demonstrated that when combined with a triple marker method (France and Siddons, 1986), the technique can allow for fairly small coefficients of variation in measurement of ruminal digestion variables. Omasal sampling experiments have provided useful data from which to build and evaluate field applicable models of rumen fermentation. Broderick et al. (2010) demonstrated the NRC (2001) overestimated RUP by 21%, and underestimated microbial-N flow by 26%. This series of studies also provided much needed data for evaluation of the CNCPS, through which post ruminal N and AA flows could be compared to model predictions (Higgs, 2014; Van Amburgh et al., 2015).

Partitioning of Post-ruminal N Flows

To better understand the different sources of AA flowing from the rumen, N must be partitioned between microbial and non-microbial sources. To do this, a NPN compound enriched with ^{15}N isotope was provided to the rumen via the blood as a marker. Microbes in the rumen take up the N and synthesize amino acids. Therefore, any ^{15}N amino acid measured in the rumen or omasum is assumed to be of microbial origin. By measuring the ^{15}N content of isolated microbes and the ^{15}N content of the rumen outflow, we can determine microbial protein synthesis. Many previous omasal studies have used this marker system, as it holds distinct advantages over other methods like purines.

Several aspects of rumen fermentation can be determined using the omasal flow method, including dry matter, organic matter and NDF digestion, VFA flows, and N flows. Using the data from the omasal experiment with fermentation byproduct (Table 1), it is evident that cows fed the EXP diet did not show an increase in microbial flow, as has been shown in vitro (Lean et al., 2005). Instead, there was a 15% decrease in rumen degraded N (68.7 vs. 58.3% of dietary N intake). Total NAN flow from the rumen

was well predicted by CNCPS v. 6.5, however the partition between microbial and non-microbial N demonstrates the need for further investigation, most likely related to the current inability to predict robust rates of digestion of protein.

Table 1. Effect of rumen available nitrogen source on omasal nitrogen flow and digestibility

	Diet[1]			
Item[2]	CON	EXP	SEM	*P*
N intake, g/d	603	613	18	0.70
CNCPS fraction PA1	61	43	-	-
CNCPS fraction PA2	171	183	-	-
CNCPS fraction PB1	304	310	-	-
Flow at omasal canal				
Total N, g/d	664	693	25	0.37
Total N flow predicted by CNCPS v. 6.5, g/d	664	674	-	-
Ammonia N, g/d	21.5	22.4	1.5	0.67
NAN				
g/d	642	670	25	0.38
% of N intake	106.6	109.1	3.4	0.58
NANMN				
g/d	191	256	26	0.09
% of N intake	31.3	41.7	3.5	0.05
Microbial NAN				
g/d	450	409	28	0.31
% of total NAN	69.9	61.5	3.5	0.11
Microbial N flow predicted by CNCPS v. 6.5, g/d	351	352	-	-
Microbial efficiency				
g of microbial CP/kg of OTDR	28.9	26.1	1.7	0.26
True ruminal N digestibility, %	68.7	58.3	3.5	0.05
aNDFom digested/g of dietary CP degraded	0.97	1.23	0.1	0.02

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

[2]NANMN = non-ammonia non-microbial N, OTDR = organic matter truly digested in the rumen.

The information on flows and partitioning of N also demonstrates that flow alone does not give a strong indication of the processes happening within the rumen (Table 1). In this case, 20 g more non-ammonia soluble N was provided by EXP diets, however 65 g additional non-ammonia non-microbial N were flowing out. This indicates that the soluble portion of the fermentation byproduct was not simply flowing out with the liquid phase. Instead, some aspect of the feed was exerting associative effects on the degradation of proteins from other feedstuffs. To fully understand this effect for model characterization, we need to better understand the dynamics within the rumen, not just the outflow. This is achieved by leveraging other data collected during the trial, namely the partitioning of protozoa flows and rumen pool sizes of microbial biomass and

digestible substrate. This is not data usually reported in other omasal flow studies, but can be very useful data for modeling purposes.

Protozoa Isolation

Protozoa flow has been quantified using a variety of methods (Ahvenjärvi et al., 2002; Sylvester et al., 2005). As investigators became more interested in protozoa and bacteria interactions, it was found that protozoa typically take up less of the ^{15}N microbial marker (Brito et al., 2006) primarily due to the lack of direct incorporation of ammonia by protozoa. The ultimate effect of this is that reported microbial AA flows are likely underestimated by approximately 10% in the literature datasets that do not quantify the protozoa flow. The most common issues to address in the isolation protocol are feed particle and bacterial contamination (Volden et al., 1999). A typical isolation procedure relies on filtration and/or centrifugation to isolate biomass that is assumed to be representative of protozoa. One of the early studies of microbial composition isolated protozoa only through repeated centrifugation (Czerkawski, 1976). For large scale separations, Storm and Ørskov (1983) used a large filtration and separation system to examine microbial biomass from animals coming into abattoirs, however feed and bacterial contamination was likely high. To address this, researchers began using flocculation and sedimentation to remove large feed particles, followed by centrifugation and filtration on nylon cloth to wash away bacteria (Williams and Strachan, 1984; Martin et al., 1994). Glucose was used to enhance flocculation, although this likely altered microbial composition as a result of competition for growth substrate. For a protozoal isolation to be representative of the population in the rumen, techniques must strive to be rapid, have limited addition of any growth promoting substances, and avoid lysis of microbial cells. Many of the previously reported studies have suffered from weaknesses in one or more of these areas.

More recent work with microbial populations has necessitated the development of a rapid technique to isolate mixed protozoa cultures with viability enough to culture. The techniques are described in the paper by Denton et al. (2015) and might provide useful data when combined with the omasal sampling technique. The procedure uses a combination of flocculation, sedimentation, and filtration to recover much of the protozoa in a sample in a form that has high viability, low feed contamination, and no addition of substrate that is known to appreciably change cell composition. For the omasal study, protozoa were isolated as quantitatively as possible from omasal fluid, and the marker system was used to calculate the flow of protozoa in the fluid phase (Table 2). Partitioning of the microbial flows also allowed for estimation of the predation of protozoa on bacteria under a couple assumptions: 1) Protozoa acquire almost all of the ^{15}N through consumption of bacterial AA (Newbold et al., 2005), and 2) protozoa retain approximately 50% of consumed AA in cell biomass (Hristov and Jouany, 2005).

Predictions using the dynamic version of the CNCPS v. 7 demonstrated the ability of the model to predict microbial flows (Table 2). Compared to the predictions from v6.5 (Table 1), microbial flow is much closer to the actual measured value. The output from v7 also predicts protozoa flow although the values appear to be slightly

under predicted in this comparison. The predicted values for CON and EXP also demonstrate that the model is not necessarily sensitive to the associative effect of the fermentation byproduct—an expected finding given the structure of the CNCPS.

Table 2. Microbial nitrogen flows and protozoa predation in lactating dairy cattle fed two different sources of rumen available nitrogen

	Diet[1]			
Item	CON	EXP	SEM	*P*
Total microbial NAN flow, g/d	450	409	28	0.31
Bacteria NAN	378	337	23.0	0.22
% of microbial NAN flow	84.2	82.1	1.0	0.12
Protozoa NAN	72.1	73.9	7.3	0.84
% of microbial NAN flow	15.8	17.9	1.0	0.12
Protozoa NAN consumed	90.6	76.3	12.9	0.45
% of bacterial N flow	23.4	22.2	2.4	0.70
CNCPS v. 7 output				
Predicted microbial N flow, g/d	412	417	-	-
Bacteria N flow	371	375	-	-
Protozoa N flow	41	42	-	-
% of microbial N flow	9.9	10.1	-	-
Predation estimate, bacterial N consumed, g/d	75	76	-	-

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.

Microbial Growth and Turnover

To improve the predictions of microbial growth and turnover, we have to move past looking at post-ruminal flows alone, and start to understand how microbial populations are interacting with their substrate. Therefore, rumen evacuations and measurements of pool sizes were critical to determine digestion kinetics and evaluate the effects of the fermentation byproduct. For this study, rumen contents were evacuated, weighed, and subsampled to get a representative sample from the rumen. This sample was analyzed for DM, OM, N, NAN, aNDFom, uNDFom, and the microbial marker, ^{15}N. Using these values, we are able to determine the pool sizes of these nutrients in the rumen. Total fermentable carbohydrate was calculated in a similar manner to the traditional non-fiber carbohydrate fraction in feeds, however potentially digestible NDF was added back to the equation. Using the same approach on the flows, it is possible to calculate the fractional rate of degradation of the digestible pools (Table 3). This value, albeit subject to some compiled error, can be evaluated against the predicted rate of degradation in CNCPS v. 7. To obtain model predictions of carbohydrate availability, samples of the forages and corn grains were analyzed for in vitro aNDFom and starch digestion rates using commercially available methods. These values were then entered into the model, and feed library digestion rates were used for

all other rates to reflect the data that would be available when using the model in the field.

Rumen microbial pool size and flows can be used to calculate growth rates of microbes in the rumen. To calculate fractional rate of microbial growth, omasal flow (g/h) is divided by the pool size in the rumen (g). Since flows are measured post ruminal values, the result is a fractional rate of growth that accounts for lysis and turnover (Wells and Russell, 1996). However, since protozoa pool size was not directly measured in the rumen fluid, and protozoa are thought to be selectively retained in the rumen, it becomes difficult to partition bacterial and protozoal N pools. Reported rumen protozoa retention in rumen vs. post-ruminal measurements vary widely, and range from < 5 % (Sylvester et al., 2005) to over 70% (Punia et al., 1992). Luckily, the total ^{15}N pool in the rumen can be measured, making it possible to evaluate the effect of several theoretical levels of selective retention on rumen pool sizes. In this way, at 0 % selective retention, we expect the protozoa to account for the same proportion of total microbial N as measured in the omasal flow. At greater levels of retention, protozoa account for larger portions of the microbial pool. Therefore, rumen protozoa ^{15}N proportion of the total rumen ^{15}N pool was calculated at 4 different levels representing 0 to 75 % retention (Table 3).

To assess which level of selective retention of protozoa is likely most correct, it is possible to use pool size and flow to estimate fractional rates of growth (Table 3). Recognizing that the main energy substrate for rumen bacteria is CHO (Russell et al., 1992), and assuming the maximum yield of cell DM / g of CHO degraded ($\mathbf{Y_g}$) is 0.5 (Isaacson et al., 1975), one can quickly determine which retention values allows for realistic growth rates. In this instance, selective retention at 50 % indicate that bacteria would have to grow at a fractional rate of 0.07 h^{-1}, corresponding to a CHO degradation rate of 0.14 h^{-1} (0.07 / 0.5). Given the estimated pool size (g) and digestion (g/h), the fractional rate of CHO availability in this study averaged 0.138 h^{-1} of the available pool; therefore theoretical maximal fractional growth rate was estimated at 0.138 x 0.5, or ~ 0.069 h^{-1}. Using the measured total microbial pool at 25 % selective retention, it was calculated that the fractional growth rate of all microbes in the rumen was 0.061 h^{-1}. This corresponds to an estimated Y_g of 0.44 g / g of CHO degraded. This is close to the theoretical maximums for individual species reported in pure cultures (Russell and Baldwin, 1979; Theodorou and France, 2005). In vitro measurements of mixed rumen microbes often give yields on the high range of those observed in pure culture (Russell and Wallace, 1997).

Also, the calculations allow for comparisons of the model predicted vs. study estimated Y_g (Table 3). This comparison serves primarily to verify several aspects of the rumen sub-model in CNCPS v. 7. When provided with feed chemistry data that is available in the field, the CNCPS was able to fairly accurately predict the fractional rate of CHO degradation. The model relates cell growth directly to CHO availability, so accurate estimates of CHO degradation are key to accurately predicting microbial yield and eventually AA supply.

Table 3. Fractional rates of microbial growth, nutrient digestion, and rumen fermentation parameters in lactating dairy cattle fed two different sources of rumen available nitrogen

Item	Diet[1]		SEM	*P*
	CON	EXP		
Fractional growth rate of bacteria[2], h^{-1}				
0% selective retention	0.061	0.061	0.004	0.99
25% selective retention	0.064	0.064	0.005	0.99
50% selective retention	0.070	0.070	0.006	1.00
75% selective retention	0.108	0.103	0.012	0.74
Fractional growth rate of protozoa[2], h^{-1}				
0% selective retention	0.061	0.061	0.004	0.99
25% selective retention	0.046	0.046	0.003	0.99
50% selective retention	0.030	0.030	0.002	0.99
75% selective retention	0.015	0.015	0.001	0.99
Omasal flows and ruminal digestion parameters				
True OM flow, kg/d	7.08	7.19	0.47	0.87
Microbial NAN flow, g/d	450	409	28	0.31
Ruminal true OM digestion rate, g/h	626	619	17	0.77
Ruminal true CHO digestion rate, g/h	518	526	15	0.72
Fractional rate of OM digestion[3], h^{-1}	0.101	0.094	0.008	0.54
Fractional rate of CHO digestion[3], h^{-1}	0.139	0.138	0.011	0.91
Microbial growth parameters				
Fractional growth rate of all microbes, h^{-1}	0.060	0.060	0.004	0.94
Theoretical maximum CHO allowable growth[4], h^{-1}	0.070	0.069	0.005	0.91
Observed Y_g, g of cells / g of CHO degraded[5]	0.44	0.44	0.03	0.99
% of theoretical maximum Y_g	88.4	88.3	6.6	0.99
CNCPS v. 7 output				
Predicted CHO degradation, g/h	484	487	-	-
Predicted fractional rate of CHO digestion, h^{-1}	0.124	0.124	-	-
Predicted Y_g, g of cells / g of CHO degraded	0.45	0.45	-	-

[1]CON = 3% of diet DM as urea control mix; EXP = 3% of diet DM as fermentation byproduct.
[2]bacteria or protozoa daily flow (g/h) / bacteria or protozoa pool size (g) at 4 levels of protozoa selective retention
[3]Measured microbial NAN flow (g/h) / measured rumen microbial NAN pool (g)
[4]Fractional rate of CHO digestion x 0.5
[5]Fractional microbial growth rate / fractional rate of CHO digestion

By dividing predicted yield of all microbes by carbohydrate degradation, we can calculate an apparent Y_g used by the model and compare it to the measured values obtained from the omasal study. The agreement between predicted vs. independently measured values indicates the structure of the model is likely adequate to provide accurate estimates of microbial yield from substrate degradation. This provides a strong basis from which to improve AA supply predictions, as microbial N represents a large portion of MP flowing from the rumen.

Overall, the model guided research approach to the non-nutritive aspects of feeds has allowed for a better understanding of how fermentation byproducts might be characterized. Investigation of the feeding effects on kinetic aspects of rumen fermentation allowed us to better understand that the byproduct did not stimulate microbial growth, but rather changed the way microbial populations interact with their substrate. Fractional rates of digestion and growth indicate that bacteria were not negatively influenced by fermentation byproduct inclusion. By studying and modeling the dynamics within the rumen, not just the outflows, we gain a deeper understanding of the system. Models, while inherently wrong, can help a great deal in guiding the research question. For complex models to be improved, a stepwise evaluation is usually necessary to identify and address offsetting errors. In this case, the stepwise evaluation demonstrated that effects observed in vitro did not occur in vivo. Further, the data generated in this study allowed us to updated AA profiles of microbial protein, and evaluate the model's ability to predict post-ruminal flows of AA when compared with a larger dataset, as described in the next two sections.

AMINO ACID PROFILES OF MICROBIAL PROTEIN

The CNCPS uses a factorial approach to calculate AA supply, so accurate profiles of AA in undegraded feed, bacteria, protozoa, and endogenous portions of post rumen protein flows are important. For the purposes of this study, it was necessary to understand principally the microbial portions, as limited data exist on microbial (especially protozoa) AA profiles from high producing lactating cows. Amino acid content of protein has historically been determined by single time point hydrolysis, as this represents a compromise between maximal release of AA from the matrix while minimizing the loss of acid labile AA (Rutherfurd, 2009). Determination at multiple time points followed by least-squares non-linear regression provides more accurate estimates of the true amino acid profile (Darragh and Moughan, 2005). To our knowledge, AA determination after multiple hydrolyses times has not been performed on rumen microbial biomass.

Microbial samples obtained from the omasum were used to determine the AA content after multiple time point hydrolysis. The AA content of all samples was determined by HPLC following hydrolysis at 110°C in a block heater (Gehrke et al., 1985) for 2, 4, 6, 12, 18, 21, 24, 30, 48, 72, 120 and 168 h. All AA except Trp were determined using 6N HCl hydrolysis, with Met and Cys undergoing an additional pre-oxidation step. Tryptophan was determined using florescence detection after hydrolysis in barium hydroxide at the same time points as the acid hydrolysis. The entire time

course was performed twice for each sample, and the reported values are the mean of the two determinations.

Table 4. Comparison of measured AA composition after single hydrolysis time point vs. estimated AA composition determined using least-squares non-linear regression after multiple hydrolysis times for omasal bacteria and protozoa isolates from trial B.

	Bacteria			Protozoa		
Item	24 h[1]	Mult[2]	% Δ	24 h[1]	Mult[2]	% Δ
Essential AA, % of AA						
ARG	4.96	4.88	1.6	5.37	5.41	-0.7
HIS	2.24	2.17	3.0	2.50	2.59	-3.6
ILE	4.25	4.77	-12.4	4.03	4.51	-12.0
LEU	5.48	5.47	0.3	6.83	6.43	5.8
LYS	7.52	7.40	1.6	8.90	8.79	1.2
MET	4.71	4.81	-2.0	3.44	3.87	-12.6
PHE	6.15	5.94	3.4	6.79	6.76	0.4
TRP	5.51	5.93	-7.7	4.26	5.49	-29.1
THR	5.67	5.70	-0.5	4.84	5.09	-5.1
VAL	6.58	7.14	-8.4	4.67	4.88	-4.6
Total EAA	53.07	51.73	2.5	51.61	51.01	1.2
Non-essential AA, % of AA						
ALA	6.68	7.15	-7.0	5.36	5.17	3.6
ASP	10.46	11.13	-6.3	9.65	10.42	-7.9
CYS	1.43	1.45	-1.4	2.37	2.22	6.5
GLU	11.25	11.39	-1.3	12.94	13.40	-3.5
GLY	5.01	4.98	0.6	4.67	4.53	2.9
PRO	2.00	1.97	1.2	2.99	2.97	0.7
SER	4.48	5.03	-12.2	5.14	5.43	-5.8
TYR	5.61	5.82	-3.6	5.27	4.83	8.3
Total NEAA	46.93	48.90	-4.2	48.39	49.22	-1.7
Total AA, % of DM	346.6	339.0	2.2	295.0	290.7	1.4

[1]AA composition after 24 h hydrolysis time

[2]AA composition determined from least-squares non-linear regression from multiple hydrolysis times.

The comparison of the multiple time point vs. single time point indicates that the AA profile is affected by the rate at which AA are hydrolyzed in the assay. This means that when using a single time point hydrolysis at 21 or 24 h, the acid labile and slower releasing AA will be underestimated, while the faster releasing and acid stable AA would be overestimated. In a quantitative sense, this might not account for much of the rumen-undegraded portion individual feed ingredient AA. However, when assigning a profile of AA to the microbial flows, error in the analysis will have a large effect on

predicted AA flows when using the factorial approach, as the microbial portion is usually responsible for 40-60 % of the total AA supply.

MODEL EVALUATION

To determine the effect of the updated AA profiles on prediction from the microbial sub-model of CNCPS v.7, an evaluation was performed in a similar matter as previous evaluations of the CNCPS (Higgs, 2014; Van Amburgh et al., 2015). In a separate paper in these proceedings, Higgs and Van Amburgh reported the v.7 predicted vs. observed values for microbial N, undegraded feed N, and total non-ammonia N from the evaluations of Higgs (2014). Amino acid predictions were also evaluated using 11 publications with 43 treatment means of individual AA flows at the omasal canal. Full descriptions of the criteria used to select and enter the studies into the database have previously been reported by Higgs (2014).

The updated bacteria and protozoa AA profiles were entered into CNCPS v.7, and the evaluation was re-run. The results from the original evaluation (Higgs, 2014) were compared with values from the updated evaluation. The regressions for Lys, Met, and His are displayed in Figure 1. A full reporting of the results of the evaluation is beyond the scope of this paper, however overall AA predictions were improved from the previous evaluation of the model. Average reported concordance correlation coefficient (CCC; a simultaneous measure of accuracy and precision) and root mean squared prediction error (RMSPE) for the Higgs (2014) evaluation was 0.66 and 28.5, respectively; while the current evaluation averaged 0.69 and 23.8 for CCC and RMSPE, respectively; indicating overall improvement in AA flow predictions. Of the AA considered most often to be first limiting in lactating dairy cattle, Lys and His predictions were improved, while Met predictions were not improved. Met analysis is technically challenging, and pre-oxidation recoveries are rarely reported in the literature. It is important to note that reported AA flows in the literature are from a single time point hydrolysis, which would likely contribute additional mean and/or systematic bias when values are compared to the predictions from the CNCPS when using the updated profile. Nonetheless, this evaluation demonstrated that as with all model development, improvements in some areas leads can lead to the realization of shortcomings in others.

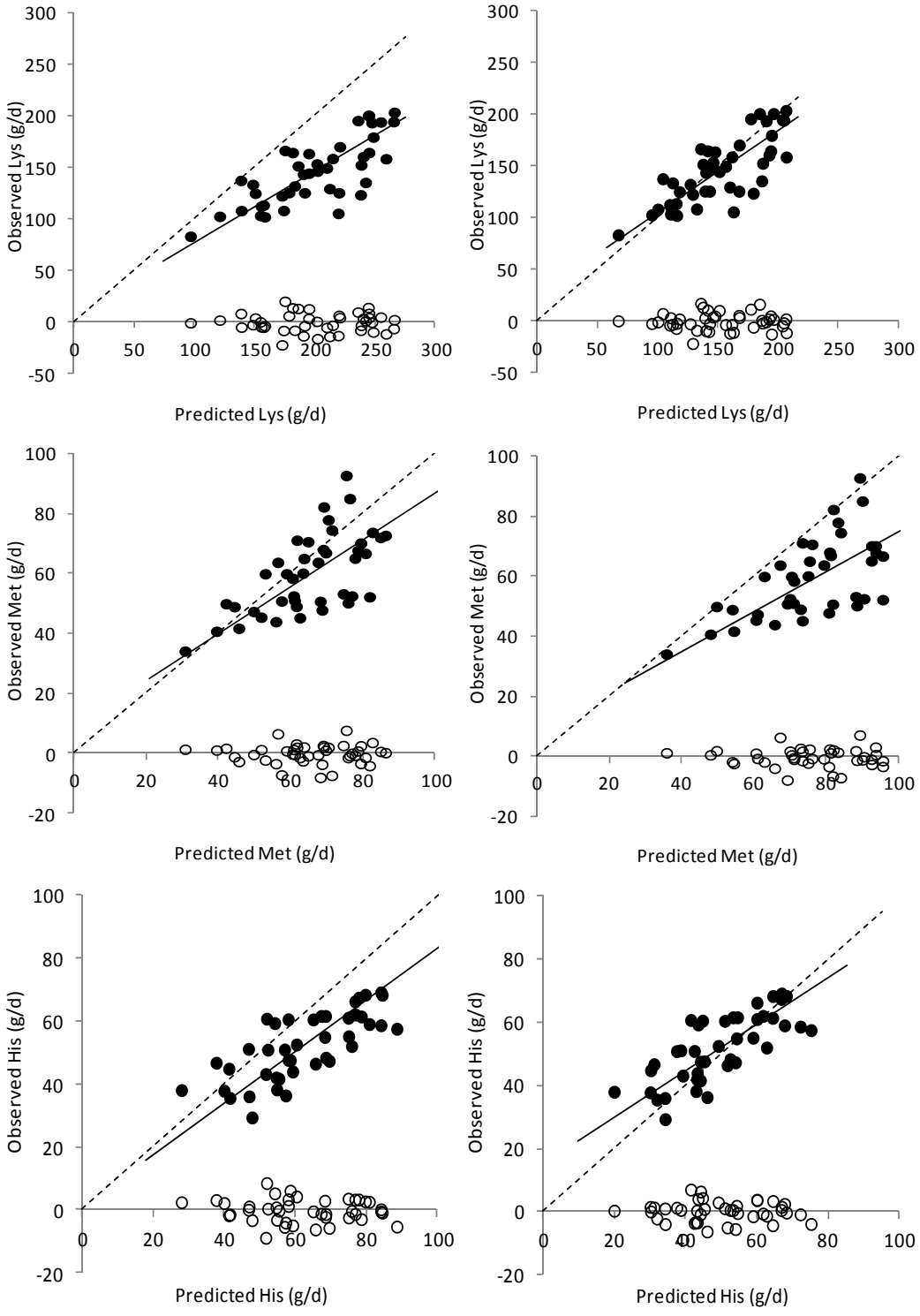

Figure 1. Predicted vs. observed values for Lys, Met, and His flow at the omasal canal (g/d). Values (●) and residuals (○) from a mixed model analysis, along with lines representing the regression (—) and unity (---) are displayed.

CONCLUSIONS

Evaluation of the AA profiles indicated the CNCPS has a good ability to predict post-ruminal AA flow in lactating dairy cattle. Further work is needed to improve predictions of some AA, especially Met. Re-evaluation of AA ratios and relationships to other dietary or animal parameters used in practical ration formulation will likely occur as supply predictions improve. Overall, the methods detailed in this paper, including omasal sampling, improved isolation of protozoa, and more accurate determination of post ruminal flows of digestible AA can allow for further development of mechanistic elements that describe the non-nutritive aspects of feedstuffs. Using the model to guide research can lead to large advances in our knowledge of the ruminant animal. This is often done through the leverage of specific techniques to better understand a complex system. However, modelers can often become quite enamored with their work when models perform well, and can fail to recognize structural issues when the models fail. This can, and often does lead to excessive complexity and decreased applicability --- a fatal outcome for any model. At all times in model development, application of the model must be considered. If more complex models are to be used in the field, training, support, and most of all, usability must be a top priority at all times.

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EFFECT OF PERIPARTUM SOURCE OF MAGNESIUM AND CALCIUM, AND POSTPARTUM FEEDING RATE OF MAGNESIUM, ON INTAKE, PERFORMANCE AND MINERAL AND ENERGY STATUS OF MULTIPAROUS HOLSTEIN COWS

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INTRODUCTION

Subclinical hypocalcemia (**SCH**) is disorder in which blood Ca concentrations fall below a critical threshold as the result of inadequate adaptation to the lactational demands for Ca that occur at the onset of colostrum production in dairy cows (Ramberg et al., 1970). Recent research has determined that 11-25% of first lactation animals and 42-60% of multiparous cows can be categorized as subclinically hypocalcemic in the day after parturition (Reinhardt et al., 2011; Caixeta et al., 2015). This is likely a conservative estimate of the true prevalence of SCH after parturition as we continue to learn more about the associations between the severity and duration of low blood Ca after parturition and negative downstream consequences. Martinez et al. (2012) demonstrated a strong association between SCH and compromised energy metabolism, risk of uterine disease and delayed reproduction. Additional work has found similar associations with reproduction (Chapinal et al., 2012) and energy metabolism (Chamberlin et al., 2013) and has further demonstrated increased risk for displaced abomasum and early lactation culling (Chapinal et al., 2011; Roberts et al., 2012) as well as decreased early lactation milk production (Chapinal et al., 2012) in cows with SCH. Taken together, the body of evidence suggests that SCH is a highly prevalent and costly disorder.

Magnesium is known to be an important mineral in the homeostatic pathway for regulating blood Ca from work conducted in cows (van Mosel et al., 1990; van Mosel et al., 1991) and humans (Rude et al., 1978; Rude et al., 1985). Feeding higher concentrations of dietary Mg prepartum (0.45-0.50% of DM) has become common practice to aid in prevention of hypocalcemia at parturition and has been supported in a meta-analysis by Lean et al. (2006). Postpartum plasma Ca concentrations have been shown to take several days to return to prepartum levels (Ramos-Nieves et al., 2009) and theoretically, feeding higher concentrations of Mg postpartum may help in the recovery of plasma Ca. To the author's knowledge, feeding varying rates of Mg postpartum to support the recovery of blood Ca has not been investigated.

Mineral status in the transition period may vary based on supplemental mineral source due to differences in bioavailability. Bioavailability can be affected by chemical structure, particle size or both (Moore et al., 1971; Xin et al., 1989). Further, mineral sources of different chemical structures have been shown to have varying buffering capacities (Schaefer et al., 1982), which may aid in intake and performance during the transition period where diet transitions have been shown to challenge rumen health

(Penner et al., 2007). Investigation of performance of cows in the transition period fed varying supplemental mineral sources may provide evidence for strategic use of mineral sources to promote successful diet transitions and optimal mineral status.

The objectives of this experiment were to determine the effects of dietary source of supplemental Ca and Mg, and postpartum dietary level of Mg, on intake, performance and aspects of energy and mineral metabolism in multiparous Holstein cows. We hypothesized that plasma mineral status would be altered by feeding supplemental minerals from a commercial Ca-Mg dolomite and feeding a higher rate of dietary Mg postpartum. If plasma mineral status was improved by either factor, it was hypothesized that intake and performance would also be improved in those cows.

EXPERIMENTAL APPROACH

All animal protocols were approved by the Cornell University Institutional Animal Care and Use Committee. Animals were enrolled in the experiment between May and July of 2015. Multiparous Holstein cows (n = 47) were enrolled in a 2 × 2 factorial design experiment starting at 28 d prior to expected parturition. Cows were fed a control diet for one week and at 21 d prior to expected parturition cows were randomly assigned to treatment with randomization restricted to balance for parity group (2nd vs. 3rd and greater lactation) and previous lactation 305 d mature equivalent milk production. Prepartum, cows were randomized to one of two source treatments in which supplemental dietary Ca and Mg were provided primarily from common sources (Mg oxide and limestone; **CS**) or a commercial Ca-Mg dolomite supplemental mineral source (MIN-AD, Papillon Agricultural Company, Easton, MD; **MA**). At the next feeding that occurred after calving, cows were further randomized to receive diets formulated to contain Mg at close to NRC (2001) recommendations or at a higher rate (**LM** = 0.30% of DM, **HM** = 0.45% of DM) within their source treatments. Cows were followed through 42 DIM. Criteria for removal from the trial included twin calving and calving with less than 10 d on the experimental prepartum diet. Cows excluded before the end of the enrollment period were replaced and in anticipation of the loss of cows from the trial, one additional cow was enrolled into each treatment group at the end of the enrollment period. The final dataset included 41 cows from which 11 were in the common source, low Mg group (**CS-LM**), 11 were in the common source, high Mg group (**CS-HM**), 10 were in the MIN-AD, low Mg group (**MA-LM**) and 9 were in the MIN-AD, high Mg group (**MA-HM**).

Cows were housed in tiestalls and fed once daily at approximately 0800 h for lactating cows and 0930 for dry cows. Individual feed intake was measured on a daily basis throughout the experiment by weighing feed delivered and refused. Cows were fed for a targeted refusal rate of 10% to allow for ad libitum intake. Rations were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS v. 6.5). Ingredient composition and analyzed diet composition of all prepartum and postpartum treatment diets are presented in Tables 1 and 2. All rations were composed of a base TMR containing forages and a base grain mix as well as a small inclusion rate grain mix (containing supplemental minerals). The base TMR was mixed in one batch for all prepartum cows and in one batch for all postpartum cows. Prior to delivery to the cow,

smaller batches were made which included the small inclusion rate grain mixes. Samples of TMR and all feed ingredients were collected weekly for determination of DM (dried at 40°C for 96 h). Weekly dry matters were used to adjust as fed inclusion rates of all forages and grain ingredients and to calculate DMI. At the end of the experiment, dried samples were ground to 2 mm in a Wiley mill and composited at 4 wk intervals for TMR samples and over the duration of the trial for all forages and grains. Composited samples were sent to a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) for wet chemistry analysis.

Cows were observed daily throughout the experiment for health disorders. Body weights were measured weekly and BCS assigned by two scorers weekly according to Edmonson et al. (1989) beginning during the week prior to assignment to treatment and continuing through 42 d postpartum. Body condition scores were averaged over two scorers before statistical analysis.

After calving, all cows were milked three times daily at 0600 h, 1400 h and 2200 h and milk weights recorded. Milk samples were collected at 3 consecutive milkings each week and analyzed at a commercial laboratory (DairyOne, Ithaca, NY) for milk fat, protein, lactose, total solids and MUN using mid-infrared techniques (Method 972.16, (AOAC International, 2006) and SCC was determined by optical fluorescence (Method 978.26, (AOAC International, 2006). Somatic cell scores were calculated from SCC (SCS = $\log_2$(SCC/100,000)+3). Milk yield at the corresponding milking was used to weight milk composition and calculate yield of fat, protein, lactose and total solids. Weekly average yield of 3.5% FCM was calculated [3.5% FCM = (0.432 × kg of wk average milk yield) + (16.216 × kg of fat)] as well as weekly average yield of ECM [ECM = (0.327 × kg of wk average milk yield) + (12.95 × kg of fat) + (7.65 × kg of true protein)]. Milk production efficiency was calculated from weekly average DMI and ECM (efficiency = kg of ECM/ kg of DMI). Weekly energy balance (**EBAL**) was calculated according to NRC (2001).

Blood samples were collected via coccygeal venipuncture between 0600 h and 0730 h 2×/wk from d -28 relative to expected parturition until parturition (Monday and Friday), within 2 h of parturition (d 0), daily from d 1 through 7 in milk, and 3×/wk thereafter (Monday, Wednesday and Friday) through 21 DIM. Plasma was harvested and snap frozen in liquid nitrogen and stored at -20°C until analysis. A subset of samples were analyzed using commercial enzymatic kits for β-hydroxybutyrate (**BHBA;** Catachem Inc., Oxford, CT) and non-esterified fatty acids (**NEFA;** HR Series NEFA HR (2), Wako Pure Chemical Industries, Osaka, Japan). Mineral concentrations were determined at the Cornell Animal Health and Diagnostic Center (Ithaca, NY) on an automated analyzer (Hitachi Modular P800, Roche Diagnostics, Indianapolis, IN).

STATISTICAL ANALYSIS

Prepartum and postpartum data were analyzed separately. All statistical analyses were conducted with the statistical software SAS (version 9.4, SAS Institute Inc., Cary, NC). All measurements repeated over time were subjected to repeated measures analysis using the REPEATED statement in the MIXED procedure of SAS (Littell et al.,

1996). The fixed effects of time, source, level (postpartum only), parity group (2nd vs. 3rd+ lactation) and the two- and three-way interactions of source, level (postpartum only) and time were included in the model.

Table 1. Ingredient composition of prepartum and postpartum diets

	Prepartum Diet[1]		Postpartum Diet[1]			
Ingredient (% of DM)	CS	MA	CS-LM	CS-HM	MA-LM	MA-HM
Brown mid-rib corn silage	37.59	37.59	38.00	38.00	38.00	38.00
Alfalfa hay	-	-	7.60	7.60	7.60	7.60
Wheat straw	23.23	23.23	6.21	6.21	6.21	6.21
Corn grain, finely ground	2.29	2.29	17.10	17.10	17.10	17.10
Wheat midds	6.54	6.54	4.71	4.71	4.71	4.71
Citrus pulp	4.31	4.31	4.75	4.75	4.75	4.75
Soybean hulls	6.50	6.50	2.13	2.13	2.13	2.13
Canola meal	3.33	3.33	3.80	3.80	3.80	3.80
Corn gluten feed	1.67	1.67	2.37	2.37	2.37	2.37
Distillers	1.09	0.62	1.29	1.02	1.13	0.58
Amino Plus[2]	2.32	2.32	5.70	5.70	5.70	5.70
Gemini Protein[3]	1.99	1.99	2.28	2.28	2.28	2.28
Energy Booster 100[4]	-	-	1.14	1.14	1.14	1.14
Biochlor[5]	5.56	5.56	-	-	-	-
Alimet[6]	0.07	0.07	0.06	0.06	0.06	0.06
Salt	0.33	0.33	0.57	0.57	0.57	0.57
Sodium bicarbonate	-	-	0.38	0.38	0.38	0.38
Limestone	2.46	1.49	1.35	1.38	1.08	0.47
Ca sulfate	-	-	0.25	0.25	0.25	0.25
Mg oxide	0.41	0.09	0.13	0.38	0.05	0.08
MIN-AD[7]	-	1.78	-	-	0.52	1.66
Mineral oil	0.02	0.02	0.02	0.02	0.02	0.02
Rumensin[8]	0.04	0.04	0.06	0.06	0.06	0.06
Trace minerals & vitamins	0.21	0.21	0.04	0.04	0.04	0.04

[1]Treatments consist of a 2 × 2 factorial arrangement of source assignments (CS = common sources of supplemental minerals, MA = supplemental minerals from a commercial source) beginning at 21 d prior to due date, and level assignments (LM = formulated diet Mg at 0.30% of DM, HM = formulated diet Mg at 0.45% of DM) beginning within 1 d after parturition. Treatments were continued through 42 DIM.
[2]Ag Processing, Inc. Omaha, NE
[3]Papillon Agricultural Company, Inc., Easton, MD
[4]Milk Specialties Global, Eden Prairie, MN
[5]Church & Dwight Co., Inc., Trenton, NJ
[6]Novus International, Saint Charles, MO
[7]Papillon Agricultural Company, Easton, MD
[8]Elanco Animal Health, Greenfield, IN. Contained 26,400 g/ton monensin.

Table 2. Analyzed nutrient composition and partitioning of mineral intake by sources

	Prepartum Diet[1]		Postpartum Diet[1]			
	CS	MA	CS-LM	CS-HM	MA-LM	MA-HM
Nutrient (mean ± SD)						
DM (%)	45.7 ± 2.0	46.4 ± 1.6	45.8 ± 1.0	46.0 ± 1.2	45.5 ± 1.0	45.5 ± 1.2
CP (% of DM)	14.3 ± 0.4	14.1 ± 0.6	14.9 ± 0.2	15.0 ± 0.26	15.2 ± 0.38	15.4 ± 0.4
ADF (% of DM)	28.1 ± 0.9	29.5 ± 0.6	20.9 ± 0.2	21.5 ± 0.6	21.2 ± 1.0	21.1 ± 0.5
NDF (% of DM)	43.4 ± 0.8	45.4 ± 0.9	32.5 ± 0.2	32.9 ± 0.3	33.2 ± 0.9	33.4 ± 0.9
Lignin (% of DM)	3.9 ± 0.2	4.1 ± 0.2	2.1 ± 1.2	3.3 ± 0.1	3.2 ± 0.1	3.1 ± 0.1
Starch (% of DM)	15.8 ± 0.8	14.5 ± 1.5	25.5 ± 0.9	25.3 ± 0.6	24.6 ± 0.4	25.2 ± 0.4
NFC (% of DM)	33.0 ± 1.2	31.2 ± 0.9	45.2 ± 0.7	43.7 ± 0.29	43.5 ± 1.3	43.6 ± 1.2
Fat (% of DM)	2.17 ± 0.08	2.22 ± 0.15	3.25 ± 0.26	3.10 ± 0.09	3.04 ± 0.13	2.87 ± 0.24
Ca (% of DM)	1.44 ± 0.00	1.40 ± 0.00	1.21 ± 0.08	1.13 ± 0.06	1.17 ± 0.07	1.24 ± 0.03
P (% of DM)	0.35 ± 0.00	0.34 ± 0.00	0.36 ± 0.01	0.34 ± 0.01	0.37 ± 0.01	0.36 ± 0.00
Mg (% of DM)	0.49 ± 0.02	0.52 ± 0.01	0.35 ± 0.02	0.40 ± 0.01	0.35 ± 0.01	0.48 ± 0.00
K (% of DM)	1.08 ± 0.02	1.08 ± 0.03	1.00 ± 0.03	0.98 ± 0.03	1.02 ± 0.03	1.01 ± 0.04
S (% of DM)	0.45 ± 0.01	0.44 ± 0.01	0.32 ± 0.01	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.02
Na (% of DM)	0.26 ± 0.01	0.25 ± 0.02	0.42 ± 0.01	0.42 ± 0.00	0.43 ± 0.01	0.43 ± 0.01
Cl (% of DM)	0.79 ± 0.04	0.80 ± 0.05	0.53 ± 0.01	0.53 ± 0.02	0.54 ± 0.01	0.53 ± 0.02
DCAD (mEq/100 g DM)	-11.2 ± 1.0	-11.1 ± 1.4	8.7 ± 1.1	7.7 ± 1.2	8.9 ± 1.7	9.2 ± 1.9
NE_L (Mcal/kg)	1.46 ± 0.02	1.43 ± 0.02	1.65 ± 0.09	1.61 ± 0.02	1.61 ± 0.02	1.61 ± 0.02
MP (g/kg DM)[2]	91.1	90.8	113.9	113.8	113.6	113.4
MP Intake (g/d)[3]	1549	1616	2312	2390	2295	2427
Mineral Intake Sources[3]						
Mg from MIN-AD (g/d)	-	36.6	-	-	12.1	40.9
Mg from Mg Oxide (g/d)	37.8	8.5	15.4	43.6	5.0	9.5
Mg from Other (g/d)	45.5	48.0	55.6	40.4	53.6	52.3
Ca from MIN-AD (g/d)	-	68.4	-	-	22.6	76.4
Ca from Limestone (g/d)	159.1	101.2	104.1	110.5	82.9	38.5
Ca from Other (g/d)	85.8	81.1	141.6	126.8	130.9	150.5

[1]Treatments consist of a 2 × 2 factorial arrangement of source assignments (CS = common sources of supplemental minerals, MA = supplemental minerals from a commercial source) beginning at 21 d prior to due date, and level assignments (LM = formulated diet Mg at 0.30% of DM, HM = formulated diet Mg at 0.45% of DM) beginning within 1 d after parturition. Treatments were continued through 42 DIM.
[2]Metabolizable protein (**MP**) intake as predicted by CNCPS (v. 6.5) based on forage composite analyzed composition
[3]Based on actual 21 d prepartum and postpartum intake, predicted diet MP supply and analyzed mineral concentrations

Cow within source (prepartum) or source and level (postpartum) was the random effect. When available, covariate measurements collected in the week prior to treatment assignment were included in all models. Previous lactation 305 d mature equivalent milk production was included as a covariate for milk yield. The Kenward Rogers method was used for estimation of denominator degrees of freedom. Four covariance structures were tested for each model and the model with the lowest Aikaike's Information Criterion was selected. When $P \leq 0.10$ for interactions with time, the SLICE option was used in the LSMEANS statement to conduct an F-test to determine at which levels of time the treatment groups differed. When non-normality of residual variance was evident (NEFA and postpartum BHBA), data were log transformed and analysis repeated. Least squares means and standard errors, or geometric mean and confidence intervals (NEFA and postpartum BHBA), are reported throughout. Significance was declared at $P \leq 0.05$ and trends are discussed at $0.05 < P \leq 0.10$.

RESULTS

Analyzed dietary Mg concentration of postpartum treatment diets were different from formulated (Table 2). Both of the LM diets had analyzed Mg concentrations of 0.35% of DM, above the targeted 0.30% of DM. The concentration of Mg in the HM diets was higher than the LM diets, however, they were different from one another with the CS-HM diet at 0.40% of DM and the MA-HM diet at 0.48% of DM. Intake of Mg and Ca from supplemental sources versus basal ingredients is also presented in Table 2. Overall, differences in level of Mg were apparent, justifying analysis of data for any effects of level. As will be discussed, level effects were minimal and this may have been due at least in part to the variation in actual diet Mg concentrations versus the formulated composition. The population of cows in this trial was exceptionally healthy presenting a challenging test of the source and level treatments. Incidence of retained placenta and displaced abomasum were both 2% (n = 1/41), incidence of metritis was 17% (n = 7/41) and only 2 cows had at least one case of clinical mastitis during the study period (5%).

Prepartum DMI was affected by supplemental mineral source and cows fed supplemental minerals primarily from MIN-AD had higher DMI (CS = 17.0 vs. MA = 17.8 kg/d, $P = 0.05$, Figure 1). Similarly, DMI as a percent of BW was higher in cows fed MA (CS = 2.13 vs. MA = 2.23% of BW, $P = 0.05$). An interaction of source, level and time was observed for postpartum DMI ($P = 0.01$; Figure 1) and DMI as a percent of BW ($P = 0.05$) and DMI appeared to be higher for cows fed MA-HM in wk 2 postpartum and for cows fed CS-HM in wk 4, however, none of the slice effects at particular weeks were significant. Previous work suggests that chemical composition and quality of processing of Mg sources can impact buffering capacity (Schaefer et al., 1982). While the buffering capacity of the mineral sources was not tested in this trial, it is possible that a higher buffering capacity of MIN-AD contributed to differences in DMI. While this is plausible postpartum as cows transition onto higher starch postpartum diets, it is unlikely during the prepartum period when cows are consuming low starch, high fiber diets. A previous trial demonstrated that cows fed dolomitic minerals had decreased fiber digestions and increased passage rate (Moore et al., 1971), which may be another potential mechanism for higher intake in cows fed MA.

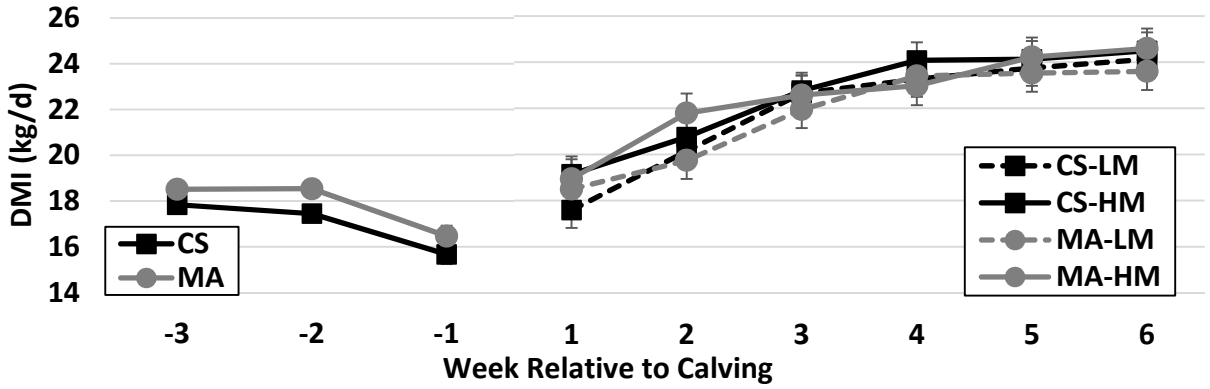

Figure 1. Least squares means and standard errors for DMI in the 3 wk prepartum period and from wk 1 through 6 postpartum

Calculated EBAL was similarly affected prepartum by supplemental mineral source and EBAL tended to be higher for cows fed MA (CS = 8.8 vs. MA = 10.0 Mcal/d, $P = 0.06$). There were no significant effects on EBAL postpartum. Prepartum and postpartum plasma concentrations of NEFA and BHBA are presented in Figure 2. As expected based on the effects on DMI, plasma concentrations of NEFA prepartum were lower in cows fed MA ($P = 0.004$). Cows fed MA also tended to have lower NEFA in the postpartum period ($P = 0.09$). There were no effects of treatment on prepartum plasma concentrations of BHBA but there was a trend for an interaction of source and level on postpartum plasma BHBA concentrations ($P = 0.09$) and BHBA concentrations were numerically lowest in cows fed MA-LM but multiple comparisons using Tukey's adjustment did not reveal differences between specific groups.

Results for milk yield, milk composition and milk production efficiency are presented in Table 3. There were no effects of source or level on milk yield, protein content, protein yield, lactose content, lactose yield, total solids content, milk production efficiency or somatic cell score. An interesting source by week effect was found for fat content ($P = 0.07$), fat yield ($P = 0.02$), fat-corrected yield ($P = 0.04$), total solids yield ($P = 0.05$) and energy-corrected yield ($P = 0.03$). These effects are driven by higher content and yield of fat in wk 1 for cows fed MA ($P < 0.05$). Considering the trend for lower plasma NEFA concentrations in the first 21 d postpartum for cows fed MA, it is hypothesized that the additional source of milk fat is not from the mobilization of body reserves as is typically expected when fat content is higher immediately after parturition. The DIM at which the first week's milk sampling occurred tended to be lower for cows fed CS compared to cows fed MA (CS = 3.3 vs. MA = 4.3 DIM; $P = 0.09$), suggesting that higher fat content due to colostrum composition is not responsible for this source effect. Controlling for the random effect of DIM within sampling week did not alter interpretation of the data and therefore was not included in the final analysis.

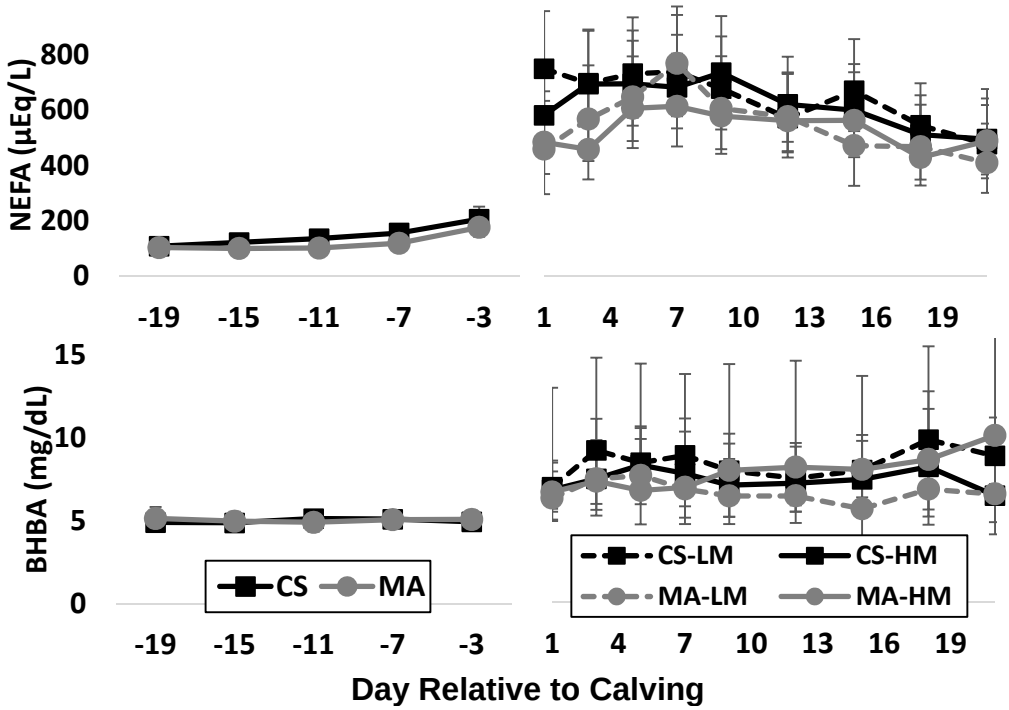

Figure 2. Geometric means and back transformed 95% confidence limits, or least squares means and 95% confidence intervals (prepartum BHBA), for NEFA and BHBA for the 3 wk prepartum and postpartum

Postpartum plasma mineral concentrations over time are presented in Figure 4. There were no overall effects of dietary supplemental mineral source on prepartum plasma concentrations of Ca (CS = 2.44 vs. MA = 2.44 mmol/L; *P* = 0.85) or Mg (CS = 0.96 vs. MA = 0.96 mmol/L; *P* = 0.59), although cows fed MA had higher concentrations of P in plasma prepartum (CS = 1.69 vs. MA = 1.79 mmol/L; *P* = 0.02). Postpartum plasma P tended to be higher for cows fed MA (*P* = 0.09), consistent with the effects in the prepartum period. Higher plasma P concentrations have been demonstrated when intake of P is increased (Barton et al., 1987) and higher intake for cows fed MA may be responsible for higher plasma P concentrations. Postpartum plasma Mg tended to be lower for cows fed LM compared to cows fed HM (*P* = 0.11) which would be expected in diets with lower Mg supply (van Mosel et al., 1991). Postpartum plasma Mg also tended to be lower for cows fed MA compared to cows fed CS (*P* = 0.10). Some work has demonstrated competitive inhibition of Mg absorption as dietary concentration, or ruminal concentration, of Ca increased (Care et al., 1984; Kronqvist et al., 2011). If ruminal concentration of Ca was higher in cows fed MA due to higher intake of Ca, or greater solubility of that Ca, this could have contributed to lower plasma Mg postpartum in cows fed MA. There is evidence from work in steers suggesting that apparent Mg absorption from Ca-Mg dolomites is lower than that of Mg oxide (Moore et al., 1971) and lower absorption coefficients are assigned to Ca-Mg dolomites by the NRC (2001). However, minimal work has been done assessing bioavailability of Mg sources in lactating dairy cows, especially that comparing different sources, particle sizes and rumen conditions. There were no effects of source or level on postpartum plasma Ca concentrations despite alterations in plasma Mg. Incidence of hypocalcemia (plasma Ca < 2.125 mmol/L) was

low in this trial. Peak prevalence of hypocalcemia was 51% at 1 DIM and was reduced to 27% at 2 DIM, 7% at 3 DIM and by 4 DIM no cows had plasma Ca < 2.125 mmol/L.

Table 3. Least squares means and standard error for milk yield, milk composition, and milk production efficiency over the first 6 wk postpartum

	Treatments[1]					*P*-values[2]					
Variable	CS-LM	CS-HM	MA-LM	MA-HM	SEM	S	L	S×T	L×T	S×L	S×L×T
Milk yield (kg/d)	45.0	46.5	45.9	44.0	1.8	0.64	0.89	0.19	0.47	0.30	0.54
Fat (%)	3.85	3.72	3.84	3.94	0.11	0.35	0.90	0.07	0.01	0.34	0.84
Fat (kg/d)	1.68	1.68	1.75	1.72	0.09	0.51	0.81	0.02	0.44	0.88	0.82
3.5% FCM (kg/d)	46.9	47.5	48.4	46.7	2.0	0.85	0.77	0.04	0.27	0.54	0.73
Protein (%)	2.86	2.81	2.83	2.87	0.07	0.78	0.90	0.64	0.50	0.49	0.55
Protein (kg/d)	1.24	1.24	1.29	1.23	0.05	0.67	0.49	0.18	0.41	0.55	0.24
Lactose (%)	4.86	4.84	4.81	4.87	0.05	0.85	0.72	0.29	0.97	0.50	0.97
Lactose (kg/d)	2.18	2.23	2.24	2.14	0.10	0.86	0.76	0.26	0.90	0.44	0.66
Total solids (%)	12.5	12.3	12.4	12.6	0.2	0.51	0.94	0.38	0.32	0.36	0.77
Total solids (kg/d)	5.53	5.56	5.72	5.50	0.25	0.78	0.69	0.05	0.68	0.61	0.51
ECM (kg/d)	46.1	46.5	47.6	45.9	1.9	0.80	0.70	0.03	0.50	0.54	0.58
ECM/DMI	2.12	2.09	2.17	2.03	0.07	0.91	0.21	0.28	0.84	0.43	0.46
MUN (mg/dL)	6.74	6.58	7.30	6.61	0.35	0.37	0.20	0.74	0.06	0.43	0.47

[1]Treatments consist of a 2 × 2 factorial arrangement of source assignments (C = common sources of supplemental minerals, M = supplemental minerals from a commercial source) beginning at 21 d prior to due date and level assignments (LM = formulated diet Mg at 0.30% of DM, HM = formulated diet Mg at 0.45% of DM) beginning within 1 d after parturition. Treatments were continued through 42 DIM.
[2]S = source; L = level; T = time

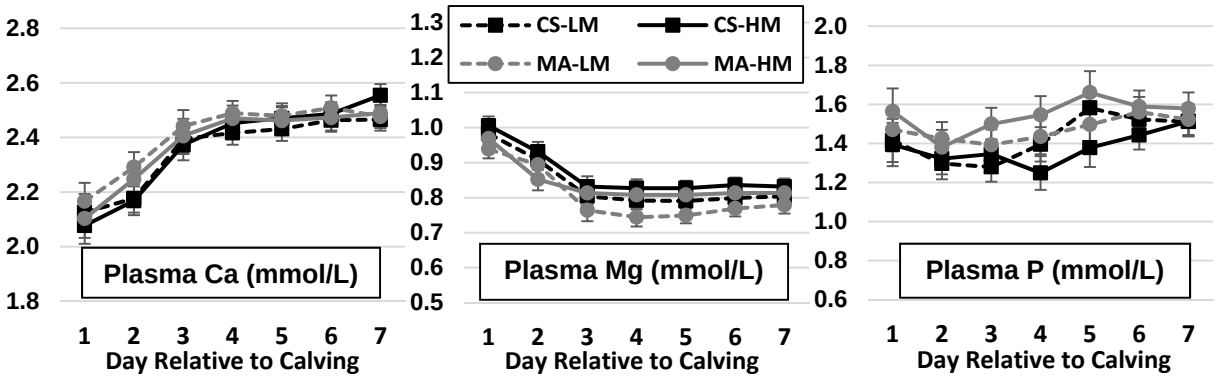

Figure 4. Least squares means and standard errors for plasma Ca, Mg and P from d 1 through 7 postpartum

CONCLUSIONS AND IMPLICATIONS

This experiment found that cows fed supplemental minerals from a commercial Ca-Mg dolomite source, compared to commonly used Mg oxide and limestone, had higher intakes in the prepartum period leading to lower concentrations of NEFA in plasma prepartum. Lower NEFA concentrations for cows fed MA tended to carry over into the postpartum period. The data also indicate potential intake advantages for cows fed MA in portions of the postpartum period. Results from this experiment suggest that there is opportunity for strategic use of mineral sources to aid in DMI and energy metabolism during the transition period.

Effects of dietary Mg level were minimal despite a tendency for cows fed LM to have lower plasma Mg concentrations. Neither dietary mineral source, nor level of Mg, influenced plasma Ca concentrations in the peripartum period. This suggests that in this study, feeding 0.35% of DM as Mg using either source was sufficient to support plasma Ca, resulting in low prevalence of hypocalcemia and a rapid recovery of blood Ca. This population of cows was exceptionally healthy and similar work in a study population with more severe mineral homeostasis challenges at parturition is warranted.

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TRANSITION COW MANAGEMENT AND OUTCOMES IN NORTHEAST HERDS

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INTRODUCTION

Many management factors contribute to cow success during the transition period through means of minimizing stressors, ration formulation and implementation, monitoring and treatment of health disorders, and cow comfort. Limited data exist that investigate the success of different management strategies that contribute to cow success in a commercial dairy setting and therefore, recommendations are often driven by field experience from concepts established through controlled research with comparatively small numbers of cows. Describing non-nutritional factors, such as pen movement, and nutritional factors, such as consumption of different nutrients, with outcome factors such as health and culling events, energy metabolism and inflammation blood biomarkers, milk production, and reproductive performance on well-managed farms, may provide an understanding of which factors contribute to transition cow success.

The objective of this study was to describe herd characteristics, management and nutritional factors pertaining to the transition cow period that may have an effect on milk production, reproductive status, and health in large, high producing Northeastern herds.

EXPERIMENTAL DESIGN

A prospective cohort study was conducted from a convenience sample of 72 farms located in New York and Vermont between November 2012 and August 2015. Inclusion criteria for herds were: 1) Holstein , 2) ≥ 400 milking cows, 3) free-stall housing, 4) TMR-fed , and 5) enrolled in monthly DHI testing or have on-farm milk recording with record management by Dairy Comp 305 (DairyComp 305, Valley Ag Software, Tulare, CA) or PC Dart (PC Dart, Dairy Record Management System, Raleigh, NC). A transition cow management survey was filled out by farm personnel which consisted of questions regarding to pen movements, fresh cow checks, pen information, and reproduction management. Farms were enrolled based on the willingness of the farm to participate and characterized into 1 of 6 transition cow nutritional strategies. Characterization was based primarily upon the formulated starch levels, provided by the farm's nutritionist before data collection commenced. We intended to enroll 12 farms in each category. The characteristics of the nutrition strategies were: 1) Low energy dry cow (<16% starch), high energy lactating (>25% starch), 2) Step-up dry cow (far-off <16% starch, close-up >16% starch), high energy lactating, 3) High energy dry cow (>16% starch), high energy lactating, 4) Low energy

dry cow, step-up fresh (fresh <25% starch, high >25% starch), 5) Step-up dry cow, step-up fresh, 6) High energy dry cow, step-up fresh. Farms were re-categorized after data collection according to the starch level in rations as predicted by the Cornell Net Carbohydrate and Protein System v. 6.1 (CNCPS version 6.1, Cornell University, Ithaca, NY) using NIR analysis of forage samples collected during herd visits and feed library values for grains and additives.

Each farm was visited 4 times over the course of 10.5 wk for data collection focused on the same cohort of animals during the far-off dry, close-up dry, fresh, and high lactation periods. Animals were scored for body condition (Edmonson et al., 1989) and locomotion (Flower and Weary, 2006) by the same experience technician, and were observed for health disorders through 30 DIM by farm personnel. Blood samples obtained from up to 24 animals per herd, 1/3 primiparous and 2/3 multiparous, were taken from the coccygeal vein or artery from a sub-sample cohort of cows during the close-up dry period visit and from the same cows during the fresh cow period visit. Prepartum samples were analyzed for non-esterified fatty acids (**NEFA**; HR Series NEFA HR(2), Wako Pure Chemical Industries, Osaka, Japan) and postpartum samples were analyzed for NEFA and β-hydroxybutyrate (**BHBA**; Abbott Laboratories, Abbott Park, IL).

STATISTICAL ANALYSIS

All descriptive analyses were calculated using SAS (SAS 9.4, SAS Institute Inc., Cary, NC). PROC UNIVARIATE was used to obtain means and standard deviations for continuous data. PROC FREQ was used to generate frequencies for categorical data. PROC MEANS and PROC FREQ was used to calculate the prevalence of elevated blood metabolites.

RESULTS AND DISCUSSION

Herd Performance

Herd size averaged 935 milking cows (range: 345 to 2900) with an annual rolling herd average of 12,674 kg (Table 1). Within the 40% of herds using recombinant bovine somatotropin (**rbST**) an average of 78% of eligible cows received rbST (Table 2). The number of herds using rbST has decreased compared to previous studies (Bewley et al., 2001; Caraviello et al., 2006; Fulwider et al., 2008; Brotzman et al., 2015). Cows entering into 2nd lactation had similar average days dry compared to cows entering 3rd or greater lactations (Table 3). This is less than what other survey studies reported which ranged 59 to 61 d (Bewley et al., 2001; Kellogg et al., 2001; Brotzman et al., 2015). Herd reported voluntary waiting period was similar between primiparous and multiparous animals (Table 3) which was greater than 52 and 53 d, respectively, as reported by Caraviello et al. (2006). Annual farm recorded transition cow health events are reported in Table 1. It is important to note that clinical ketosis and metritis values are likely underreported as not all herds recorded these disorders consistently.

Table 1. Annual herd reported calving related health events and herd level production

Production Characteristics	n herds	Mean ± SD
Herd size, n milking cows	72	935 ± 486
Dairy Herd Improvement herd milk average, kg	50	12,283 ± 1,051
Annual rolling herd average, kg	69	12,674 ± 1,220
Herd average milk yield/cow, kg/d	69	37.8 ± 3.8
Health Events		
Stillborn heifer rate, %	72	5.9 ± 1.8
Twinning, %	72	4.1 ± 1.4
Retained Placenta, %	71	6.5 ± 3.8
Metritis ≤ 30 DIM, %	71	6.4 ± 8.5
Displaced Abomasum ≤ 60 DIM, %	71	2.0 ± 1.6
Ketosis ≤ 30 DIM, %	71	6.6 ± 8.9

Management Strategies

A key management strategy to optimize postpartum cow performance is to minimize stress during the transition period. Decreasing antagonistic interactions between cows by reducing pen moves and separating primiparous and multiparous animals is a putative strategy to address minimizing stress for a more successful transition period. Over 25% of herds were found to move animals into the close-up dry cow pen more than 1× per wk. Almost 28% of herds moved animals into a maternity pen 0 to 3 d before expected calving. Field investigations by Cook and Nordlund (2004) suggests that cows spending ≥ 3 d in the maternity pen have elevated NEFA concentrations. Even though it is reported that only 18% of herds have separate calving locations for primiparous and multiparous animals, many farms utilize individual calving pens so animals are segregated at the time of calving (Table 2). Herds moved cows out of the maternity or calving pen an average of 4 h after calving. Primiparous animals remained in the first pen they moved to after calving for an average of 23 d compared to about 15 d for multiparous animals (Table 3).

Densities for stocking, water space, and feed bunk space are reported in Table 4. Noteworthy, the stocking density in the close-up dry period was the lowest; however, it had the greatest variability. The variability may be related to the fluctuations in cow numbers that may be seen in the close up pen from cows leaving due to calving or cows entering due to routine pen moves. Cows in the close-up and fresh periods had the most access to water and most access to the feed bunk.

The majority of herds had 2 dry cow groups, i.e. a far off pen and close-up pen, and similar results were found during early lactation. About 93% of herds had 2 early lactation groups, i.e. a fresh cow pen and a high lactation pen. Despite these grouping strategies during the dry and early lactation periods, only 65.3% of herds implemented a 2-ration feeding strategy, i.e. a far-off and close-up ration, during the dry period and 80.6% of herds implemented a 2-ration feeding strategy postpartum, i.e. a fresh and high lactating ration (Table 2). Since the objective of this study was to enroll herds

based on different feeding strategies, these results do not represent a random sample, but describe a specific demographic.

Table 2. Frequencies of rBST administration, pen movement, facility type, and grouping and feeding systems in 72 herds.

Item	Level	Frequency
Use of recombinant bovine somatotropin (rbST)		40.3%
Herd moves animals to maternity pen 0 to 3 d before calving		27.8%
Herd moves animals to calving pen when showing signs of calving		72.2%
Separate calving location for primiparous and multiparous cows		18.0%
Dry cow grouping system	1-group	9.7%
	2-group	90.3%
Early lactation grouping system	1-group	6.9%
	2-group	93.1%
Dry cow feeding strategy	1-ration	34.7%
	2-ration	65.3%
Early lactation feeding strategy	1-ration	19.4%
	2-ration	80.6%
Percentage of herds separating primiparous and multiparous animals	Far-off period	71.3%
	Close-up period	31.5%
	Fresh period	25.7%
	High period	86.8%
Facility type for far-off animals	Freestall	92.0%
	Bedded pack	8.0%
Facility type for close-up animals	Freestall	82.4%
	Bedded pack	17.6%
Times per week animals moved into pens housing close-up animals	< 1 ×	2.1%
	1 ×	71.6%
	> 1 ×	25.3%

Table 3. Annual herd reported reproductive performance, culling, and pen movements for primiparous and multiparous cows.

		Primiparous	Multiparous
Item	N herds	Mean ± SD	Mean ± SD
Average days dry, d	72	56.0 ± 6.7	57.0 ± 6.3
Voluntary waiting period, d	72	58.0 ± 9.3	58.7 ± 9.8
Herd mean cull and death rate ≤ 60 DIM, %	71	5.9 ± 4.5	8.4 ± 4.3
Overall herd mean cull and death rate, %	71	20.6 ± 7.8	35.7 ± 7.2
Time spent in calving or maternity pen after calving, h	72	3.9 ± 5.4[1]	4.0 ± 5.6
Time spent in first moved to pen after calving, d	72	23.1 ± 49.4[1]	14.9 ± 18.5

[1] n = 71 herds due to heifers not being raised on 1 farm

Table 4. Densities for stocking, water space, and feed bunk space, based on the number of cows present during each respective visit in 72 herds (mean ± SD).

Item	Period			
	Far-off (n pens)	Close-up (n pens)	Fresh (n pens)	High (n pens)
Stocking density (cows / stall), %	94.4 ± 21.2 (102)	92.9 ± 34.5 (75)	102.5 ± 21.5 (90)	118.8 ± 16.4 (187)
Linear Water Space, cm/cow	6.6 ± 4.4 (110)	9.2 ± 6.4 (90)	10.0 ± 4.6 (93)	7.1 ± 2.2 (187)
Overall bunk density (cows/headlock spaces[1]), %	122.5 ± 41.1 (110)	98.8 ± 44.1 (90)	121.8 ± 37.7 (93)	156.5 ± 34.7 (187)

[1] Headlock spaces = (length of neck rail (cm) / 60.96 cm) or 1 headlock (1 headlock = 60.96 cm of neck rail space)

Feeding Management

It was more common to find feed bunks with walls, to eliminate the need to pushup feed, during the dry cow period than the lactating period (15.5% of dry cow pens vs. 5.4% of lactating pens). The dry cows were fed fewer times per day than the lactating cows (Table 5).

Table 5. Percentage of pens per period at varying feeding frequencies for 72 herds.

Feeding Frequency / d	Period			
	Far-off n = 112	Close-up n = 91	Fresh n = 94	High n = 190
≤ 1	92.9%	93.4%	68.1%	53.7%
1 ≤ 2	7.1%	6.6%	28.7%	38.4%
3	–	–	–	1.6%
4	–	–	3.2%	3.2%
5	–	–	–	1.6%
6	–	–	–	1.6%

The frequency of herds using different feed additives and specific nutrients in rations fed to cows during the close-up, fresh, and high lactating periods are presented in Figure 1. Despite only 65.3% of herds implementing a 2-ration feeding strategy, almost 78% of herds incorporated commercial anionic supplements in the rations fed to the close-up dry cows. Over 80% of herds used rumen-protected amino acids, not including analogs of amino acids, in the rations fed to the fresh and high cows. Over 90% of herds added rumen-protected fat to rations fed to the fresh and high cows.

The chemical composition of rations as formulated and fed in the far-off, close-up, fresh, and high lactating periods, was evaluated using the Cornell Net Carbohydrate and Protein System v. 6.1 (CNCPS version 6.1, Cornell University, Ithaca, NY) and is reported in Table 6. As expected, ME, MP, CP, and starch all increased from the far-off period to the high lactating period. Fermentable starch increased from the far-off period to the high lactating period and the opposite occurred with fermentable NDF, resulting in

the total fermentable carbohydrates, on a DM basis, to remain similarly for prepartum and postpartum diets. Although the fermentable total carbohydrate remained about the same across all periods, lactating cows have higher intakes and will consume more total fermentable carbohydrate compared to dry cows, and will have a different profile of carbohydrate intake fractions. Because each farm was visited once while the study cohort was in each phase of the transition from far dry to high lactation, we were unable to ascertain accurate DMI for all pens, so diet assessment is reported on a density basis.

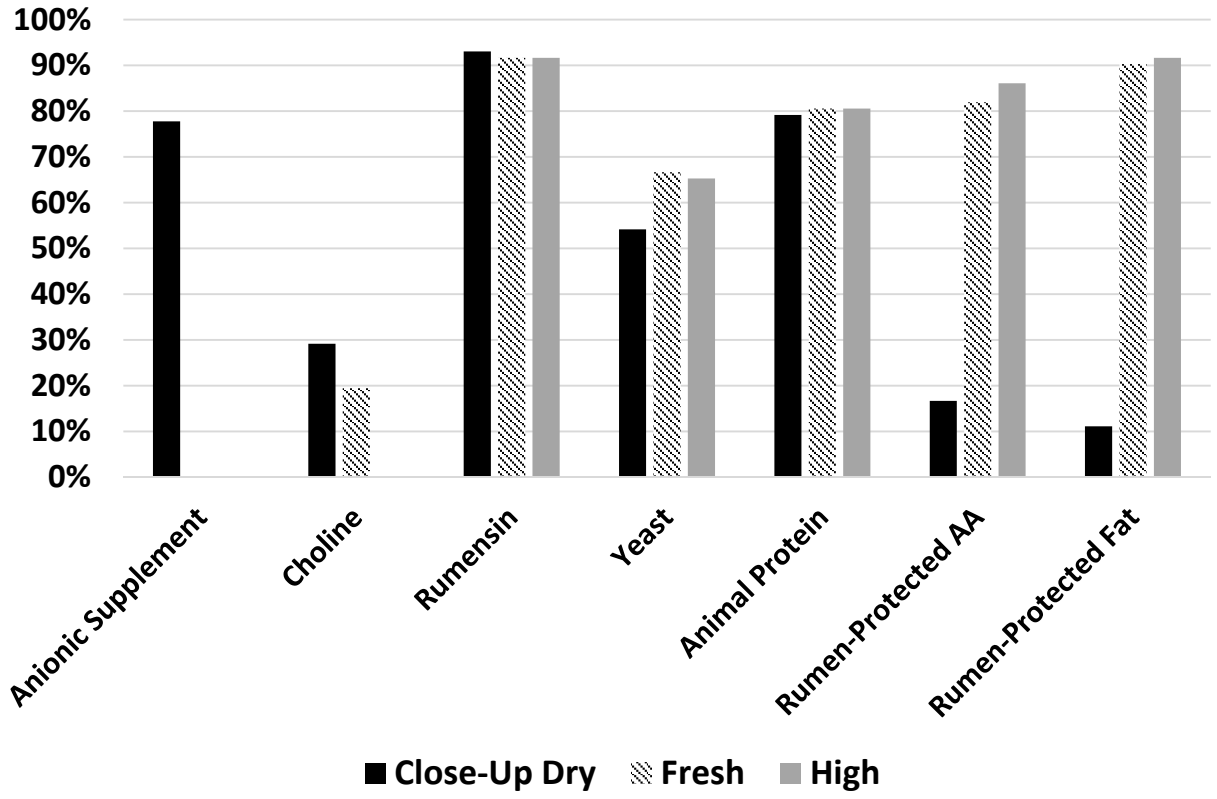

Figure 1. Percentage of herds (n = 72) that feed additives and specific nutrients during the close-up, fresh, and high lactating periods.

Table 6. Predicted chemical composition (CNCPS v. 6.1 using NDS software; mean ± SD) of rations associated with pens respective to period, as formulated from NIR forage analyses for 72 herds. Forage samples were obtained at each visit.

Nutrient	Period			
	Far-off n = 91 pens	Close-up n = 79 pens	Fresh n = 74 pens	High[1] n = 92 pens
ME, Mcal/kg DM	2.07 ± 0.10	2.11 ± 0.10	2.49 ± 0.07	2.51 ± 0.07
MP, g/kg DM	77.2 ± 10.6	89.2 ± 7.7	107.4 ± 6.5	110.1 ± 5.5
Met, %MP	2.12 ± 0.19	2.20 ± 0.21	2.36 ± 0.20	2.33 ± 0.18
Lys, %MP	6.90 ± 0.39	7.03 ± 0.29	6.69 ± 0.23	6.60 ± 0.21
His, %MP	2.60 ± 0.32	2.86 ± 0.28	2.81 ± 0.16	2.80 ± 0.16
CP, %DM	13.5 ±1.9	14.5 ± 1.4	16.4 ± 0.9	16.5 ± 0.8
Starch, %DM	14.5 ± 4.5	17.8 ± 2.7	26.3 ± 2.8	27.8 ± 1.8
Sugar, %DM	3.0 ± 1.0	3.3 ± 1.0	4.1 ± 1.3	4.2 ± 1.3
EE, %DM	3.3 ± 0.4	3.4 ± 0.7	5.1 ± 0.7	5.1 ± 0.7
NDF, %DM	47.1 ± 4.9	43.1 ± 3.8	31.8 ± 2.2	30.7 ± 1.9
Forage NDF, %DM	44.4 ± 6.4	37.5 ± 4.7	23.9 ± 2.2	22.8 ± 2.1
Fermentable total carbohydrate, %DM	38.4 ± 3.0	39.4 ± 2.3	39.9 ± 1.6	39.4 ± 1.7
Fermentable starch, % DM	12.9 ± 4.0	15.8 ± 2.4	21.1 ± 2.2	21.7 ± 1.6
Fermentable sugar, %DM	2.3 ± 0.8	2.6 ± 0.8	3.0 ± 0.9	3.0 ± 0.9
Fermentable NDF, % DM	17.7 ± 2.4	15.9 ± 1.7	11.2 ± 1.3	10.4 ± 0.8

[1]High rations obtained from 68 herds.

Energy Metabolites

Table 7 describes the prevalence of all sampled cows, within the sampling time frame, with elevated prepartum NEFA and postpartum NEFA and BHBA, using established metabolic thresholds (Ospina et al., 2010a). Almost 20% of cows, within the sampling time frame, had elevated metabolites. These elevated metabolites have been associated with a decreased risk of pregnancy, decreased ME305 milk yield, and increased disease incidence (Ospina et al., 2010b). The prevalence of elevated metabolites in herds above the herd alarm level of 15% is displayed in Figure 1 (Ospina et al., 2010c). Almost half the herds had < 15% of cows with elevated prepartum NEFA, and postpartum NEFA and BHBA. As an industry, we still have room for improvement and can decrease the incidence of hyperketonemia further.

Table 7. Prevalence of cows above metabolic threshold as identified by Ospina et al. (2010a).

Metabolite Cut-Point	Sampling time relative to parturition	Cows, n	Prevalence
NEFA ≥0.27 mmol/L	2 to 14 d prepartum	1232	19.2%
NEFA ≥0.60[1] or 0.70[2] mmol/L	3 to 14 DIM	1100	19.8%
BHBA ≥1.0[1] or 1.2[2] mmol/L	3 to 14 DIM	1100	19.7%

[1] Metabolic cut-point used for primiparous cows.
[2] Metabolic cut-point used for multiparous cows.

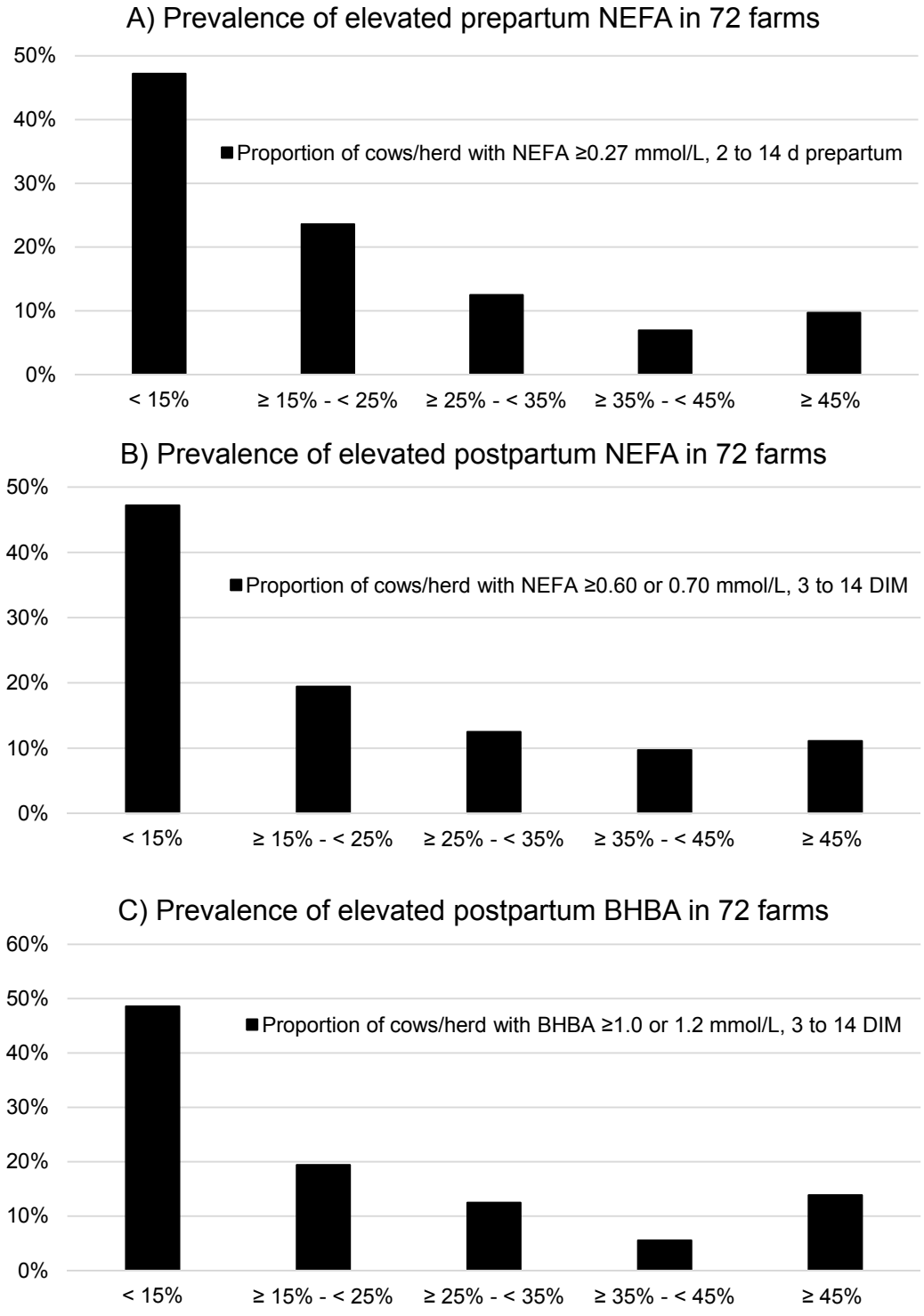

Figure 2. Prevalence of elevated metabolites as a proportion of cows sampled per herd. A) Elevated NEFA (≥0.27 mmol/L) 2 to 14 d prepartum, B) elevated NEFA (≥0.60 mmol/L for primiparous and ≥0.70 mmol/L for multiparous) 3 to 14 DIM, and C) elevated BHBA (≥1.0 mmol/L for primiparous and ≥1.2 mmol/L for multiparous) 3 to 14 DIM.

CONCLUSIONS AND IMPLICATIONS

These results demonstrate the variability in current practices and health related outcomes in large, progressive dairies in the Northeast and can be used for comparison and advisement purposes. Further analyses will determine if associations exist between different management and nutritional factors with health, culling, milk production, reproductive performance outcomes, and energy and inflammation blood biomarkers.

ACKNOWLEDGEMENTS

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METHANE MITIGATION STRATEGIES FOR DAIRY HERDS

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INTRODUCTION

The U.S. dairy industry has committed to decreasing methane emissions by 25% by 2020 (Tricarico, 2014). This is a value for the total dairy industry from feed production to consumption by consumers. In terms of the total dairy supply chain, milk production accounts for about 51.5% of the methane emissions while on-farm feed production accounts for 20.3%. Thus, the primary area for lowering dairy industry methane emissions is nutritional and management factors related to milk production. The U.S. EPA publishes a report on greenhouse gas (GHG) emissions that provides data by individual gases and the contributions by various sectors (U.S. EPA, 2016). Key points from this most recent report are

- Total U.S. GHG emissions were 6,870 MMT of CO_2 equivalents in 2014 compared with 6,397 in 1990.
- Total methane emissions were 730.8 MMT of CO_2 equivalents in 2014 compared with 773.9 in 1990. Methane emissions were 10.6% of the total GHG emissions.
- Enteric methane emissions were 164.3 MMT of CO_2 in 2014 which is similar to the 1990 value. Enteric methane emissions are 22.5% of the total methane emissions.
- Methane emissions from dairy cattle in 2014 were 41.9 MMT of CO_2 equivalents. This is 5.7% of the total methane emissions and 0.6% of the total U.S. GHG emissions.
- Methane emissions from beef cattle were 116.7 MMT of CO_2 equivalents in 2014. A large portion of this is the enteric methane emissions from cow-calf operations.

MEASUREMEMT

There are 2 main ways to express methane emissions from dairy cattle. One is as grams/lb. of milk produced while the other is actual grams (or liters). Table 1 contains methane emissions at varying levels of milk production using these measures. A ration with 53% forage and 47% concentrate (DM basis) was used. The forage mix 56% corn silage and 44% alfalfa silage. Ration nutrient values were 17.6% CP, 31.5% NDF, 26% starch and 4.7% fat. As milk production increases, the methane emissions per pound of milk produced decrease. However, total daily methane emissions (g/day) increase with higher levels of milk production. This is logical since more feed is being consumed and processed in the rumen. It is suggested that methane emissions be reported using both methods in future papers.

Table 1. Methane Emissions for Dairy Cattle at Varying Milk Production Levels[a]

Milk, lbs./day	Dry Matter Intake, lbs./day	Methane Emissions, g/day	Methane Emissions, g/lb. milk
40	41	373	9.32
60	47	409	6.82
80	52.5	439	5.49
100	61	482	4.82
120	67	509	4.24

[a] Calculated using the CNCPS version 6.5 model (Van Amburgh et. al., 2015)

DISTRIBUTION OF METHANE EMISSIONS IN DAIRY HERDS

Herd structure will impact both total methane emissions and the opportunity to adjust practices to decrease methane emissions. Factors such as the number of milk cows, number of dry cows and the number of replacement heifers are key factors that influence this. Some preliminary work has been done using herds with 150 and 1,500 total cows. In these herds dry cows were about 13% of the total cows while replacement heifers were about 85-87% of the total cow numbers. Methane emissions from the dry cows represented about 7 -7.5% of the total herd emissions. Replacement heifers accounted for 18.5- 20.7% of the total herd emissions. Additional work needs to be done in other herds to better quantify these values and better estimate the variability that exists. The result is that the net effects of adjusting milking cow rations to lower methane emissions will be impacted and reduced at the herd level by the number of dry cows and replacement heifers.

COMMERCIAL DAIRY HERD RATIONS

As part of a larger study, a database of commercial dairy herd rations is being developed. All rations are run through the CNCPS 6.5 model to estimate methane emissions and nitrogen and phosphorus excretion (Van Amburgh et. al., 2015). The initial database contains 199 rations. The average target milk was 83.7 lbs. per day with range of 50 to 128 lbs. Average dry matter intake was 51.4 lbs. with a range of 35.2 to 69.8 lbs. An initial evaluation was to examine correlation coefficients between ration factors and daily methane emissions (g/day). Dry matter intake (DMI) had a correlation coefficient of +0.795 with daily methane production (g/day). The correlation of DMI and methane emissions (g/lb. milk) was -0.65. A second evaluation was done with a constant DMI for all rations to examine the potential relationships of nutrients with daily methane emissions. The correlation coefficients in this evaluation were 0.1, 0.636, -0.27 and 0.23 for CP, NDF, starch and fat. This indicates that higher NDF rations tend to increase methane emissions while higher starch rations tend to decrease methane emissions. Additional herds are being added to increase the number of diversity of the rations used. An additional dataset using rations from peer reviewed journal papers will also be developed.

REVIEW PAPERS

Some excellent review papers have been published regarding options for methane reduction in dairy herds (Hristov, 2013a, and 2013b and Knapp et.al. 2014). The papers by Histov used a ranking system of low, medium and high to rank options to lower GHG emissions. Low was a <10% reduction, medium was 10-30% and high was >30% in terms of mitigation potential. The options listed were:

- Low, rBST, ionophores, plant bioactive compounds, grazing management, genetic selection, improved animal health and reduced animal mortality.
- Low to medium – Improved forage quality, feeding grain, precision feeding.
- Medium – Dietary lipids.
- High – Increased productivity.

The paper by Knapp et.al. (2014) listed the following management options and the maximum potential GHG emission reduction associated with each:

- Genetic selection = 18%.
- Feeding and nutrition = 15%.
- Rumen modifiers = 5%.
- Other management approaches = 18%.
- All approaches combined = 30%.

HERD MANAGEMENT OPTIONS

There are a number of herd management options that can assist in reducing GHG emissions from dairy herds. These include:

1. Increased productivity and efficiency –
    - o A one standard deviation increase in feed efficiency would lower CO_2 equivalent emissions by 6.5% (Bell et.al. 2011). Bauman and Capper (2010) reported that the use of rBST could lower methane emissions by 8.3% compared to not using rBST. This decrease is a combination of more milk per cow and fewer cows to produce a set quantity of milk.
2. Calf and heifer management –
    - o Dairy replacement heifers can account for up to 27% of the total CH4 emissions in a dairy herd (Garnsworthy, 2004). Decreasing the age at 1st calving lowers the number of heifers needed to maintain current herd size and would provide some decrease in GHG emissions.
3. Reproduction –
    - o Garnsworthy (2004) indicated that if herds in the United Kingdom could improve herd fertility levels to those of 1995 that methane emissions could be lowered by 10-11%.
4. Forage type and quality –
    - o. A 5% decrease in methane emissions could result from improving total tract NDF digestibility (Knapp et.al. 2014). Methane emissions when C4 grasses were fed had 17% higher methane emissions than when C3 forages were fed (Archimede et.al. 2011). A number of other reports indicate lower methane emissions on higher corn silage diets.

5. Herd grouping strategy
    o. A recent paper reported on the impact of grouping strategies on the economic efficiency of dairy herds (Cabrera and Kalantari, 2014). The efficiency of feed energy use was increased as the number of feeding groups increased from 1 to 4. There was little benefit to >4 feeding groups in this study. Even though GHG emissions were not estimated, they would be expected to decrease as the efficiency of feed energy use increases.
6. Nutrition and feeding management factors –
    o. Table 2 provides an overview of some of the key factors.

Table 2. Nutrition and Feeding Management Impacts on Methane Emissions[a]

Change	**CH4/Energy Corrected Milk**
Increase dry matter intake	Decrease of 2 – 6% for each 2.2 lb. increase
Decreased forage particle size	Neutral
Processing of grain	Decrease about 1-2.5% for a 5% increase in total tract starch digestibility
Rumen pH <5.5	Decrease of 15-20%
Feeding higher grain levels	Decrease about 2% for a 1% increase in ration NFC (maximum credit about 15%)
Increased forage quality	Decreases about 5% with a 5 unit increase in total tract NDF digestibility
Fat feeding	Decreases by about 5% for each unit of fat in the ration
Forage type and selection	Decreased by 0 to 4%

7. Rumen modifiers –
    o. There have been many papers examining the in vitro potential of compounds added to the rumen that could decrease methane production. Some of these decrease methane production by 30 – 80% but have not been evaluated in animal trials. This step is critical and especially important to examine the question of long-term efficacy. There has been a recent paper using one compound in rations for dairy cows over a 12 week period (Hristov et.al. 2015). The compound used was 3-nitrooxypropanol (3NOP). This was added at levels of 0, 1,120, 1,662 and 2,200 mg/day to a diet with 60% forage and 40% grain. Dry matter intake and milk production were not different between the diets. However, there was a reduction in methane emissions of 24.5 to 31.6% on a grams per day basis. At least over a 12 week period, there seemed to be little indication of adaptation by rumen microorganisms to this compound. This model can be used to test other compounds for potential use to lower methane emissions in dairy cattle.

WHOLE FARM FACTORS

The use of whole farm models is another tool that can be used to assess changes in farm management strategies on methane emissions and carbon footprint (Rotz, 2014). The IFSM (Integrated Farm System Model) was used to simulate the effects of varying levels of milk production and feeding strategies. Feeding strategies used were full confinement, summer grazing and an all grass, low input system. Milk production levels from 16,000 to 30,000 pounds of milk per cow were used for the full confinement system. This analysis indicated a 1% decrease in carbon footprint (lb. CO_2 equivalents/lb. milk) for each 2,000 lb. increase in milk production. A herd using summer grazing with a milk production of 20,000 lbs. had a carbon footprint similar to a confinement herd producing 26,000 lbs. of milk or more. The all grass herd with a milk production level of 16,000 lbs. of milk had a carbon footprint similar to the summer grazing and confinement herds described above. This type of approach needs to be more frequently used by the industry to examine alternative farm management approaches.

SUMMARY

The dairy industry has made great strides in lowering methane emissions and the carbon footprint of milk production. The challenge is to continue lowering the total industry carbon footprint. From a nutrition and herd management perspective, the adjustments to be made in the future can be divided into 2 basic categories. These are:

Long-Term:

- These will be applicable over the next 5 to 15 years and will require additional data and research information to provide a base for application. Examples include:
    a. Genomics and genetic selection to improve feed efficiency.
    b. Opportunities to alter the rumen microbial population.
    c. Added compounds that can alter methane emissions in the rumen.
    d. Using whole farm models to integrate crop production, manure handling systems and animal productivity.

Short-Term:

- These are technologies that can be implemented now and will help the industry to improve the efficiency of feed nutrient use and rumen fermentation. Examples include:
    a. Continue to manage animals for higher levels of productivity and improved feed efficiency.
    b. Balance rations using the new concepts of fiber and starch digestibility.
    c. Implement feeding management practices that improve consistency and minimize variability.
    c. Utilize feed additives and production technologies based on research.
    d. Select forages based on yield, quality and digestibility.
    e. Provide facilities and herd management systems that improve cow comfort and reduce stress.
    f. Improve herd health and reproductive performance.

g. Lower the age at 1st calving in replacement heifers.
h. Implement herd grouping and ration formulation strategies to improve the efficiency of nutrient use.

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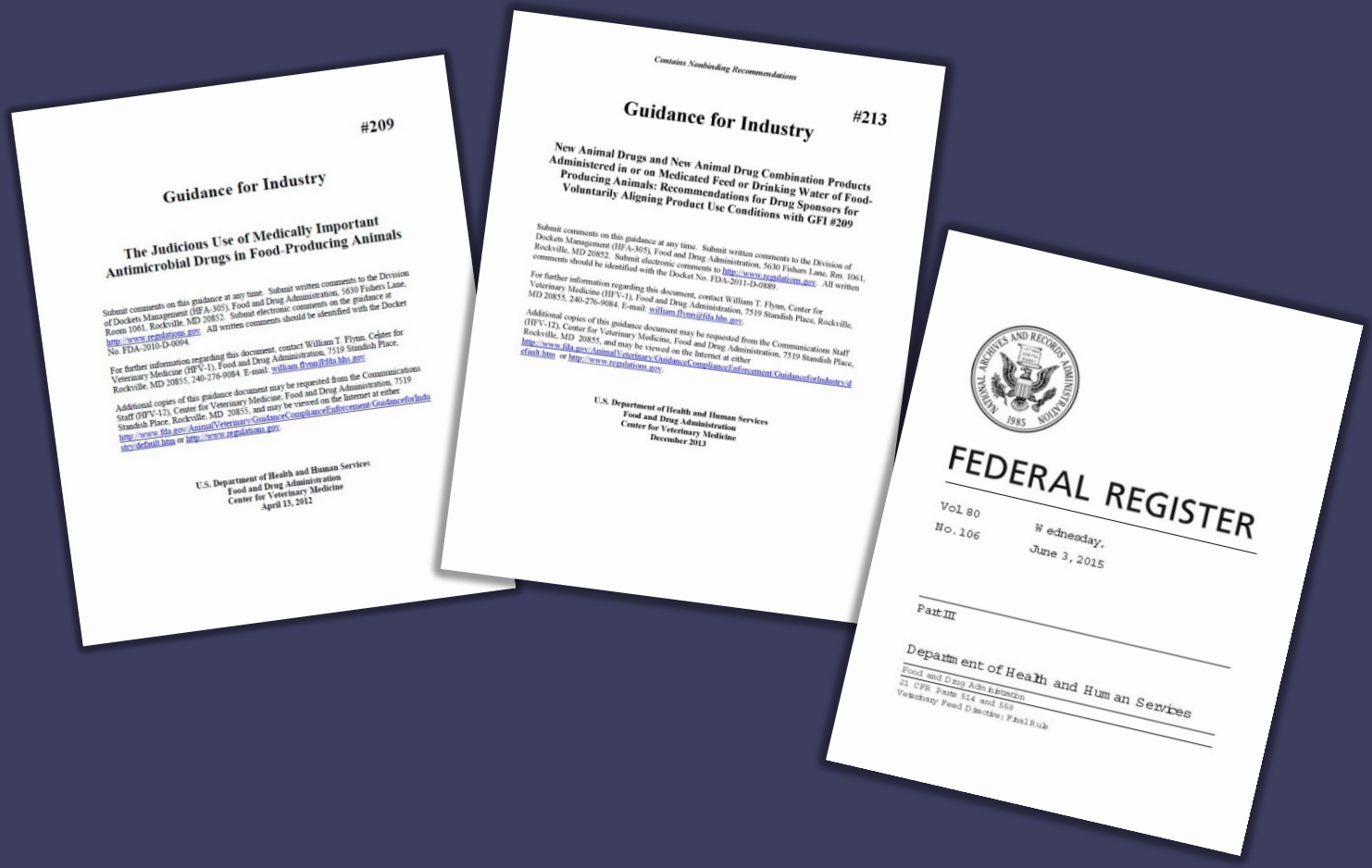
Medically Important Antimicrobials in Animal Agriculture

Outline – Questions to Be Addressed

- What changes are being made and why?
- What drugs are affected, which ones are not?
- What is a veterinary feed directive?
- What are key elements of VFD regulation?
- When will this go into effect?

What changes are being made and why?



#209

Guidance for Industry

The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals

Submit comments on this guidance at any time. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. Submit electronic comments on the guidance at http://www.regulations.gov. All written comments should be identified with the Docket No. FDA-2010-D-0094.

For further information regarding this document, contact William T. Flynn, Center for Veterinary Medicine (HFV-1), Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, 240-276-9084. E-mail: william.flynn@fda.hhs.gov.

Additional copies of this guidance document may be requested from the Communications Staff (HFV-12), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm or http://www.regulations.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
April 13, 2012

Contains Nonbinding Recommendations

Guidance for Industry #213

New Animal Drugs and New Animal Drug Combination Products Administered in or on Medicated Feed or Drinking Water of Food-Producing Animals: Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209

Submit comments on this guidance at any time. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.regulations.gov. All written comments should be identified with the Docket No. FDA-2011-D-0889.

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Part III

Department of Health and Human Services

Food and Drug Administration

21 CFR Parts 514 and 558

Veterinary Feed Directive; Final Rule

Antimicrobial Resistance – In Perspective

- Complex, multi-factorial issue
  - Acquired vs. naturally occurring
- Use as a driver of resistance
  - All uses (human, animal, horticultural, other) are part of the picture

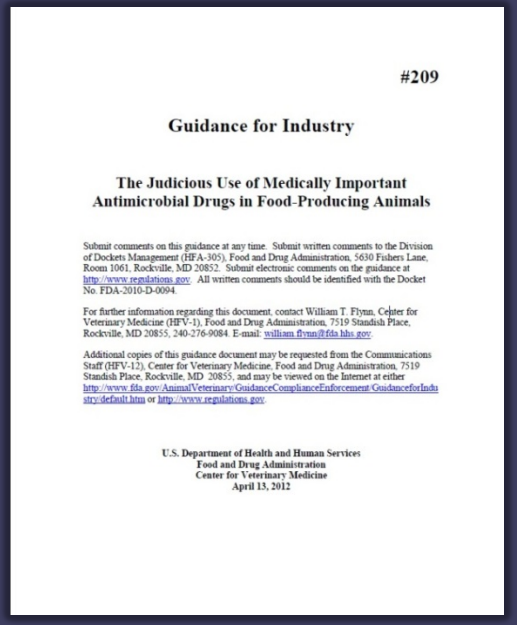



Antimicrobial Use in Animal Agriculture

- Subject of scientific and policy debate for decades
- The science continues to evolve
- Despite complexities and uncertainties steps can be identified to mitigate risk
- The intent is to implement measures that address public health concern while assuring animal health needs are met

Guidance #209: Outlined AMR policy

- Describes overall policy direction



#209

Guidance for Industry

The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals

Submit comments on this guidance at any time. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. Submit electronic comments on the guidance at http://www.regulations.gov. All written comments should be identified with the Docket No. FDA-2010-D-0094.

For further information regarding this document, contact William T. Flynn, Center for Veterinary Medicine (HFV-1), Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, 240-276-9084. E-mail: william.flynn@fda.hhs.gov.

Additional copies of this guidance document may be requested from the Communications Staff (HFV-12), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm or http://www.regulations.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
April 13, 2012

FDA's Judicious Use Strategy

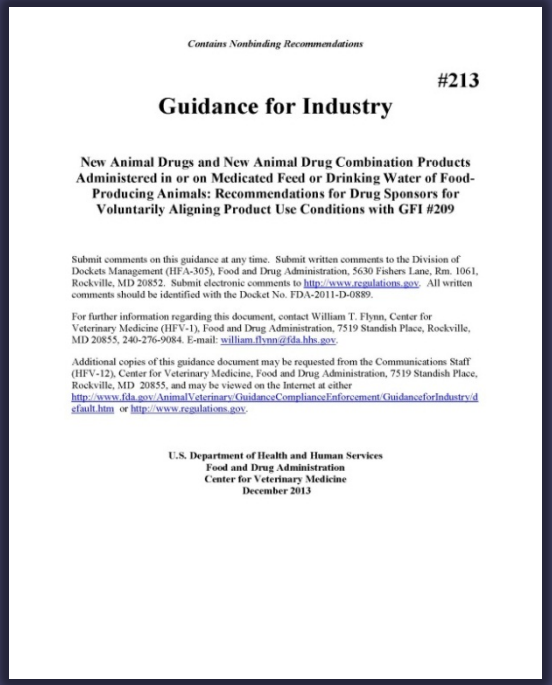
Two key principles outlined in Guidance #209:

Limit use of medically important antimicrobial drugs in food-producing animals to those uses

1. considered necessary for assuring animal health. (therapeutic uses)
2. that include veterinary oversight or consultation

Guidance #213: Implementation

- Finalized December 2013
- More detailed guidance on implementing key principles in Guidance #209
  - Timeline
  - Defines medically important



Contains Nonbinding Recommendations

#213

Guidance for Industry

New Animal Drugs and New Animal Drug Combination Products Administered in or on Medicated Feed or Drinking Water of Food-Producing Animals: Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209

Submit comments on this guidance at any time. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.regulations.gov. All written comments should be identified with the Docket No. FDA-2011-D-0889.

For further information regarding this document, contact William T. Flynn, Center for Veterinary Medicine (HFV-1), Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, 240-276-9084. E-mail: william.flynn@fda.hhs.gov.

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
December 2013

Guidance #213: Overview

- December 2016 - Target for drug sponsors to implement changes to use conditions of medically important antimicrobials in food and water to:
  - Voluntarily withdraw approved production uses
    - such as “increased rate of weight gain” or “improved feed efficiency”
  - After the label changes these production uses will no longer be legal

Guidance #213: Overview

- However, therapeutic uses are to be retained
- treatment, control, and prevention indications
- Require transition to veterinary oversight

Guidance #213: Veterinary Oversight

- Key principle is to include veterinarian in decision-making process
  - Does not require direct veterinarian involvement in the drug administration
  - Does require use to be authorized by a licensed veterinarian in the context of a VCPR
- This means changing the marketing status from OTC to Rx or VFD
  - Water soluble products to Rx – “medicated drinking water”
  - Products used in or on feed to VFD – “medicated feed”

What drugs are affected, which ones are not?

Guidance #213: Scope

- Only affects antimicrobials that are:
  - "Medically important"
  - Administered in feed or drinking water
    - Other dosage forms (e.g., injectable, bolus) not affected **in this transition**.

“Medically Important” antimicrobials

- Includes antimicrobial drugs that are considered important for therapeutic use in humans
- Guidance #213 defines “medically important” to include:
  - All antimicrobial drugs/drug classes that are listed in Appendix A of FDA’s Guidance #152
  - For a complete list of affected applications see:

http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/JudiciousUseofAntimicrobials/ucm390429.htm

Affected feed-use antimicrobials

Antimicrobial Class	Specific drugs approved for use in feed
Aminoglycosides	Apramycin, Hygromycin B, Neomycin, Streptomycin
Diaminopyrimidines	Ormetoprim
Lincosamides	Lincomycin
Macrolides	Erythromycin, Oleandomycin, Tylosin
Penicillins	Penicillin
Streptogramins	Virginiamycin
Sulfas	Sulfadimethoxine, Sulfamerazine, Sulfamethazine, Sulfaquinoxaline
Tetracycline	Chlortetracycline, Oxytetracycline

Affected water-use antimicrobials

Antimicrobial Class	Specific drugs approved for use in water
Aminoglycosides	Apramycin, Gentamicin, Neomycin, Spectinomycin, Streptomycin
Lincosamides	Lincomycin
Macrolides	Carbomycin, Erythromycin, Tylosin
Penicillins	Penicillin
Sulfas	Sulfachloropyrazine, Sulfachlorpyridazine, Sulfadimethoxine, Sulfamerazine, Sulfamethazine, Sulfaquinoxaline
Tetracycline	Chlortetracycline, Oxytetracycline, Tetracycline

Drugs not affected by Guidance #213

- Antimicrobials
  - that are already VFD – avilamycin, florfenicol, tilmicosin;

    or Rx - Tylosin.
  - that are not medically important, for example:
    - Ionophores (monensin, lasalocid, etc.)
    - Bacitracin (BMD, bacitracin zinc)
    - Bambermycins
    - Carbadox
- Other drugs (that are not antimicrobials), for example:
  - Anthelmentics: Coumaphos, Fenbendazole, Ivermectin
  - Beta agonists: Ractopamine, Zilpaterol
  - Coccidiostats: Clopidol, Decoquinate, Diclazuril

What is a veterinary feed directive?

VFD Definitions

- **VFD drug**

- **Veterinary Feed Directive (VFD) -**

VFD Definitions

- **VFD drug** –
- (6) A ‘‘veterinary feed directive (VFD) drug’’ is a drug intended for use in or on animal feed which is limited by a [CVM] approved application … to use under the professional supervision of a licensed veterinarian. …

VFD Definitions

- **VFD drug** - …

- Use of animal feed bearing or containing a VFD drug must be authorized by a lawful veterinary feed directive.

VFD Definitions

- **Veterinary Feed Directive (VFD) –**
- (7) A ‘‘veterinary feed directive’’ is a written (nonverbal) statement issued by a licensed veterinarian in the course of the veterinarian’s professional practice that orders the use of a VFD drug or combination VFD drug in or on an animal feed. …

VFD Definitions

- **Veterinary Feed Directive (VFD)** – …
- This written statement authorizes the client (the owner of the animal or animals or other caretaker) to obtain and use animal feed bearing or containing a VFD drug or combination VFD drug to treat the client's animals only in accordance with the conditions for use approved … by the Food and Drug Administration.

Veterinary Feed Directive

- Existing framework for veterinary oversight of feed use drugs is the *veterinary feed directive* (VFD)
- In 1996 Congress passed the ADAA stating that a drug intended for use in animal feed which requires professional supervision (oversight) of a licensed veterinarian is a VFD drug
- In 2000 FDA finalized regulations for authorization, distribution and use of VFDs
- Although a similar concept, (… *by or on the order of a licensed veterinarian*) VFD **drugs** are not Rx **drugs**

Updates to VFD regulation

- Changes intended to make the process more efficient while continuing to provide public health protections
- VFD Final Rule
  - June 3, 2015 – VFD final rule published
  - October 1, 2015 – VFD final rule became effective

Current VFD Drugs

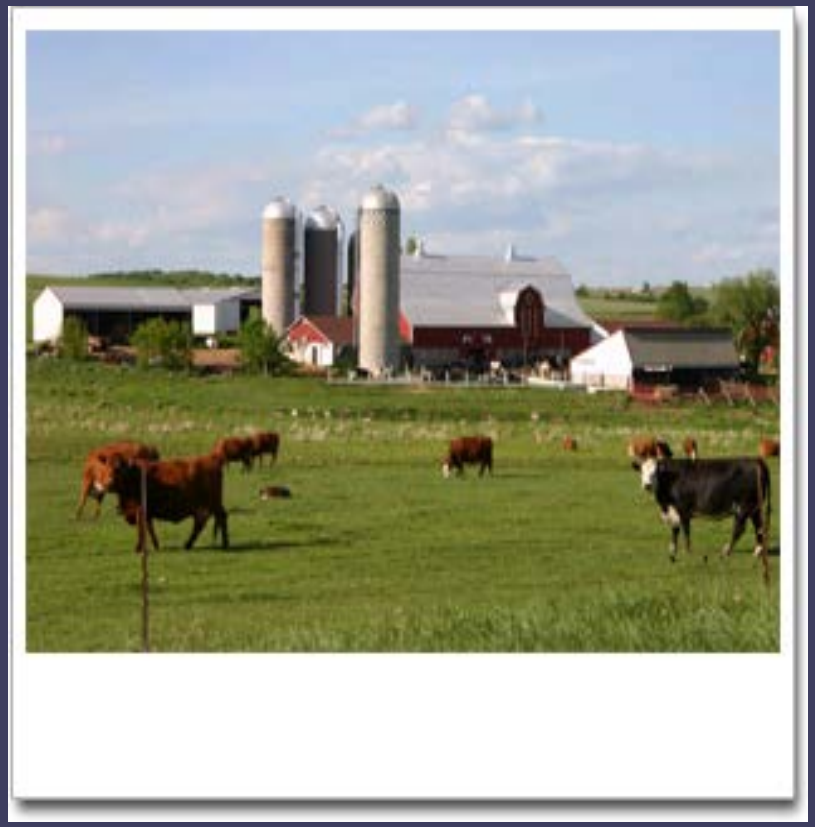
Currently Approved VFD Drugs	Approved for Use in the Following Species
Avilamycin	Swine – reduction of diarrhea – E. coli.
Florfenicol	Fish – control of mortality (various diseases by fish type) Swine – control of SRD
Tilmicosin	Cattle – control of BRD Swine – control of SRD

Note: The three drugs above are affected by the VFD regulation which went into effect 1 October 2015, because they are currently approved as VFD drugs. The medically important antimicrobials will be affected by the VFD Rule when they transition from OTC to VFD beginning 1 January 2017. (See the next slide.)

Examples of medicated feed-use antimicrobials that are expected to **be voluntarily withdrawn or** transition **from OTC** to VFD status

Antimicrobial Class	**Specific drugs approved for use in feed**
Aminoglycosides	Apramycin, *Neomycin*, Streptomycin
Diaminopyrimidines	Ormetoprim
Hygromycin B	Hygromycin B
Lincosamides	*Lincomycin*
Macrolides	Erythromycin, Oleandomycin, *Tylosin*
Penicillins	Penicillin - Currently only production uses.
Streptogramins	*Virginiamycin*
Sulfas	Sulfadimethoxine, Sulfamerazine, Sulfamethazine, Sulfaquinoxaline
Tetracycline	*Chlortetracycline, Oxytetracycline*

What are key elements of VFD regulation?



Information Required on the Veterinary Feed Directive

- The regulation lists all information that must be included on the VFD in order for it to be lawful
- The veterinarian is responsible for making sure the form is complete and accurate
- See brochures for listing of required information:
  - Veterinary Feed Directive Producer Requirements
  - Veterinary Feed Directive Requirements for Distributors (Who Manufacture VFD Feed)
  - Veterinary Feed Directive Requirements for Distributors (Who Do Not Manufacture VFD Feed)
  - Veterinary Feed Directive Requirements for Veterinarians
  - Veterinary Feed Directive Requirements for Veterinarians - For Veterinary Students

VFD Final Rule: Distributors

- A ‘‘distributor’’ means any person who distributes a medicated feed containing a VFD drug to another person.
  - Such other person may be another distributor or the client-recipient of the VFD medicated feed.

  There are two kinds of distributors:

  1. Only distributes VFD feed
  2. Manufactures and distributes VFD Feed
- Distributors must notify FDA:
  - Prior to the first time they distribute animal feed containing a VFD drug
  - Within 30 days of any change of ownership, business name, or business address

To notify FDA, please contact: FDA, Division of Animal Feeds
7519 Standish Place, HFV-220
Rockville, MD 20855
FAX: 240-453-6882

VFD Final Rule: Drug Categories

- Feed-use drugs are assigned to one of two categories:
  - **Category I -** drugs having the lowest potential for residues
  - **Category II -** drugs having the highest potential for residues
- Category determines whether a facility needs to be licensed to handle the drug in the Type A form
- Definition of **Category II** has been revised to eliminate the automatic classification of VFD drugs into Category II
- This change applies to the existing approved VFD drug products, in addition to the products that will become VFD under GFI #213

VFD Expiration Date and Duration of Use

- **VFD Expiration Date –**
  - Specifies the period of time for which the VFD authorization is valid
  - A VFD feed should not be fed after the expiration date (i.e., after VFD authorization expires)
  - May be specified on the product label; if not – it cannot exceed 6 months after the date of issuance.
  - The veterinarian can use his or her medical judgment to determine whether a more limited period is warranted

VFD Expiration Date and Duration of Use

- **The Duration of Use –**
  - A separate concept from the expiration date
  - The length of time that the animal feed containing the VFD drug is allowed to be fed to the animals
  - Established as part of the approval, conditional approval, or index listing process
  - If the VFD order will expire before completing the duration of use on the order, the client should contact his/her veterinarian to request a new VFD order

Current VFD Drugs

Currently Approved VFD Drugs	Approved for the Following Uses	VFD Expiration Date	Duration of Use
Avilamycin	Swine – reduction of diarrhea – E. coli.	90 d	21 d
Florfenicol	Fish – control of mortality (various diseases by fish type)	15 d	10 d
	Swine – control of SRD	90 d	5 d
Tilmicosin	Swine – control of SRD	90 d	21 d
	Cattle – control of BRD	45 d	14 d

Medically important antimicrobials used in animal feed expected to transition from OTC to VFD marketing status.

- Expiration Date: not to exceed 6 months
- Duration of Use: See CVM Blue Bird Label website
- http://www.fda.gov/animalveterinary/products/animalfoodfeeds/medicatedfeed/bluebirdlabels/default.htm

Refills

- Refills (reorders) – Are only permitted to be authorized by veterinarians if the drug approval, conditional approval, or index listing expressly permits a refill (or reorder)
  - If a label is silent on refills, a refill may not be authorized
  - Currently, there are no approved VFD drugs that allow refills or reorders as a condition of their approval, conditional approval, or index listing

Approximate Number of Animals

- VFD must include an **approximate** number of animals:
  - The potential number of animals of the species and production class identified on the VFD that will be fed the VFD feed or combination VFD feed manufactured according to the VFD at the specified premises by the expiration date of the VFD

Approximate Number of Animals

- VFD no longer requires the amount of feed to be fed
  - Expectation is that feed mill will work with the client and veterinarian to determine an appropriate amount of feed to manufacture and distribute under the VFD
    - based on the approximate number of animals, duration of use, and expiration date

Combination VFD drugs

- **“Combination VFD drug”** - (12) A ‘‘combination veterinary feed directive (VFD) drug’’ is a combination new animal drug … intended for use in or on animal feed which is limited by a [CVM] approved application … to use under the professional supervision of a licensed veterinarian, and at least one of the new animal drugs in the combination is a VFD drug.
  - The new VFD rule requires the issuing veterinarian to include one of three “**affirmation of intent**” statements to affirm his or her intent as to whether the VFD drug being authorized can or cannot be used in approved combinations

Current VFD Drugs

Currently Approved VFD Drugs	Approved for Use in the Following Species – abbreviated indication	Combinations/ Affirmation
Avilamycin	Swine – reduction of diarrhea – E. coli.	None/ 1
Florfenicol	Fish – control of mortality (various diseases by fish type)	None/ 1
	Swine – control of SRD	None/ 1
Tilmicosin	Swine – control of SRD	None/ 1

Current VFD Drugs

Currently Approved VFD Drug	Currently Approved Combination	Approved for Use in the Following Species - abbreviated indication	Affirmation
Tilmicosin (pioneer)	Tilmicosin only	Cattle – control of BRD	1
	+ Monensin	Cattle – control of BRD + Coccidiosis	2 or 3
	+ Monensin	Cattle – control of BRD + Feed efficiency	2 or 3

Substitution of VFD drugs

Use of an approved generic VFD drug as a substitute for an approved pioneer VFD drug in cases where the pioneer VFD drug is identified on the VFD.

- If the veterinarian does not specify that a substitution is not allowed, the feed manufacturer may use either the approved pioneer or an approved generic VFD drug to manufacture the VFD feed.
- However, the feed manufacturer may not substitute a generic VFD drug for a pioneer VFD drug in a combination VFD feed if the generic VFD drug is not part of an approved combination VFD drug.

Current VFD Drugs

Currently Approved VFD Drug	Approved for Use in the Following Species - abbreviated indication	Pioneer	Generic
Avilamycin	Swine – reduction if diarrhea – E. coli	Yes	No
Florfenicol	Fish – control of mortality (various diseases by fish type)	Yes	No
	Swine – control of SRD	Yes	No
Tilmicosin	**Swine – control of SRD**	**Yes**	**Yes**
		Substitution Option	
	Cattle – control of BRD	Yes	No

Veterinary Client Patient Relationship (VCPR)

- Veterinarian issuing a VFD is required to be licensed to practice veterinary medicine and operate in compliance with either, the:
  - **State-defined VCPR** – if the VCPR defined by such State includes the key elements of a valid VCPR defined in § 530.3(i); or
  - **Federally-defined VCPR** – if a VCPR is not required to write a VFD in that state or the key elements are not met.

Veterinary Client Patient Relationship (VCPR)

- The State-defined VCPR must at least address these key element concepts that the veterinarian:
  1) engage with the client to assume responsibility for making clinical judgments about patient health;
  2) have sufficient knowledge of the patient by virtue of patient examination and/or visits to the facility where patient is managed; and
  3) provide for any necessary follow-up evaluation or care

Veterinary Client Patient Relationship (VCPR)

- FDA worked with State regulatory authorities to verify whether that state has VCPR requirements in place that:
  - apply to the issuance of a VFD, and
  - include the key elements of the federally-defined VCPR

Veterinary Client Patient Relationship (VCPR)

- **FDA has provided an online list of VCPR requirements by state on the VFD website**
  - This list will be updated periodically as FDA receives and verifies information from states if they change their VCPR definition or its applicability
  - For the current list of state or federal VCPR see http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/ucm460406.htm

When will this go into effect?

Implementation Timeline Summary

- October 1, 2015 – VFD Final Rule went into effect
  - Applies to current VFD drugs
- January 1, 2017 – Target for implementation that all medically important antimicrobials for use in or on feed to require a VFD and for those for use in drinking water to require a Rx
  - December 2016 – Target for drug sponsors to implement changes to use conditions of products affected by GFI #213

References and Resources

NOTE:

- **As the industry transitions, CVM anticipates additional changes during the coming months to the information in this presentation, please check the following links for the most recent updates.**

References and Resources

- **Veterinary Feed Directive,** http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/ucm071807.htm
- **Judicious Use,** http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/JudiciousUseofAntimicrobials/default.htm
- **Blue Bird Labels,** http://www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/MedicatedFeed/BlueBirdLabels/default.htm

To Receive Information Updates

- Follow CVM on Twitter @FDAanimalhealth
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  - CVM FR Notices:
    Federal Register notices issued by the Center for Veterinary Medicine, excluding drug approvals.
  - What's New:
    RSS Feed for new items posted to the Animal & Veterinary section of FDA's website.

Thank You

TRACE MINERALS (Zn, Cu, Se, and Mn) AND THE ACQUIRED IMMUNE RESPONSE IN CATTLE

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INTRODUCTION

The immune system consists of two main branches; the innate and the adaptive immunity. These two components have specific functions, but work integrated to protect the animal from infections. Trace minerals (TM) are crucial for the development of an adequate immune response in cattle, especially in stressed animals, since some of these micro-elements activate or are structural components of metabolic pathways, DNA replication, antioxidant protective systems, etc. The benefits of administering trace minerals on animal health and performance have been previously assessed in dairy (Harrison et al., 1984; Machado et al., 2013), and beef cattle (Arthington et al., 2014; Berry et al., 2000; Genther, and Hansen, 2014; Richeson and Kegley, 2011). Several studies have evaluated the effects of injectable TM on the immune function of cattle (Arthington and Havenga, 2012; Chirase et al., 1994; Clark et al., 2006; Droke and Loerch, 1989), some of which will be discussed in this manuscript.

Field and experimental studies focused on improving the TM status of feeder calves have shown positive effects of administration of TM improving feed efficiency, decreasing morbidity and treatment costs and improving performance and production traits. Our studies at University of Georgia revealed that administration of injectable TM (Se, Zn, Cu and Mn) concurrently with modified-live virus vaccination in dairy and beef calves resulted in earlier and increased antibody titer and leukocyte proliferation upon stimulation with viruses compared to the control group. Therefore, addition of injectable TM supplements to calf management protocols might represent a promising tool to improve livestock health on commercial farms.

THE IMMUNE SYSTEM IN CATTLE

Innate Immunity

The innate immune system represents the first line of defense against a pathogen before the adaptive system can develop the appropriate response. The innate immunity is not antigen specific and does not include anamnestic or memory response. Components of nonspecific immunity are present both prior to and following antigen exposure and they do not discriminate against most foreign substances. These components include natural barriers (skin, epithelial lining, anti-microbial substances, enzymes, etc.), complement proteins, and white blood cells (granulocytes, macrophages) that engulf and eliminate pathogens (phagocytosis and killing).

Further, the innate immunity also includes type I interferon (IFN) antiviral response and natural killer cells (NKC) which are lymphocytes able to destroy infected cells. Granulocytes are also called polymorphonuclear leukocytes (PMN), mainly neutrophils. The main function of neutrophils is to monitor for infection to perform phagocytosis and killing of pathogens. Neutrophils identify pathogens by the recognition of pathogen-associated molecular patterns (PAMPs) using specific receptors, called Toll-like receptors. Microbes have molecules not typically found in mammalian cells (e.g. double-stranded RNA, CpG DNA sequences, and unusual carbohydrate residues), so that neutrophils are able to detect these molecules to eliminate the infectious agents. After binding of PAMPs to Toll-like receptors, neutrophils activate and initiate the phagocytosis and killing. Microbial destruction by phagocytosis involves the production of reactive oxygen species (ROS) which are bactericidal.

Adaptive Immunity

The adaptive or acquired immunity has the capacity to recognize specific antigens and has memory. The primary components of the adaptive immune system involve humoral (antibody production by B lymphocytes) and cell mediated immunity (developed mainly by $CD4^+$ helper T cells, $CD8^+$ cytotoxic T cells and $WC1^+$ γδ T cells).

B-cells mature in bone marrow and are released into blood, where they circulate and populate lymphoid tissues. These cells also act as antigen presenting cells (APC) so that they can recognize antigens and present them to helper T cells, which enhance further antibody production. B lymphocytes activate and undergo proliferation and differentiation, a process termed “clonal expansion”. Each clone of B lymphocytes can recognize a specific target antigen. Production of antibodies by B lymphocytes following interaction with the antigen requires input of interleukins-2 (IL-2), IL-4 and IFN-γ. These cytokines also cause formation of B memory cells from the activated B-cell population. Antibodies are Y-shaped proteins, containing a constant (C) region and a variable (V) region. The V region contains the antigen binding site. The constant region is assembled from distinct genes that yield different antibody isotopes, such as IgG, IgM, IgD, IgA and IgE. Each of these isotopes has different physical properties and specific roles in the animal’s immune system. The roles of antibodies in adaptive immune response include: neutralization or microbe and toxins, opsonization and phagocytosis, antibody-dependent cellular cytotoxicity, and complement activation.

T-cells are produced in the bone marrow and mature in the Thymus (hence the name “T”-cell). T-cells are responsible for “cell-mediated immunity”. Similar to B cells, clonal expansion and differentiation of T-cells results in the development of effector and memory T-cells. During cell-mediated immunity, T lymphocytes recruit and stimulate phagocytic (nonspecific) activity as well as participate in direct lysis of infected cells (e.g. viral infected cells). Helper T-lymphocytes ($CD4^+$) orchestrate the adaptive immune response through the production of cytokines and costimulatory molecules, while cytotoxic T-cells ($CD8^+$) have the capacity to destroy infected cells (e.g. during virus infection).

ROLE OF TRACE MINERALS IN THE IMMUNE RESPONSE

Nutritional status, and particularly mineral levels, have been demonstrated to impact cattle health and performance (Enjalbert et al., 2006; Galyean et al., 1999; Underwood and Suttle, 1999). Trace minerals such as Zinc (Zn), Manganese (Mn), Copper (Cu), and Selenium (Se) are important for optimal immune function (Chirase et al., 1991; Percival, 1998; Underwood and Suttle, 1999) and growth (Spears and Kegley, 2002) in cattle, particularly in highly stressed, and newly received feeder calves (Duff and Galyean, 2007). Zinc contributes with the structure and function of more than 2,500 enzyme systems involved in metabolism (Andreini et al., 2009; Cousins and King, 2004). Zinc activates the enzyme superoxide dismutase, which plays a crucial role in stabilizing cell membranes against reactive oxygen species (ROS) (Bonaventura et al., 2015; Haase and Rink, 2014). Zinc is involved in DNA replication through the actions of ribonucleotide reductase, and is necessary for lymphocytes proliferation and differentiation. Zinc's major roles in the immune response involve signaling and adhesion of neutrophils and macrophages (Bonaventura et al., 2015), production of pro-inflammatory cytokines by monocytes (Rink and Kirchner, 2000), regulation of IL-2 secretion, signal transduction for T cell activation, clonal expansion, differentiation and T_H cells polarization (Haase and Rink, 2014), B-cell function, and antibody production (Pinna et al., 2002; Tomlinson et al., 2008).

Copper is important in the mitochondrial metabolic cascades for energy production to supply different organs, including those of the immune system (Failla, 2003). Copper also plays a role in superoxide dismutase activity and neutralization of ROS (Maggini et al., 2007), and contributes to the process of phagocyte killing (Linder, 1991). Ceruloplasmin is a copper-containing enzyme whose production increases dramatically during inflammation in response to the necessity of scavenging oxygen radicals released by immune cells (Percival, 1998). In rodents, copper deficiency is associated with decreased IL-2 production, lymphocyte proliferation and T cells counts (Bala and Failla, 1993; Bonham et al., 2002; Klotz et al., 2003; Linder and Hazegh-Azam, 1996; Minatel and Carfagnini, 2000; O'Dell, 1993; Pan and Loo, 2000; Percival, 1998). Similarly, studies in cattle fed a copper-deficient diet showed a significant reduction in B-lymphocytes and impaired neutrophil activity (Cerone et al., 1998).

Selenium appears to be very important to the migration of neutrophils into tissues and subsequent inflammation (Maddox et al., 1999). Selenium is a component of the enzyme glutathione peroxidase that inactivates ROS production and prevents released ROS from causing cellular damage (Maddox et al., 1999; Neve, 1991). Selenium deficiencies have been associated with depressed neutrophil migration and killing ability, and reduced B-cell response and antibody production. Moreover, Se supplementation enhanced both humoral and cell-mediated and immune responses (Maggini et al., 2007). The level of Se in tissues and blood affected the total IgM levels and BHV1-specific antibody titers after challenge (Reffett et al., 1988). Evidence that Mn plays a role in the immune system is limited. However, Mn has an essential function in removing ROS produced by active phagocytic cells (Tomlinson et al., 2008).

EFFECT OF INJECTABLE TRACE MINERALS (ITM) ON THE IMMUNE RESPONSE TO VACCINATION AGAINST BOVINE RESPIRATORY DISEASE (BRD).

Bovine respiratory disease (BRD) has a major impact on the profitability of the dairy and beef industries in North America, resulting in substantial economic losses that exceed $1 billion annually (Griffin, 1997; McVey, 2009). The infectious agents most consistently implicated in BRD include *Bovine viral diarrhea virus* (BVDV), *Bovine herpes virus 1* (BHV1), *Bovine respiratory syncytial virus* (BRSV), *Parainfluenza 3 virus* (PI3V), *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni,* and *Mycoplasma bovis*. Appropriate biosecurity measures and vaccination program are crucial to prevent and control BRD.

Arthington and Havenga (2012) assessed the effect of administration of ITM on the humoral immune response after BRD specific MLV vaccination in cattle. That study demonstrated that ITM given concurrently with viral vaccination enhanced the production of neutralizing antibodies to BHV1 in beef calves. Additionally, recent studies have shown that treatment with ITM concomitantly with MLV vaccination induced a faster BVDV-specific antibody response in newly received, highly stressed calves (Roberts et al., 2015).

A growing body of evidence suggests that both humoral and cell mediated immune (CMI) responses are critical in protection against viral agents involved in BRD (Collen and Morrison, 2000; Howard 1990; Nobiron et al., 2003). A more complete evaluation of the immune responses induced by MLV vaccination requires the use of methods to assess both humoral (antibody response) and cellular effector mechanisms (recall antigen induced proliferation and induction of IFN-γ as the core Th1 cytokine).

A study was performed at University of Georgia to evaluate the effect of an injectable trace mineral (ITM) supplement containing zinc, manganese, selenium, and copper on the humoral and CMI responses to vaccine antigens in dairy calves receiving a modified-live viral (MLV) vaccine containing BVDV, BHV1, PI3V and BRSV (Palomares et al., 2016). A total of 30 dairy calves (3.5 months of age) were administered a priming dose of the MLV vaccine containing BHV1, BVDV1 & 2, BRSV, PI3V, and an attenuated-live *Mannheimia-Pasteurella* bacterin subcutaneously (SQ). Calves were randomly assigned to 1 of 2 groups: (1) administration of ITM SQ (ITM, Multimin 90® Multimin USA®, n = 15) or (2) injection of sterile saline SQ (Control; n = 15). Three weeks later, calves received a booster of the same vaccine combination SQ, and a second administration of ITM, or sterile saline, according to the treatment group. Throughout the study, the calves grazed Bermuda grass (*Cynodon dactylon*) and Fescue grass (*Festuca arundinacea*) with no access to mineral supplementation. Animals had access to hay (Bermuda grass and Fescue grass) and water *ad libitum*. Additionally, calves received 2.7 kg/head/day of concentrate supplement (Bulk Cattleman's Special; Godfrey's Warehouse; Madison-GA; Palomares et al., 2016) divided into two meals.

In this study, calves had adequate liver tissue concentration of all the study trace minerals assessed at all sampling dates according to standard reference values (Herdt and Hoff, 2011). Administration of ITM resulted in increased concentrations of liver Se (on days 21 and 56), Cu (on day 56) and Mn (on day 56) compared to saline injected calves (Palomares et al., 2016).

Administration of ITM concurrently with MLV vaccination resulted in higher antibody titers to BVDV1 on day 28 after priming vaccination compared to the control group (*P* = 0.03). This higher BVDV1 specific humoral immune response was associated with both higher production of antibody (Fig. 1A) and a numerical tendency of greater proportion of sero-conversion (≥ 4-fold increase compared to day 0) to BVDV1 on day 28 in the ITM group (12/15; 80.0%) compared to the control group (8/15; 53.3%; *P* = 0.13; Fig. 1B).

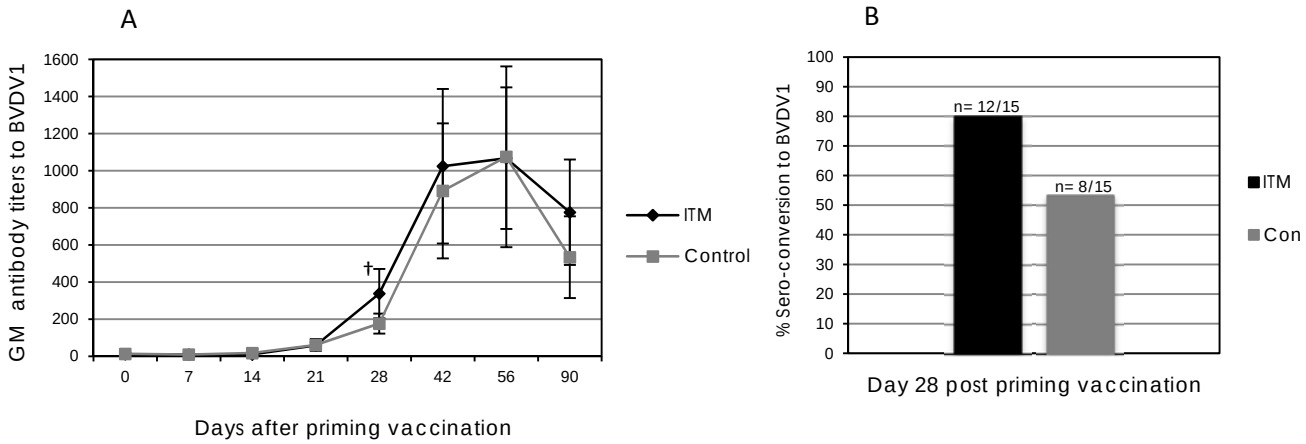

Figure 1. Geometric means serum neutralizing (SN) antibody titers to BVDV1 (A), and proportion of sero-conversion or ≥4-fold increase in SN antibody titer to BVDV1 on day 28 relative to the day of priming vaccination (B) in dairy calves that received injectable trace minerals (ITM) or saline (Control) concurrently with a MLV vaccine. †Significant differences between groups for each day (P = 0.03).

This quicker BVDV-specific antibody response elicited by an MLV vaccine delivered concurrently with ITM might be of significant value by conferring earlier protection following vaccination. This could be particularly important in newly received, highly stressed cattle that are at high risk of respiratory viral infections. A rapid increase in serum neutralizing antibodies against BVDV after vaccination may be beneficial to prevent infection and disease development. The antibodies may neutralize extracellular virus particles, inhibiting attachment of the virus to host cells, and contribute to antibody dependent cell mediated cytotoxicity (Forthal, 2014). Similarly, previous reports have shown that ITM enhanced humoral immune response to pathogens of clinical significance in cattle production, including BVDV (Roberts et al., 2015), BHV-1 (Arthington and Havenga 2012), *E. coli* (Panousis et al., 2001) and *Pasteurella*

haemolytica (Droke and Loerch, 1989). In the study performed by Arthington and Havenga (2012), administration of trace minerals concurrently with an MLV vaccine to steers induced a significant increase in BHV-1 serum neutralizing antibody titers on days 14, 30, and 60 post-vaccination compared to the base line titers on day 0 and to the titers in the saline injected steers.

Calves treated with ITM showed an earlier enhancement in mononuclear leukocyte proliferation to BVDV1 following vaccination compared to the control group (peak of leukocyte proliferation occurred 14 days later). Proliferation of mononuclear leukocytes after BVDV stimulation tended to be higher on day 14 after priming vaccination in calves treated with ITM than in the control group (P = 0.08; Fig. 2A). Calves that received ITM showed higher mononuclear leukocyte proliferation to BRSV stimulation on day 7 after priming vaccination compared to the control group (P = 0.01; Fig. 2B). Moreover, calves in the ITM group also had an enhanced production IFN-γ by PBMC after stimulation with BRSV on day 21 after priming vaccination compared to day 0 (P < 0.01).

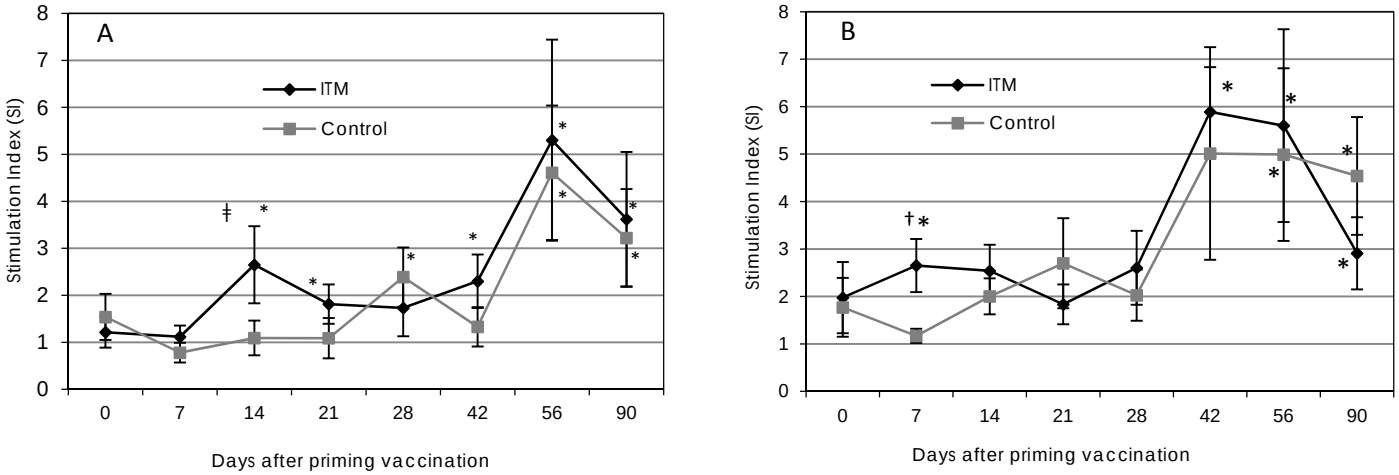

Figure 2. Peripheral blood mononuclear leukocyte proliferation (expressed as stimulation index; SI) in response to BVDV1 (A), and BRSV (B) in dairy calves injected with a trace mineral supplement (ITM) or saline (Control) concurrently with administration of a MLV vaccine. * Value differs significantly from the value on day 0 for each group (3A: P < 0.05; 3B: P < 0.01). ‡Suggests a tendency of higher leukocyte proliferation compared to the control group (3A, P = 0.08). †Significant difference between groups (3B, P= 0.01).

Adequate supply of Zn, Cu, Se and Mn has been documented to be essential for cell signaling and cytokine production during lymphocyte activation (Puertollano et al., 2011; Spears, 2000). These trace minerals are fundamental elements in the structure and function of several metalloproteins that participate in general housekeeping processes involved in cellular clonal expansion including metabolic cascades for energy production, DNA replication and transcription, as well as protection against ROS (Failla,

2003). The enhancement of these general cellular functions might be contributing to the higher leukocyte proliferation response observed in the ITM group.

Trace minerals are crucial for the development of an adequate immune response in cattle, especially in stressed animals. Field and experimental studies focused on improving the trace mineral status of stressed feeder calves have shown positive effects of ITM improving feed efficiency, decreasing morbidity and treatment costs and improving performance and production traits (Arthington et al., 2014; Berry et al., 2000; Clark et al., 2006; Genther, and Hansen, 2014; Richeson et al., 2011). The results of the current study support our hypothesis, and recapitulate the findings of previous studies demonstrating the benefits of trace mineral supplementation on the immune response to MLV vaccines in cattle. This suggests that addition of ITM to calf management protocols might represent a promising tool to improve livestock health on commercial farms.

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KEY ROLES OF AMINO ACIDS IN COW PERFORMANCE AND METABOLISM – CONSIDERATIONS FOR DEFINING AMINO ACID REQUIREMENTS

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INTRODUCTION

Traditionally, maintenance protein requirement (rqt) included integumental, endogenous urinary and metabolic fecal protein loss; NRC (2001) has added a rqt for duodenal endogenous protein. Most of the current models used to balance dairy rations for metabolizable protein (MP; e.g. NRC (2001), CNCPS (Fox et al., 2004), van Duinkerken et al. (2011)) base their estimation of maintenance MP rqt on an excellent but relatively old review from Swanson (1977). However, recently rqt for metabolic fecal protein (MFP) has been revisited in Norfor (2011) and Systali (Sauvant et al., 2015) as well as rqt for endogenous urinary in Systali (Sauvant et al., 2015). Regarding individual amino acid (AA) rqt, the NRC (2001) subcommittee agreed that "current knowledge is too limited, both for model construction and model evaluation, to put forth a model that quantifies AA requirements for dairy cattle" (in a factorial approach). They have therefore adopted a proportional approach, similar to the French system (Rulquin et al., 2001). This approach determines the AA rqt based on empirical relationships between observed milk protein concentration or yield relative to the proportion of the AA in MP supply.

In contrast, other models (e.g. CNCPS (Fox et al., 2004), AminoCow (Evonik AG Industries, Hanau, Germany)) have adopted a factorial approach. Estimation of AA rqt using the factorial approach can be viewed as a 3-step procedure: 1) identify and quantify the quantity of true protein (TP) excreted out of the cow daily or protein accretion as body weight gain or conceptus: the "exported" proteins need to be balanced by an exogenous supply; 2) determine the AA composition of these TP excretions or accretions; and 3) determine the efficiency of utilization of the digested AA to support the protein functions. As our knowledge on AA metabolism has increased over the last decades, it becomes appropriate to use this knowledge to update the factorial estimation of MP and AA rqt of high producing dairy cows. Therefore the current review aims to integrate knowledge developed in recent years on AA metabolism to update the estimation of MP and AA rqt to allow a better usage of the factorial approach to improve the formulation of rations. Discussion will be limited to essential AA (EAA) and simplified to a lactating, mature, non-gestating cow without changes in body weight (BW, expressed in kg through the text) and composition. Numerical examples will be given using a 700 kg cow producing 45 kg milk/d at 3.2% CP (3.0% TP) and with a daily dry matter intake (DMI, expressed in kg/d through the text) of 27 kg.

EXPORTED PROTEINS

Metabolic fecal loss is by far the largest component of the maintenance rqt, representing 67 and 87% of maintenance rqt in our example cow using NRC (2001) and CNCPS (Fox et al., 2004) models, respectively (Table 1). However, its estimation is based on DMI and as such, does not truly represent what is meant by maintenance. Therefore, we suggest using the term “non-productive functions” proposed by Sauvant et al. (2015) to refer to this group of functions inherent to the biology of the cow but not supporting productive functions as growth, gestation or milk production.

Integumental proteins include loss and growth of hair, scurf and scales rubbed from the skin surface, along with some N containing compounds in skin secretions. They represent less than 2 % of MP maintenance rqt (Table 1) and as such have received limited attention further than Swanson (1977). There is not enough information to update this estimation, which will be retained: integumental exported proteins were estimated at 0.20 g CP/$BW^{0.60}$ per day (Swanson, 1977). To transfer this relation into MP rqt, we need, however, to convert it to TP and divide it by the efficiency with which the MP supply is being used to support the protein function. Although the efficiency of converting MP supply to exported proteins is variable depending on the supply of MP relative to rqt (this will be discussed in a following section), the value of 0.67 is commonly used. This value seems to correctly represent the biology when MP supply is in a relative equilibrium with the rqt and will be used, when needed, to present the changes proposed to the estimation of the MP rqt. Therefore, in the current model, **daily MP rqt (g MP/d) for integumental proteins = 0.26 g MP × $BW^{0.60}$**, using a TP/CP ratio of 0.859 (based on AA composition: see next section) and an efficiency factor of 0.67 (0.20 × 0.859 / 0.67).

The estimation of endogenous urinary MP rqt used by most models is also based on Swanson (1977) estimating daily CP losses at 2.75 g/$BW^{0.50,}$ which divided by an efficiency of 0.67 yields a rqt of 4.1 MP g/$BW^{0.50}$ (the TP/CP ratio is not taken into account). However, it was not clear which AA composition should be assigned to this exported “protein” (in fact what is exported is N-containing compounds, derived from AA, but not protein anymore). The CNCPS (Fox et al., 2004) proposed using the AA composition of the empty body. In an attempt to better define the AA composition of this exported “protein”, a literature review was conducted to quantify the composition of urinary-N. Force is to admit that literature is scarce on that domain (Dijkstra et al., 2013). The major N-fractions in urine that contributed to the non-productive functions are: endogenous urea, endogenous purine derivative (PD), creatinine and creatine, hippuric acid and 3-methyl-His (considered because of its potential demand on His). Daily excretion of endogenous urea has been quantified as 10 mg N/BW per day (Hutchinson and Morris, 1936; Biddle et al., 1975; Marini and Van Amburgh, 2005; Wickersham et al., 2008a and b). To estimate creatinine excretion, we built a database using exclusively dairy breeds, growing or lactating (141 treatment means from 27 publications from 1979 to 2015): urinary excretion of creatinine averaged 9.46 mg N/BW per day (25.5 mg creatinine/BW). Creatine excretion was evaluated as 0.37 that of creatinine (Blaxter and Wood, 1951; Nehring et al., 1965; Bristow et al., 1992). Urinary

excretion of endogenous PD was estimated to average 27.1 mg N/$BW^{0.75}$ per day (483 µmol: reviews from Tas and Susenbeth, 2007; Fujihara and Shem, 2011). Daily urinary excretion of 3-methyl-His (µmol) was estimated as 50.5 + 3.54 × BW (Harris and Milne, 1981). Using the database from Spek et al. (2013), the "measured" endogenous urinary-N excretion was calculated as non-urea urinary excretion minus estimation of PD from absorbed MCP plus endogenous urea, estimated as described above. The sum of the estimates described above represented 54% of the "measured" endogenous urinary N excretion. Another important N-fraction excreted in urine is hippuric acid. Hippuric acid is formed in the liver to detoxify benzoic acid originating from rumen fermentation of dietary phenolic compounds. Although this excretion cannot be purely defined as "endogenous", it has probably been included in previous estimates of endogenous urinary excretion. When determined, it averaged 25.7% of non-urea N urinary excretion (Nehring et al., 1965; Bristow et al., 1992; Kool et al., 2006). With this addition to the "endogenous" urinary excretion, the estimated and measured values were in a similar range (29.7 vs. 33.4 g N/d, for estimated vs. measured, respectively), but there was a strong slope bias. The potential hippuric acid excretion was best related, in the database, to the proportion of urea-N in urinary N excretion. Using this relationship to evaluate the hippuric acid excretion, the estimated endogenous urinary N excretion averaged 33.2 g N/d compared with 33.4 g N/d for the 84 treatment means "measured" as described above. There was a less important slope bias, but there is not enough information to correct it, indicating an important gap in our knowledge on the composition of urinary-N excretion. The BW range in this database is too small to find a significant relationship between the endogenous urinary-N excretion and BW. However, as beside hippuric acid, most of the estimations were based on BW, the endogenous urinary-N excretion was expressed relative to BW and averaged 53 mg N/BW. Interestingly, using a totally different approach, in the new French model, Systali, the daily endogenous urinary-N loss averaged 50 mg N/BW (Sauvant et al., 2015). As these compounds are 1) expressed in g N/d, there is no need to convert from CP to TP; 2) end-product of metabolism, we consider, as Sauvant et al. (2015), that an efficiency of 1.0 should be used. Therefore, in the current model, **daily MP rqt (g MP/d) for endogenous urinary loss = 0.33 g MP × BW** (0.053 × 6.25 × 1).

The estimations of rqt to cover MFP are probably those varying the most between models: Sauvant et al. (2015) reported that, using cow characteristics of their database "Bovidig_PDI", estimates of MFP rqt (g MP/d) varied among models:18.2 ± 3.4 (NorFor, 2011), 19.8 ± 3.7 (Sauvant et al., 2015), 23.1 ± 5.4 (Van Duinkerken et al., 2011), 23.5 ± 0.5 (NRC, 2001) and 38.0 ± 8.9 (CNCPS, Fox et al., 2004). The lowest SD observed for NRC (2001) is due to the fact that this estimation is solely based on DMI whereas the other models included, one way or the other, an estimation of the digestibility along the gastro-intestinal tract. One striking observation in the estimation of MFP rqt in NRC (2001) and CNCPS (Fox et al., 2004), is that for both models, there has been no conversion of the estimation of CP excretion in feces into TP and also, no utilization of an efficiency factor to obtain the final estimate of MP rqt. In fact, the definition of what is included in MFP has been somewhat vague and changing over the years. Our belief is that MFP loss should include all endogenous proteins secreted along the gastro-intestinal tract and not digested in the small intestine. Endogenous proteins can flow to

the duodenum either as free proteins or incorporated into rumen-synthesized microorganisms. However, endogenous proteins should exclude bacteria-N synthesized from urea, as utilization of urea does not impose a demand on AA per se. With this concept in mind, the estimation of the ileal flow of endogenous protein in dairy cows obtained by Ouellet et al. (2.39 g N/kg DMI: Ouellet et al., 2002 and 2010) plus an estimation of the endogenous secretion into the large intestine excreted in feces would adequately represent the MFP. Endogenous secretion into the large intestine excreted in feces was estimated to be equivalent to 0.6 times the endogenous small intestinal flow passing at the ileum, based on observations in sheep (Sandek et al., 2001): it is assumed that half of the endogenous flow secreted in the large intestine originates from urea and half from AA. Endogenous proteins flowing to the ileum from small intestinal secretion were estimated at 0.81 g N/d (Ouellet et al., 2007). However, these results are limited and expressed relative to DMI. It has been clearly shown that MFP is better related to the indigestible DM than the DMI (Swanson, 1977). In an attempt to account for diet digestibility, the equation published by Marini et al. (2008) where the intercept of the relation between total tract digested N and dietary N was interpreted as the metabolic fecal N was used: this equation includes NDF (%OM in the diet) and the carbohydrate fermentation rate. However, this MFP estimation includes undigested bacteria protein synthesized from urea-N: therefore, to only account for undigested bacteria synthesized from endogenous AA-N, the intercept and the slope were scaled to yield MFP estimates obtained by Ouellet et al. (2002 and 2010) with an average carbohydrate fermentation rate. Overall the **MP rqt (g MP/d) for MFP = [12.7 + 0.15 × NDF (%DM)] × DMI**, using a TP/CP ratio of 0.732 (based on AA composition of MFP: see next section) and an efficiency factor of 0.67.

As previously discussed (e.g. Lapierre et al., 2007), we consider that there is no need to include the duodenal flow of endogenous proteins into the rqt, as long as they are also excluded from the net supply of MP and AA. Indeed, these proteins are mainly synthesized from AA provided by the arterial supply, and as such the fraction of that flow which is digested into the small intestine does not represent a rqt as it is returned blood circulation. Therefore, only the portion of that flow reaching the ileum undigested represents a rqt and is already included in the MFP fraction. As well, this flow does not represent a new input of MP and AA into the cow and needs to be removed from the duodenal flow to estimate the true net supply of MP and AA. Based on 12 studies conducted between 1980 and 2013, the duodenal endogenous protein flow was re-evaluated to be (g CP/d)= 96.1 + 7.54 × DMI (Lapierre et al., 2016).

In summary, in the current model, using an efficiency of 0.67, **MP rqt for non-productive functions (g MP/d) = [0.26 g MP × $BW^{0.60}$] + [0.33 × BW] + [(12.7 + 0.15 × NDF (%DM)) × DMI]**, with BW and DMI in kg. Supply of MP needed to cover milk protein secretion is relatively straightforward. Milk protein yield (MPY) is easily measured and should be expressed as TP. Using an average efficiency of 0.67, **MP rqt for milk (g MP/d)= MPY_{TP} / 0.67**. The MP rqt for non-productive functions and milk MP rqt for the example cow are compared in Table 1: rqt are lower in the current model than in NRC or CNCPS but we have to keep in mind that the estimation of the duodenal flow of endogenous protein needs to be removed from the estimated MP supply.

Table 1. Comparison of metabolizable protein (MP) requirements (rqt; g/d) between different models[a].

Protein function	Model[b]	Exported CP[c]	TP/CP[c]	Exported TP	Efficiency	MP rqt
Integumental loss	NRC, CNCPS	10	ND[d]	ND	0.67	15
	Current	10	0.86	9	0.67	13
Endogenous	NRC, CNCPS	73	ND	ND	0.67	109
urinary	Current	232	1.0	232	1	232
Metabolic	NRC	631	ND	ND	ND	631
fecal protein	CNCPS	810	ND	ND	ND	810
	Current	444	0.73	324	0.67	484
Duodenal	NRC	257	0.5	128	0.67	191
endogenous	CNCPS	ND	ND	ND	ND	ND
	Current	-	-	-	-	-
Milk	NRC	-		1350	0.67	2015
	CNCPS	1440	0.93	1339	0.65	2060
	Current			1350	0.67	2015
Total MP rqt	NRC					2961
	CNCPS					2994
	Current					2746

[a]Based on cow averaging 700 kg, 27 kg/d DMI, 45 kg milk/d at 3.0% TP; NDF of the ration: 36% of DM.
[b]NRC (2001); CNCPS (Fox et al., 2004); current : as detailed in this paper.
[c]TP: true protein; CP: crude protein.
[d]ND: not determined.

AMINO ACID COMPOSITION

Correction factors

Most of the values available on AA composition of proteins are concentrations obtained after a 21-h or 24-h hydrolysis; then the sum of these AA relative to the CP of the protein is assumed to represent the TP/CP ratio. In fact, these 2 assumptions are not correct. First, it is well recognized that a period of 21 to 24 h for the hydrolysis of a protein is a compromise to reduce time and cost related to laboratory analysis. It has been known for a long time that acid-labile AA like Ser and Thr are partially destroyed after their release from the protein during a 24-h hydrolysis (Rees, 1946). On the other hand, because peptide bonds involving the branched-chain AA (BCAA) Ile, Leu and Val are difficult to cleave, a hydrolysis lasting 24 h is insufficient to release all the BCAA (Blackburn, 1968). Concentrations obtained with a 24-h hydrolysis are useful to rank or compare feed ingredients. However, when we want to link the digestive flow of AA obtained by hydrolysis with, for example, their net portal absorption measured as free AA into blood circulation, there might be discrepancy due to the low concentration of some AA obtained with the 24-h hydrolysis compared with their true concentration in the digestive flow (Pacheco et al., 2006). On the other hand, often, milk AA composition used in models to determine AA rqt is obtained from calculation based on the protein composition of milk and AA composition of each milk protein fraction based on its primary structure (e.g. CNCPCS, Fox et al., 2004). So, when setting up a factorial approach to balance AA supply and rqt, it is not coherent to use, in the same

calculation, AA composition obtained with one method (24-h hydrolysis underestimating some AA) for all the supply and all the rqt except milk and use a totally different theoretical approach for milk, the major component of the rqt, which is assumed to have the true AA composition.

To obtain the true amount of an AA in the protein in the original material, before the start of hydrolysis, a method of extrapolation with multiple hydrolysis times involving simultaneous release and decay has been proposed (Robel and Crane, 1972): they were using 5 times of hydrolysis, ranging from 4 to 141 h. This method was further explored with up to 19 times of hydrolysis, ranging from 2 to 141 h (e.g. Rutherfurd et al., 2008). The results obtained at 24 h were compared with the extrapolated values estimating the "true" AA composition of the protein. Others have compared the maximal AA concentration obtained after different times of hydrolysis ranging from 4 to 10 times with the 24-h value (e.g. Rowan et al., 1994). In addition, comparison has also been made with the theoretical value calculated based on the primary structure of the protein (e.g. lysozyme: Darragh et al., 1996; our unpublished work with bovine serum albumin). We have also conducted multiple hydrolysis times (13 times in triplicate, ranging from 2 to 168 h; unpublished) on 6 feeds. The ratio of the "true" concentration relative to the 24-h measurement was calculated combining the ratios of the maximal value, theoretical value and extrapolated value relative to 24-h measurement (Table 2).

Table 2. Correction factors (CF) proposed for individual AA to estimate the true AA concentration of the anydrous AA (AAA) from concentrations obtained after a 24-h hydrolysis.

AA	Missing in 24-h hydrolysis	MW[a] AAA / MW AA	global CF_{AA}[b]
Ala	1.05	0.798	0.84
Arg	1.03	0.897	0.93
Asx	1.03	0.865	0.89
Cys	1.23	0.850	1.05
Glx	1.06	0.878	0.93
Gly	1.09	0.761	0.83
His	1.02	0.884	0.90
Ile	1.12	0.863	0.97
Leu	1.07	0.863	0.92
Lys	1.06	0.877	0.93
Met	1.05	0.879	0.92
Phe	1.09	0.891	0.97
Pro	1.05	0.844	0.88
Ser	1.13	0.829	0.94
Thr	1.08	0.903	0.98
Trp	1.12	0.849	0.95
Tyr	1.08	0.912	0.98
Val	1.11	0.901	1.00

[a]MW: molecular weight.
[b]The global CF_{AA} is used to calculate the corrected AAA (AAAc) composition from the 24-h hydrolysis.

The second consideration relates to the chemistry of the protein. When a peptide bond in cleaved, one molecule of water is added to each released AA: complete hydrolysis of 1 kg of protein should yield ± 1.15 kg of free AA. Therefore, summing the AA concentrations obtained after a hydrolysis and expressing this result over total CP overestimates the TP/CP ratio by approximately 15%. The weight of each AA should be corrected by the ratio of the molecular weight (MW) of the AA without a molecule of water (anhydrous AA = AAA)/ MW of the AA. This approach is currently used in the NorFor system (2011). Multiplying the ratio of the AA missing because of the 24-h hydrolysis with the ratio the MW AAA/ MW AA yields a global correction factor for each AA. This global correction factor can be applied to 24-h hydrolysis concentrations to obtain the "true" corrected value of the AAA (AAAc). Although tedious, more work is needed to determine if the same factors are valid across proteins analyzed when building models (e.g. RUP, duodenal protein). Preliminary results from our laboratory indicate that the RUP fractions share the same factors than the feed ingredients.

AA Composition of the Protein Functions

In Table 3, the AA composition of each protein functions was revisited, based on the metabolic definition of each protein type and the global corrections factors of Table 2. Data are given for all AA, but rqt can only be established for the EAA, which are not synthesized by the cow. Therefore, Arg rqt will not be determined because there is substantial synthesis of Arg by the cow. The AA composition of the integumental proteins was estimated using the head, hide, feet and tail combined composition reported by Williams (1978) and van Amburgh et al. (2015). This is not totally accurate, but with the low contribution of this function to the total AA rqt, this was the best we could find. For the endogenous urinary excretion, the endogenous urea excretion is assumed to have the AA composition of the whole empty body (Williams, 1978; Rohr and Lebzien, 1991; Ainslie et al., 1993; Van Amburgh et al., 2015). The excretion of 3-methyl-His requires His whereas the excretion of the other N-fractions of the endogenous urinary does not require a direct input of EAA: PD are synthesized from Asp, Glu and Gly; creatine and creatinine from Arg and Gly (as many other metabolic functions, it requires S-adenosyl Met, but as for the other metabolic pathways this does not represent a net Met rqt); hippuric acid is synthesize from Gly. The AA composition of the MFP is based on the AA composition of ruminal and abomasal isolates from Ørskov et al. (1986) and the endogenous flow at the ileum in pigs (Jansman et al., 2002), assuming that 70% of the MFP is from undigested duodenal flow and the remaining 30% from the intestine (Ouellet et al., 2002 and 2010). Milk AA composition is based on the primary structures of the different proteins in milk (Farrell Jr et al., 2004). The protein fractions in milk were distributed as 81.4% casein (as % of total protein: 34.5% α_{s1}-casein; 7.6% α_{s2}-casein; 29.9% β-casein; 9.4% κ-casein) and 18.6% whey (as % of total protein: 4.0% α-lactalbumin; 10.7% β-lactoglobulin; 1.15% albumin; 1.90% IgG; 0.29% IgA; 0.26% IgM; 0.29% lactoferrin), based on the means reported in 14 manuscripts published between 1986 and 2012.

Table 3. Amino acid (AA) composition of the different protein functions involved in the determination of metabolizable protein and AA requirement.

	Integumental		Endogenous urea-urinary		Metabolic fecal		Milk	
AA	g AAAc[a]/ 100 g CP[b]	g AAAc/ 100 gTP	g AAAc/ 100 g CP[b]	g AAAc/ 100 g TP	g AAAc/ 100 g CP[b]	g AAAc/ 100 g TP	g AA / 100 g TP[c]	g AAAc/ 100 gTP
Ala	6.27	7.30	5.93	6.84	3.67	5.01	3.63	2.89
Arg	7.14	8.31	6.16	7.09	3.73	5.09	3.73	3.35
Asx	6.22	7.25	7.20	8.29	4.76	6.51	8.20	7.09
Cys	2.09	2.44	1.36	1.57	2.18	2.98	0.98	0.83
Glx	11.04	12.86	11.97	13.79	10.01	13.68	22.43	19.68
Gly	13.52	15.75	9.37	10.80	4.61	6.29	2.04	1.55
His	1.25	1.46	2.20	2.53	1.96	2.68	2.90	2.56
Ile	2.19	2.55	2.76	3.18	3.39	4.63	6.17	5.32
Leu	5.18	6.04	6.25	7.20	5.84	7.98	10.50	9.06
Lys	4.18	4.87	5.92	6.82	4.80	6.56	8.81	7.72
Met	1.03	1.20	1.77	2.04	1.09	1.48	2.99	2.63
Phe	2.81	3.27	3.47	4.00	3.49	4.77	5.21	4.64
Pro	8.92	10.39	7.15	8.24	5.17	7.07	10.22	8.62
Ser	4.59	5.35	4.12	4.75	4.67	6.37	6.70	5.55
Thr	3.12	3.64	3.81	4.38	4.87	6.65	4.68	3.97
Trp	0.55	0.65	0.81	0.93	1.16	1.58	1.67	1.52
Tyr	2.06	2.40	2.45	2.83	3.11	4.25	5.82	5.25
Val	3.67	4.28	4.10	4.73	4.69	6.41	6.88	5.82
ratio TP/CP	0.859				0.732			

[a]AAAc: anhydrous AA corrected for the missing concentration with a 24-h hydrolysis (see table 2).
[b]Initial concentrations obtained after 24-h hydrolysis of the protein source; CP: crude protein.
[c]Initial concentrations obtained from the primary structure of the milk protein fractions; TP: true protein.

EFFICIENCY

Efficiency of Utilization of MP

For comparison purpose between NRC (2001), CNCPS and our new estimates, MP rqt in the section above has been calculated using an efficiency of 0.67. However, it is well recognized that the efficiency of utilization of MP varies with MP supply relative to rqt (Doepel et al., 2004, Metcalf et al., 2008). Indeed, Sauvant et al. (2015) proposed a variation of the efficiency around a pivot of 0.67; however the variation was not related solely to the MP supply but to the ratio of MP supply/DMI. In this section, we will have a closer look at the variation of the efficiency using the database developed in Martineau et al. (2016) reporting studies where casein was infused post-ruminally in lactating dairy cows. Diet characteristics have been estimated using NRC (2001). As previously proposed (Lapierre et al., 2007) based on AA metabolism, a single “combined” efficiency will be used for the non-productive functions and the milk protein yield. Also, as proposed in the new French system Systali (Sauvant et al., 2015), an efficiency of 1.0 is assigned to the endogenous urinary excretion, these products being end-products

of metabolic pathways. The combined efficiency is calculated as the ratio of exported TP divided by MP supply (with the endogenous urinary removed from both components). In this database, the combined efficiency averaged 0.64 ± 0.13 (range 0.35 to 1.01): the mean agrees with the usual average efficiency used but the range clearly indicates how this efficiency is indeed variable. The relationship between total MP supply and total exported TP, with the endogenous urinary rqt removed from both components, was first examined. As expected for a variable efficiency, the relationship has a significant quadratic component ($P<0.05$; Figure 1A). We then studied the linear relationship between the combined efficiency and either MP supply, MP/NE_L supplies or MP supply/DMI. When study was not included into the equation, both MP/ NE_L supplies (adj. R^2=50%) and MP supply/DMI (R^2=60%) had a better linear relation with the combined efficiency than MP supply alone (R^2=31%). The quadratic component was significant but only slightly improved the equations for MP/NE_L supplies (R^2=55%) or MP/DMI (R^2=63%), but it was not significant for MP supply alone. The similar pattern of the relation between the combined efficiency and the ratios MP/ NE_L supplies or MP supply/DMI certainly holds due to the high correlation (r=0.98) between these 2 parameters. Because, biologically, energy supply should have more relevance to AA utilization than DMI per se, we prefer to use the MP/NE_L supplies ratio. In addition, with the ratio MP/NE_L, the intercepts and the slopes were very similar with and without study included into the linear model, indicating more robustness for the linear than quadratic model. Therefore, using the dietary characteristics estimated with NRC (2001), in this database, the combined efficiency of utilization of MP supply to support exported TP linearly declines ($P<0.001$) with an increment of the ratio MP/NE_L supplies: efficiency = 1.06 (±0.04) - 0.0078 (±0.0007) × MP/NE_L supplies (g/Mcal). This relation needs to be validated with a larger database, but the essence of the relation will remain.

Figure 1A.

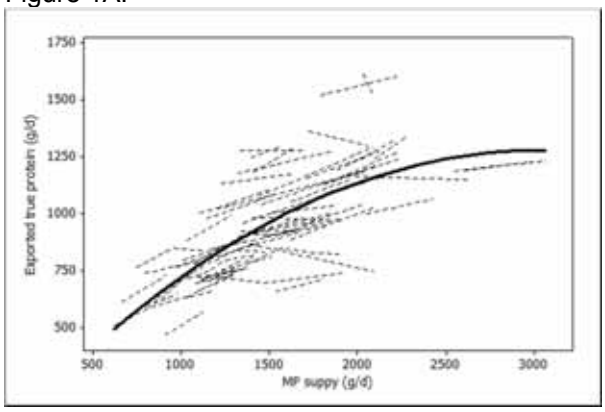

Figure 1B.

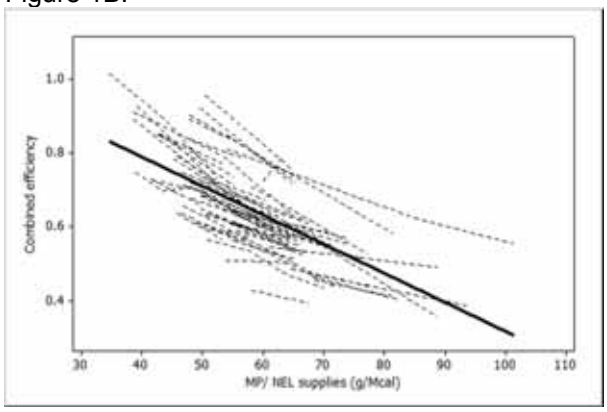

Figure 1. Relation between: *Panel A*: exported true protein and MP supply (both excluding endogenous urinary secretion); *Panel B*: Combined efficiency of utilization of MP and the ratio MP/NE_L supplies. See text for explanation.

Efficiency of Utilization of AA

The idea of using a combined efficiency for the utilization of MP was strongly suggested by AA metabolism. Indeed, examining AA metabolism in dairy cows, general trends were observed: Group 1 AA (His, Met, Phe+Tyr) are mainly catabolized by the liver and very little, if any, catabolism occurs in the peripheral tissues including the mammary gland; at the opposite, Group 2 AA (Ile, Leu, Lys and Val) are barely removed by the liver on a net basis but are catabolized by the gut, peripheral and mammary tissues (Lapierre et al., 2012). Because of the anatomical localization of the enzymes responsible of AA catabolism, the catabolism of AA does not occur at the site of the protein synthesis, certainly not for those exported proteins. For example, there was clearly no mammary catabolism of Phe even under excess supply (Lemosquet et al., 2010). Therefore, there is no biological reason to assign an efficiency of utilization for Phe, or other Group1 AA, different for non-productive functions and lactation, as its removal occurs at one site in the body, in the liver. Therefore, using a similar approach to that used for MP, the efficiency of utilization of EAA was also evaluated. The same database and assumptions as for the examination of MP efficiency were used. The exported AA were estimated from exported proteins × AA composition presented in Table 3 whereas the supply were estimated from the digestive flow (NRC, 2001); as for MP, the endogenous urinary rqt was subtracted from both components. The mean efficiency observed and variations are detailed in Table 4. The high maximal efficiencies observed might be related to the type of studies included in this database, where the control treatment was MP-deficient. However, the efficiencies higher than 1 noted for His and Met might be related to underestimation of their respective digestive flows. The averaged efficiencies observed for each AA are in the same range as those adopted by CNCPS v6.5 (van Amburgh et al., 2015) and derived from Lapierre et al. (2007).

Table 4. Combined efficiency of utilization of AA[a]

AA	Mean	SD	Min	Max	CNCPS v6.5[b]
Arg	0.56	0.21	0.30	0.89	0.58
His	0.82	0.22	0.41	1.34	0.76
Ile	0.63	0.18	0.35	0.93	0.67
Leu	0.67	0.21	0.35	0.99	0.61
Lys	0.72	0.18	0.38	1.05	0.69
Met	0.78	0.20	0.40	1.22	0.66
Phe	0.57	0.19	0.31	0.84	0.57
Thr	0.57	0.16	0.33	0.81	0.66
Val	0.63	0.17	0.35	0.92	0.66

[a]Estimated from the database of Martineau et al. (2016) according to description detailed in the text.
[b]From Van Amburgh et al. (2015) and Lapierre et al. (2007).

Results for Lys and Met are detailed to illustrate the variation of their efficiency. In fact, efficiency for these individual AA follows pretty much the same pattern as for MP. The AA exported are quadratically related to the supply (Figure 2). The combined efficiency is linearly better related to AA/NE_L (R^2: Lys=45 and Met=47%) supply than to AA supply by itself (R^2: Lys=25 and Met=29%). However, at the difference of MP, the

relationship between efficiency of utilization and the ratio of AA/NE_L supply has a significant quadaratic component, which slightly increases the R^2 to 49 and 52% for Lys and Met, respectively (Figure 3). However, in the range of the majority of the observations, the linear and quadratic relations yielded quite similar efficiencies. As for MP efficency, these relations need to be assessed with recent estimates of AA supply and validated with a larger database, but the essence of the relations will remain.

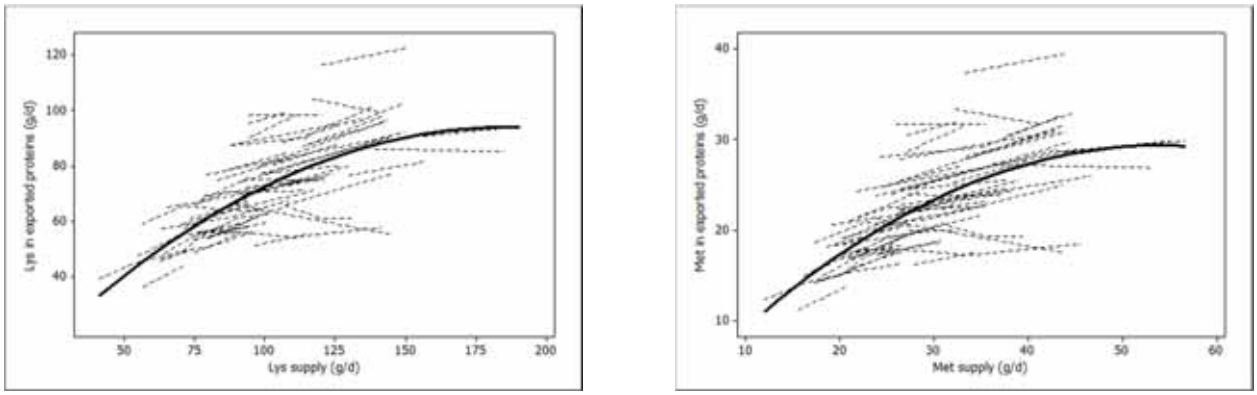

Figure 2. Relation between Lys and Met exported in true proteins and AA supply (both excluding endogenous urinary secretion).

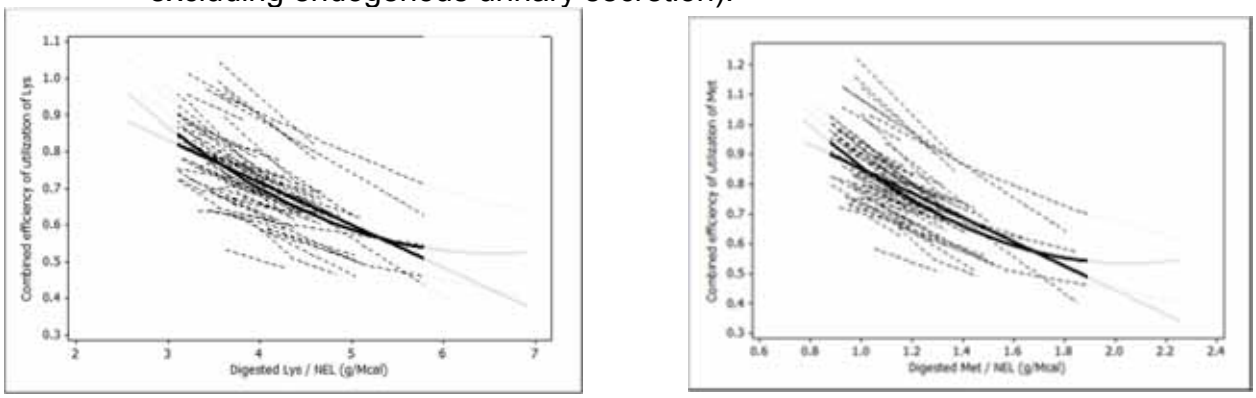

Figure 3. Combined efficiency of utilization of Lys and Met and the ratio AA/NE_L supplies. See text for explanation.

CONCLUSION

Overall, a better knowledge of AA metabolism has improved quantification of the daily amount of exported AA, either as non-productive functions or milk protein production. In addition, knowledge of AA metabolism has suggested 1) using a combined efficiency for these functions (except endogenous urinary excretion) and 2) a using a variable efficiency to convert these exported AA into requirements. Although it was first suggested that the efficiency of MP or individual AA was related to their digestive flow, it seems that the ratio of MP or AA supply to NE_L supply is better related to the efficiency: as the ratio AA/NE_L supplies increases, the efficiency decreases. In a whole model, optimization of the efficiency of the different EAA should allow a better estimation of the rqt and a better prediction of milk protein yield under known supply.

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BENEFITS OF AMINO ACID BALANCING ON METABOLISM, INFLAMMATION, AND OXIDATIVE STRESS

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KEY AMINO ACIDS AND CHOLINE FOR TRANSITION COWS

Due to extensive microbial degradation in the rumen, dietary availability of key methyl donors [(e.g., Methionine (MET) and choline (CHOL)] to mammary and liver is limited (Sharma and Erdman, 1989; Girard and Matte, 2005). Consequently, the mobilization of body protein in dairy cows close to calving compensates in part for this shortfall (Komaragiri and Erdman, 1997). Supplementing rumen-protected methyl donors may help fulfill the daily methyl group requirement, and possibly improve the overall production and health of dairy cows during the transition period (Zom et al., 2011; Osorio et al., 2013; Osorio et al., 2014).

The availability of MET and CHOL is important for various biological functions. For instance, MET together with Lys are the most-limiting amino acids (AA) for milk synthesis (NRC, 2001). Being the only essential sulfur-containing AA, MET acts as the precursor for other sulfur-containing AA such as cysteine (Cys), homocysteine and taurine (Brosnan and Brosnan, 2006). It has been estimated in lactating goats that as much as 28% of absorbed MET could be used for CHOL synthesis (Emmanuel and Kennelly, 1984). Hence, it is thought that rumen-protected CHOL supplementation could spare MET to help cows achieve better overall performance (Hartwell et al., 2000; Pinotti, 2012). Current recommendations for duodenal supply of Lys and MET to maximize milk protein content and yield in established lactation are 7.2 and 2.4% of MP, respectively (NRC, 2001). In terms of production performance, a Lys:Met ratio close to 2.8:1 of MP during the periparturient period by supplementing rumen-protected MET was beneficial (Osorio et al., 2013).

As a lipotropic agent, MET is directly involved in very low density lipoprotein (VLDL) synthesis via the generation of S-adenosylmethionine (SAM), the most important methyl donor (Martinov et al., 2010). In turn, SAM can be used to methylate phosphatidylethanolamine (PE) to generate PC, which is essential for VLDL synthesis (Auboiron et al., 1995). In the context of VLDL synthesis and liver lipid metabolism, CHOL-containing nutrients (mainly in the form of PC) are indispensable for the synthesis and release of chylomicrons and VLDL (Pinotti et al., 2002). Thus, supplementation of rumen-protected MET and/or CHOL (Zom et al., 2011) may increase hepatic triacylglycerol (TAG) export and consequently decrease lipidosis.

The immune system benefits greatly from proper nutrition, which in turn prepares the cow for periods of stress, reducing adverse effects and enhancing recovery. These concepts become of central importance when applied to the transition cow, as a

successful transition to lactation sets the stage for a profitable lactation, with optimal production, reproduction, and health, avoiding premature culling. Metabolic disorders are common during this time and can easily erase the entire profit potential for dairy cow farms (Drackley, 1999). The immune dysfunction during transition (Kehrli et al., 1989; Waller, 2000) and the state of oxidative stress (Abuelo et al., 2015) can lead to a cow that might be hyposensitive and hyporesponsive to antigens, hence, more susceptible to infectious diseases such as mastitis (Mallard et al., 1998).

RUMEN-PROTECTED METHYL DONORS AND PRODUCTION PERFORMANCE

To date, the reported effects of rumen-protected MET and/or CHOL supplementation on dairy cow production performance have been inconsistent. Although previous studies from our group and others have observed beneficial effects from MET (Chen et al., 2011; Osorio et al., 2013) or CHOL (Pinotti et al., 2003; Zom et al., 2011) supplementation, other studies did not detect significant improvements on peripartal production performance with MET (Socha et al., 2005; Ordway et al., 2009; Preynat et al., 2009) or CHOL (Guretzky et al., 2006; Leiva et al., 2015) supplementation. Similarly, studies evaluating whether CHOL alone or in combination with MET provide equal or different benefits in terms of production performance also yielded different results. For instance, MET and CHOL supplementation both led to greater DMI in previous studies (Ardalan et al., 2010; Sun et al., 2016). In contrast, only a MET effect was observed in the most recent transition cow study from our group (Zhou et al., 2016c). Production performance results from peripartal MET and CHOL supplementation studies are summarized in Table 1.

Production performance benefits in response to MET supplementation during the periparturient period are likely associated with an enriched AA and sulfur-containing antioxidant pool. Inadequate MET availability could limit the utilization of other circulating AA according to von Liebig's hypothesis which is commonly described with the analogy of the water barrel with broken staves (Mitchell and Block, 1946). The fact that circulating MET concentration decreased markedly through parturition and was not restored to prepartum levels until 28 d postpartum (Zhou et al., 2016b) suggest increasing MET availability during this period could potentially benefit production performance. Additionally, enhancing MET availability (Graulet et al., 2005) is likely to increase its entry into the 1-carbon metabolism cycle in liver which consequently increases the production of downstream compounds such as Cys. Glutathione (GSH) is another downstream compound arising in the MET cycle that can supply AA such as Cys to the mammary gland for milk synthesis (Pocius et al., 1981).

Apart from providing MET, GSH (as a potent intracellular antioxidant) may contribute to better overall performance by alleviating oxidative stress and subsequent inflammation. Previous work has demonstrated a positive effect of MET supplementation on intrahepatic GSH concentration during the peripartal period (Osorio et al., 2014; Zhou et al., 2016a). Such effect may be directly associated with MET supplementation considering that it can be incorporated upstream in the *de novo* synthesis pathway for GSH (Halsted, 2013). Both in vitro (Hartman et al., 2002) and in

vivo (Tabachnick and Tarver, 1955) studies using radioactive-labelled MET demonstrated hepatic incorporation of [^{35}S] into GSH. Therefore, the higher hepatic GSH concentration observed in MET-supplemented cows helps alleviate oxidative stress and contributes to greater DMI through an overall alleviation of the inflammatory status (Zhou et al., 2016a).

Although CHOL does not contain sulfur, MET can be generated in tissues like the liver from CHOL when homocysteine accepts a methyl group from CHOL through betaine (Wong and Thompson, 1972; Li and Vance, 2008). Hence, if comparable MET can be generated in response to CHOL supplementation, similar production performance benefits would be expected. Despite the fact that milk yield, DMI, and milk composition benefits were not observed in CHOL-supplemented cows in a recent transition cow study from our group (Zhou et al., 2016c), lactation performance benefits were detected in previous studies, indicating that CHOL may exert its lactation benefits through various means. For instance, the increase in hepatic mRNA expression of carnitine transporter suggested an increase in fatty acid uptake capacity and intracellular transport in CHOL cows, which was associated with reduced liver TAG accumulation (Goselink et al., 2013). Additionally, the CHOL supplementation from precalving through early lactation led to increased glycogen in liver tissue, implying a benefit to liver metabolism (Piepenbrink and Overton, 2003). Furthermore, CHOL can be used to generate PC, which is essential for VLDL synthesis and help reduce liver lipidosis by promoting TAG export (Pinotti et al., 2002).

RUMEN-PROTECTED METHYL DONORS AND METABOLISM

During the transition period cows will normally experience an increase in adipose tissue lipolysis due to changes in hormones such as insulin (decrease) and growth hormone (increase), and consequently blood non-esterified fatty acid (NEFA) concentrations increase. Once NEFA reach the liver these can be oxidized to provide energy, partially oxidized to produce ketone bodies, or esterified to triglyceride (TAG). A major organelle within hepatocytes where NEFA oxidation takes place is the mitochondria, and carnitine is essential for transport of NEFA from cytosol into mitochondria for subsequent β-oxidation (Drackley, 1999). Methionine is essential for carnitine synthesis (Carlson et al., 2007), thus, the greater hepatic concentration of carnitine (82.1 vs 37.5 nmol/g of tissue) that was detected in MET-supplemented cows indicates a greater bioavailability of MET to methylate Lys (Osorio et al., 2014).

Previous studies reported no significant effect of CHOL on blood glucose or BHBA concentrations (Guretzky et al., 2006; Zahra et al., 2006; Zom et al., 2011). In a recent study from our group (Zhou et al., 2016c), the tendency for lower BHBA in response to CHOL supplementation agreed with the greater glucose concentration. Although speculative, the pattern of BHBA and glucose detected in CHOL-supplemented cows was associated with numerically lower negative energy balance as a result of lower milk production. The exact mechanisms for the lower milk yield in these cows that maintained greater blood glucose is not known.

Table 1. Summary of production performance results from transition cow studies supplementing rumen-protected MET or CHOL.

Study	DMI	Milk	Protein	Fat	Dosage	Product	Duration	Cows
Methionine studies								
Overton et al., 1996	-	+	-	-	17 g	RPM (Degussa)	-7 to 126	24
Piepenbrink et al., 2003	-	+	-	-	2.34% or 2.7% MP pre; 2.36 or 2.63% MP post	Alimet	-21 to 84	48
Socha et al., 2005	-	-	-	-	10.5 g	SM	-14 to 105	48
Ordway et al., 2009	-	-	-	-	Met:Lys = 3.0:1	MS and SM	-21 to 140	60
Preynat et al., 2009	-	-	-	-	15.3 g	Mepron-85	-21 to 112	60
Ardalan et al., 2010	+	-	/	/	14.4 g	SM	-28 to 70	40
Osorio et al., 2013	+	+	+	+	0.19% MS or 0.07 % SM DM	MS and SM	-21 to 30	56
Zhou et al., 2016	+	+	+	+	0.08 % DM	SM	-21 to 30	81
Sun et al., 2016	+	+	+	+	15 g	Mepron-85	-21 to 21	48
Choline studies								
Hartwell et al., 2000	-	-	-	-	0,6,12 g	Capshure	-21 to 120	48
Pinotti et al., 2003	-	+	-	+	20 g	Overcholine	-14 to 30	26
Piepenbrink et al., 2003	-	+	-	+	11, 15, 19 g	ReaShure	-21 to 63	48
Guretzky et al., 2006	-	-	-	-	15 g	ReaShure	-21 to 21	42
Lima et al., 2007	+	+	+	+	15 g	ReaShure	-25 to 80	369
Elek et al., 2008	-	+	+	+	25/50 g pre/post	Norcol-25	-21 to 60	32
Ardalan et al., 2010	+	+	/	/	14 g	Col 24	-28 to 70	40
Zom et al., 2011	+	-	+	-	15 g	ReaShure	-21 to 42	38
Leiva et al., 2015	-	-	+	-	9.4/18.8 g pre/post	CholiPearl	-21 to 45	23
Zhou et al., 2016	-	-	-	-	15	ReaShure	-21 to 30	81
Sun et al., 2016	+	+	+	+	15	ReaShure	-21 to 21	48

IMMUNONUTRITIONAL ROLE OF METHYL DONORS

In addition to being considered one of the most-limiting AA for milk production, MET and several of its metabolites display an immunonutritional role, i.e. they help support and boost certain activities of the immune system in humans (Grimble, 2006; Li et al., 2007). Since these properties have been tested on immune-suppressed human subjects with positive outcomes (Van Brummelen and du Toit, 2007), we hypothesize that enhancing MET supply would have a positive effect on immune function in the transition period, where cows seems to be in an immuno-compromised state. A study with mid-lactation cows reported that supplementation with 30 g/d of rumen-protected MET compared with 0 or 15 g/d led to greater T lymphocyte proliferation in vitro in response to various mitogens (Soder and Holden, 1999). Since human lymphocytes seem to have an absolute requirement for MET to proliferate (Hall et al., 1986), these results were not unexpected.

In one of our previous studies (Osorio et al., 2013) where transition cows were fed either Smartamine (0.07 %DM) or Metasmart (0.19 %DM) from -21 to +30 days relative to parturition, we observed increased phagocytosis (pathogen killing ability) in neutrophils, the cells that make up the first line of defense in the animal immunity. In a follow-up study feeding Smartamine (0.08 %DM) between -21 and 30 days relative to parturition (Zhou et al., 2016a) we detected both greater in vitro blood neutrophil phagocytosis and oxidative burst (another pathogen-killing mechanism) from day 1 post-calving through day 28 postpartum. Furthermore, supplementation with rumen-protected MET optimized the response to lipopolysaccharide (LPS) (a component of bacteria cell walls), by controlling the inflammatory ability of the immune cells (Vailati-Riboni et al., unpublished). This is very relevant during the transition period, as cows might mount an excessive inflammatory response to pathogens, creating more damage than benefit (Jahan et al., 2015). One possible mechanism could be related to the ability of MET to influence the oxidative status of periparturient cows as it is a precursor for glutathione and taurine (Atmaca, 2004). Through its chloramine metabolites, taurine has a well-known immuno-modulatory capacity (Schuller-Levis and Park, 2004).

Despite the interconnection between MET and CHOL (via the one-carbon metabolism), there is a paucity of data on CHOL and the bovine immune response. Immune cells lack the ability to convert CHOL into MET through the betaine pathway, which in bovine is present only in liver and kidney (Lambert et al., 2002). In a recent study (Zhou et al., 2016a), compared with feeding MET feeding 60 g/d of Reashure (rumen protected CHOL) from -21 to 30 days in milk had no effect on immune cell killing capacity of neutrophils and monocytes (another cell type of the animal immune response). However, there are data generated using other animal models. For example, supplementation of choline in the diet improved immune indices in both fish (Wu et al., 2013) and suckling rats (Lewis et al., 2016). Authors did not speculate on the mode of action, but most probably CHOL efficacy is mediated by betaine (a choline derivate). Data from broilers revealed that dietary betaine supplementation improved intestinal health, and induced a boost in the intestinal immune response to a coccidiosis challenge (Klasing et al., 2002).

METHIONINE AND LIVER FUNCTION: BALANCE BETWEEN INFLAMMATION AND OXIDATIVE STRESS

The periparturient inflammatory response is characterized by an increase in the hepatic production of positive acute-phase proteins (posAPP), such as haptoglobin and serum amyloid A (SAA), and a concomitant decrease in the production of negative APP (negAPP) such as albumin (Bertoni et al., 2008). At the level of liver, the well-established triggers of these responses are the pro-inflammatory cytokines IL-6, IL-1β, and TNF-α (Kindt et al., 2007). In contrast, oxidative stress is driven by the imbalance between the production of reactive oxygen metabolites (ROM) and the neutralizing capacity of antioxidant mechanisms in tissues and in blood. Pro-inflammatory cytokines have also been identified as a cause of oxidative stress, hence, linking the two conditions (Sordillo and Mavangira, 2014).

Both inflammation and oxidative stress reduce liver function in periparturient dairy cows (Bionaz et al., 2007; Trevisi et al., 2012). Using changes in plasma concentrations of albumin, cholesterol, and bilirubin, Bertoni and Trevisi (2013) developed the liver functionality index (LFI), which characterizes the extent of the inflammatory response and helps predict consequences on health and well-being of the cow. For instance, a low LFI value is indicative of a pronounced inflammatory response, suggestive of a more difficult transition from gestation to lactation, whereas a high LFI is suggestive of a smooth transition. In our experiments, supplementation of MET consistently increased blood albumin (Osorio et al., 2014; Zhou et al., 2016a) well above the concentration range in cows with adequate postpartum liver function (Bionaz et al., 2007). Furthermore, supplementation with rumen-protected MET decreased the concentrations of inflammation-related biomarkers such as ceruloplasmin, haptoglobin, IL-1β, and SAA (Osorio et al., 2014; Zhou et al., 2016a). MET supplementation increased liver glutathione and antioxidant capacity (Osorio et al., 2014; Zhou et al., 2016a). Using the cow data from the Zhou et al. experiment, compared with 35% of the cows without MET supplementation ending-up in the Low LFI group, only 10% of the MET-supplemented cows ended-up in the Low LFI group, hence, supporting the existence of a positve effect of MET supplementation on liver function.

THE LINK BETWEEN INTAKE AND HEALTH

The transient inflammatory-like status around parturition appears to be a “normal” aspect of the adaptations to lactation (Bradford et al., 2015), with its positive or negative impact depending on its degree. Cows that approach parturition with a greater (but still subclinical) level of circulating cytokines have greater inflammation and oxidative stress, and lower liver function often through 30 days in milk together with lower milk yield and lower postpartum DMI (Bertoni et al., 2008; Trevisi et al., 2015). In addition to their fundamental function in immunity, cytokines (ILs), interferons (IFNs) and TNF-α also elicit pathophysiological effects. This leads to what is commonly known as “sickness behavior”, whose primary manifestation is satiety. Similar to how cows react during an inflammatory state around parturition, the reduction in DMI around calving is an example of this behavior. In mice, these cytokines have been shown to reduce meal

size and duration, as well as decrease meal frequency and prolong inter-meal intervals (Plata-Salaman, 1995). Furthermore, cytokines directly affect the hypothalamus; IL-1β and IFN act directly and specifically on the glucose-sensitive neurons in the brain "satiety" and "hunger" sites (Plata-Salaman, 1995). Thus, the increased DMI observed when feeding rumen-protected MET (Smartamine or Metasmart) can be partly explained by a reduction in inflammation, as it directly (at the hepatic level and by dampening the immune cell overresponse) and indirectly (reducing oxidative stress) decreases circulating pro-inflammatory cytokines.

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HISTORY OF CHROMIUM AND ITS RELATIONSHIP TO GLUCOSE METABOLISM

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INTRODUCTION

Studies by Schwartz and Mertz (1959) showed that trivalent chromium (Cr) was an essential component of a factor in brewers yeast (referred to as glucose tolerance factor) that corrected impaired glucose metabolism in rats fed a torula yeast-based diet. This discovery led to a number of studies in the 1960's evaluating Cr supplementation in humans with impaired glucose tolerance. Results obtained in these studies were variable but some individuals did show improvements in glucose metabolism following Cr supplementation. For example in one study 3 out of 6 adult-onset diabetics exhibited improved glucose tolerance following CrCl supplementation for 7-13 weeks (Glinsmann and Mertz, 1966).

Subsequent studies demonstrated that Cr functioned in insulin-sensitive tissues to potentiate the action of insulin. The mechanism by which Cr enhances insulin action is still unclear. In humans Cr is poorly absorbed (0.4 to 2.5%) and absorbed Cr is rapidly excreted in the urine (National Academies, 2001). Chromium in the blood is transported bound to the protein transferrin. There is believed to be little storage of Cr in the body. Based on research in humans, the Institute of Medicine established an adequate intake of Cr for humans (National Academies, 2001).

CHROMIUM IN ANIMAL NUTRITION

Chromium research in domestic animals was limited prior to the early 1990's because practical diets fed to domestic animals were generally assumed to provide adequate Cr to meet animal requirements. Steele et al. (1977) found that intravenous administration of a synthetic glucose tolerance factor, containing Cr, potentiated the hypoglycemic response following an insulin challenge in pigs. This study indicated that a Cr containing glucose tolerance factor was active in swine. The first animal production responses to Cr supplementation were reported in turkey poults. In a series of studies with turkey poults (Steele and Rosebrough, 1979, 1981; Rosebrough and Steele, 1981) supplementation of 20 mg Cr/kg (from CrCl) to a corn-soybean meal based diet increased body weight gain, liver glycogen, and hepatic lipogenesis from glucose. The first report in ruminants evaluating the effects of Cr on measures of insulin sensitivity was with growing lambs fed a high concentrate or high fiber diet (Samsell and Spears, 1989). Supplementing 10 mg Cr/kg diet (from CrCl) reduced fasting glucose concentrations in lambs fed a high concentrate diet and reduced serum free fatty acids after feeding in lambs fed a high fiber diet. Both of these responses are consistent with Cr affecting insulin sensitivity.

Chromium research with domestic animals accelerated in the 1990's. Research at the University of Guelph indicating that Cr supplementation could improve performance and reduce morbidity in stressed calves generated considerable interest in Cr in ruminant nutrition (Moonsie-Shageer and Mowat, 1993; Mowat et al., 1993). Chromium addition to swine diets was found to increase muscling and decrease fat thickness in grow-finish pigs (Page et al., 1993), and increase litter size in sows (Lindemann et al., 1995). Studies in calves (Bunting et al., 1994) and pigs (Amoikon et al., 1995) also demonstrated that Cr supplementation could increase glucose clearance rate following a glucose tolerance test. Research with broilers showed that Cr supplementation to practical diets often increased gain and improved carcass characteristics, especially under heat stress conditions (Lien et al., 1999; Sands and Smith, 1999).

Responses to supplemental Cr are greatest under conditions that decrease insulin sensitivity. Insulin resistance occurs in late gestation and continues during early lactation in both dairy and beef cows (Sano et al., 1993). Chromium supplementation (approximately 0.20 to 0.50 mg Cr/kg DM or 4 to 10 mg Cr/cow/day) of dairy cows during late gestation and early lactation has increased DM intake and milk production in a number of studies (McNamara and Valdez, 2005; Smith et al., 2005).

Hormones produced during stress (heat stress, weaning, shipping, etc) also decrease insulin sensitivity. Stress has also been shown to increase urinary losses of Cr in humans and rats (Spears and Trivedi, 2013). In dairy cows exposed to heat stress, Cr supplementation, at a level of 6 mg/cow/day, increased DM intake and milk production, and decreased serum cortisol concentrations (Soltan, 2009). Increased release of cortisol during stress is known to suppress a variety of immune responses, and Cr supplementation of stressed calves has increased immune responses in a number of studies (Spears and Trevedi, 2013). In a recent study stressed calves supplemented with 0, 0.10, 0.20, or 0.30 mg Cr/kg DM exhibited a linear improvement in gain and gain:feed during a 56-d receiving period (Bernhard et al., 2012). Morbidity during the receiving period was lower in calves supplemented with 0.30 mg Cr/kg compared with controls (7.5 vs. 25.9% of calves treated).

APPROVALS OF CHROMIUM IN ANIMAL DIETS

Until recently, Cr was not considered as a substance generally recognized as safe for addition to animal diets in the United States. Therefore, U.S. Food and Drug Administration (FDA) permission or approval is required for any Cr source to be supplemented to animal diets. Two Cr sources are permitted for supplementation to swine diets at a concentration up to 0.20 mg/kg diet (Lindemann, 2007). In 1996 FDA stated that it would not object to Cr picolinate being supplemented to swine diets. The FDA indicated in 2000 that it would not object to Cr propionate being supplemented to swine diets. Permission from the FDA to use these two Cr sources was based on changes in glucose metabolism and related safety data.

The FDA issued a regulatory discretion letter in 2009 which permitted the use of Cr propionate in cattle diets. Chromium propionate is the only Cr source currently permitted for supplementation to cattle diets in the US, and can be added at levels up to 0.50 mg Cr/kg DM. Permission to use Cr propionate in cattle diets was largely based on utility and human food safety studies. The utility study was a dose-titration study examining glucose and insulin metabolism in growing heifers supplemented with 0, 3, 6, or 9 mg supplemental Cr per animal daily (Spears et al., 2012). These daily doses of supplemental Cr corresponded to 0 (control diet analyzed 0.20 mg Cr/kg DM), 0.47, 0.94, and 1.42 mg supplemental Cr/kg DM. All levels of supplemental Cr reduced insulin release and insulin:glucose ratios following intravenous glucose infusion. The lower release of insulin and decreased insulin:glucose ratio in Cr-supplemented heifers clearly indicated that their tissues were more sensitive to insulin. Furthermore, insulin concentrations and insulin:glucose ratios did not differ among heifers supplemented with 0.47, 0.94, and 1.42 mg Cr/kg DM.

The human food safety study involved demonstrating that Cr propionate supplementation would not increase Cr concentrations in meat and milk to levels that might cause a human health concern. Supplementation with Cr propionate for 120 days at 4 times (2 mg Cr/kg) the permitted level did not increase Cr concentrations in milk, muscle, or fat, the major animal products consumed by humans (Lloyd et al., 2010). Chromium supplementation did result in small increases in liver and kidney concentrations. Based on the maximum intake of liver and kidney that would be consumed by humans, it was determined that Cr propionate supplementation even at 4 times the permitted level would have minimal effect on Cr intake by humans.

Recently (June, 2016) Cr propionate was approved by U.S. FDA for supplementation to broiler diets at a level not to exceed 0.20 mg Cr/kg diet. This action was in response to a food additive petition submitted by Kemin Industries, Inc. The food additive petition included a utility study showing increased insulin sensitivity in broilers supplemented with Cr propionate (Brooks et al., 2016), animal and human food safety studies, environmental assessment, identity of chemical structure, stability of Cr propionate, manufacturing chemistry, and a mixing and homogeneity study, among other required regulatory components. Chromium is the first new trace mineral approved for use in U.S. broiler diets since selenium in 1974.

CHROMIUM IN ANIMAL FEEDSTUFFS

Little is known regarding Cr concentrations naturally present in practical feedstuffs, and even less is known regarding bioavailability of Cr from common feedstuffs. Chromium analysis of feedstuffs and total diets is challenging due to the low levels of Cr normally present and problems with Cr contamination of samples during collection, processing, and laboratory preparation of samples for analysis (NRC, 2005). Soil contamination of samples during harvesting will increase Cr concentrations in feedstuffs. Chromium derived from metal during harvesting and processing of feedstuffs is likely a major source of Cr contamination.

Li et al. (2005) reported Cr concentrations in homegrown and imported feeds from 54 dairy farms in Wisconsin. Mean Cr concentrations in homegrown feedstuffs ranged from 0.33 mg/kg DM for corn grain to 0.91 mg/kg DM for alfalfa haylage. Of the imported feed ingredients mineral supplements contained by far the highest concentrations of Cr (69 mg Cr/kg). Chromium would be expected to occur as a contaminant in most mineral ingredients. Average Cr concentrations in unground wheat samples from different areas in Australia ranged from 0.013 to 0.041 mg/kg (Jones and Buckley, 1977). They also reported Cr concentrations in oats and barley of 0.020 and 0.024, respectively.

We recently analyzed a number of feedstuffs obtained from different areas of the US for Cr. Samples of corn, soybeans, wheat, and oats analyzed whole averaged 0.026, 0.063, 0.041, 0.025 mg Cr/kg DM, respectively. Grinding whole samples of corn, soybeans, and wheat in a Wiley mill using a stainless steel screen more than doubled analyzed Cr concentrations. Samples of processed corn also analyzed considerably higher than whole corn samples. Corn silage samples averaged 0.22 mg Cr/kg DM and samples of alfalfa hay and haylage averaged 0.52 mg Cr/kg DM. Of the major feed ingredients that we have analyzed only beet pulp and corn steep liquid analyzed over 1.0 mg Cr/kg DM. These results indicate that Cr concentrations naturally occurring in most feedstuffs are low, and that much of the Cr in feedstuffs is due to contamination.

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NUTRIENTS AND THE INNATE IMMUNE RESPONSE

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INTRODUCTION

Several researchers have focused on the influence of stress and physiological state on immune system function (Ingvartsen and Moyes, 2013). This presentation will primarily focus on the effect of stress and dietary vitamin/mineral supplementation on the innate immune response with a focus on the benefits of dietary chromium on the innate immune response. The immune system is defined as host resistance to invading microorganisms. The immune system is comprised of the innate and adaptive immune response. The innate immune response is non-specific and consists primarily of phagocytic cells such as resident macrophages and circulating neutrophils. The primary functions of the innate immune response are to detect invading microorganisms, recruit circulating neutrophils to phagocytose (i.e. engulf) and kill invading microorganisms. Resident macrophages also bridge the innate with the adaptive immune response via antigen presentation for the priming of T cells (Rainard and Riollet, 2006). The adaptive immune response is specific to invading microorganisms and primarily consists of lymphocytes. Lymphocytes consists of T (i.e. cytotoxic or helper) and B (i.e. antibodies) lymphocytes. This review will focus on the innate immune response.

IMPACT OF STRESS ON IMMUNE FUNCTION

Any stressor can impact the immune response. The major stressors that impact dairy production include heat, transportation, crowding as well as physiological stressors such as parturition (Ingvartsen and Moyes, 2013). Although many different stressors can impact dairy management and production, this presentation will primarily focus on the stressors associated with the transition period, i.e. ± 3 weeks relative to parturition (Drackley, 1999).

The transition period is considered the most critical time period regarding its influence on health and immune function. During this time, complex changes in the endocrine, neurological, digestive and immune systems occur that increase risk for disease (Ingvarsten and Moyes, 2015). As a result, ~75% of all diseases occur during the transition period. Mortality rates are also high for cows in early lactation with 50% of deaths occurring by 30 days after calving (Dechow and Goodling, 2008; Thomsen et al., 2006). Natural immunosuppression has been observed at this time that partly explains the decreased immune system function and risk of disease (Burton et al., 2005).

Natural immunosuppression that occurs around the transition period is multifactorial. Major factors associated with immunosuppression include, but are not limited to, endocrine changes, milk yield, management and genotype. All are associated

with increasing risk of disease. Mastitis, defined as an inflammation of mammary gland, occurs more frequently at the time of parturition and is the most common and most economically significant disease affecting dairy cattle (Cha et al., 2011). The decrease in economic returns and animal welfare can be partly associated with the rise in milk somatic cell count (~90% neutrophis), i.e. the primary indicator of mastitis (Sordillo et al., 1997). Although the immune response to invading microorganisms in the mammary gland has been well-documented, the metabolic response including the utilization of nutrients by phagocytic cells has received little attention. Identifying the metabolic response and the utilization of nutrients during the transition period may partly explain the high risk for disease at this time.

NUTRIENT UTILIZATION DURING INFLAMMATION

Studies of fuel use have indicated that 1) only 5% of glucose is completely oxidized via the Krebs' cycle and the rest is either directed towards the pentose phosphate pathway for the generation of reducing equivalents required for phagocytoses or is converted to lactate due to the low oxygen availability in immune cells; 2) glutamine is the preferred amino acid utilized by phagocytes, with ~74% of glutamine being completely oxidized; 3) fatty acids, such as oleate, are primarily incorporated into cellular lipids; and 4) ketone bodies are not utilized as an energy source by phagocytic cells (Ingvartsen and Moyes, 2013). However, the partitioning and benefits of other nutrients, such as chromium, by bovine phagocytic cells is currently unknown and warrants further investigation.

IMPACT OF VITAMINS/MINERAL SUPPLY ON IMMUNE RESPONSE

Vitamin E as one of the most important antioxidants extensively studied in humans and animals. Previous studies have shown that inadequate vitamin E concentration may partly explain the increased risk of metabolic and infectious diseases in dairy cows shortly after calving (Heinrichs et al., 2009). Selenium, a mineral primarily found in soil, also has antioxidant properties. Increasing dietary vitamin E with or without selenium may improve the innate immune response (Hogan et al., 1990). Growing evidence suggests that additional nutrients can alter the immune response for dairy cows during the transition period.

Dietary chromium supplementation primarily modulates the host response to insulin (Vincent, 2004). Few researchers have examined the effect of dietary chromium on the immune response, especially for dairy cows during the transition period. Researchers have shown that chromium supplementation improves insulin response, energy metabolism, dry matter intake and milk yield for lactating dairy cows (Hayirli et al., 2001; Soltan, 2010). Regarding the innate immune response, chromium supplementation has been shown to improve monocyte and neutrophil function (Burton et al., 1993; Yasui et al., 2014) as well as the anti-inflammatory response for lactating cows experiencing heat stress (Zhang et al., 2014). Immune cells primarily use insulin independent glucose transporters, i.e. GLUT-1 and GLUT-3, yet insulin receptors have been identified on bovine immune cells (Nielsen et al., 2003). Although the function of

insulin receptors on bovine immune cells remains unknown, researchers theorize that the insulin receptors are involved in monocyte function such as neutrophil recruitment. The role of dietary chromium supplementation on insulin receptor signaling as well as the overall innate immune response for transition dairy cows, especially during mastitis, has not been elucidated and warrants further investigation.

SUMMARY AND CONCLUSION

In summary, most cows are immunosuppressed around calving, primarily attributed to changes in the endocrine, neurological, digestive, and immune systems. Nutrient supply can alter the immune response. For phagocytic cells, glucose enters either the pentose phosphate pathway or is converted to lactate. Immune cells primarily oxidized glutamine for energy in the citric acid cycle. To my knowledge, ketones are not utilized or produced by phagocytic cells and fatty acids can be incorporated into cellular lipids and have been shown to alter the immune response. Vitamins and minerals, such as vitamin E and selenium, improve the immune system response, especially during the transition period. Although few studies have investigated the effects of chromium on the immune response, results suggest that dietary chromium supplementation may benefit the innate immune response. In conclusion, the benefits of dietary chromium supplementation on the innate immune response for dairy cows and how this effect is altered by age, physiological state and stage of lactation is vital for the development of new management strategies that improve the immune response and reduce risk of disease during the transition period through dietary chromium supplementation.

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EVALUATION OF CHROMIUM PROPIONATE (KEMTRACE®) ON REPRODUCTIVE PERFORMANCE OF HOLSTEIN COWS IN A PENNSYLVANIA DAIRY HERD.

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INTRODUCTION

Reproductive management influences farm profitability through effects on milk produced per cow per day, calves born per year, and herd replacement (Ferguson and Galligan, 2000). Reproductive efficiency is best measured as pregnancy rate (PR), the rate at which cows become pregnant from the voluntary waiting period (VWP), days in milk insemination begins post-calving (Ferguson and Galligan, 2000). Since cows have an average inter-estrus interval of 21-d, even in herds employing synchronized, timed artificial insemination programs (TAI), pregnancies in open cows occur in 21-d event clusters from the VWP. Survival curves or event time curves of pregnancy by days in milk reflect the 21-d PR and may be referred to as a PR curve. Herds using Presynch-OvSynch and Postsynch OvSynch (Pursley et al. 1997) programs have PR curves which decline in steps, reflecting the pattern of synchronization in groups of cows. Herds inseminating cows on visual estrus typically have PR curves that have a regular decline over sequential 21-d periods, whereas in synchronized herds they are clustered in 7-d or 14-d events within 21-d (first insemination) and 42-d periods (repeat inseminations).

Since insemination rate (IR) and conception rate (CR) influence PR, a change in either of these rates can alter the slope of the PR curve within the period of change, creating an inflection in the rate at which the curve declines. In many herds, CR to first insemination (FSTCR) is greater than CR to second, third, or fourth and higher services (repeat CR, RPTCR), due to infertile cows concentrating as service number progresses. This change in CR from FSTCR to RPTCR causes a slower decline in the PR curve following first insemination and can decrease the potential efficiency of reproductive programs.

In herds using a post-synchronization program, PR may decline for repeat inseminations due to a delay in reinsemination to 42-d periods from the prior insemination. The sequencing of GnRH-PGF-GnRH injections cannot occur until open status is confirmed, which may not be possible until 28 to 42 days postinsemination depending on method and frequency of pregnancy diagnosis. Some herd managers employ visual estrus detection to maintain inseminations at 21-d post prior inseminations and only synchronize cows not seen in estrus by 28 to 32 days following a prior insemination. Management programs influence the periodicity of repeat inseminations.

However, PR at repeat insemination may also be reduced due to the reduction in CR with increasing service number. Reduction in CR at repeat services may be due to cows that had health problems post-calving (retained placenta, metritis, ketosis, displaced abomasum, lameness, or mastitis among other conditions), or may be those cows which have experienced greater metabolic stress and lost more body condition, or may be cows that experienced later first ovulation post-calving. Greater milk production has also been identified as a risk factor for reduced CR, and higher producing cows may have lower FSTCR and become a greater proportion of cows inseminated at higher services, thus lowering CR to these services. There is not one reason which can be linked to reducing CR to increasing service number in dairy herds and analysis of risk factors needs to be undertaken to identify the potential factors.

The result is that PR curves in a dairy herd can take altered shapes and may not decline uniformly following the VWP. Overall mean PR can be very misleading as an index of overall reproductive efficiency due to alterations in the shape of the PR curve by 21-d periods due to the above mentioned factors. PR and CR should be examined for first insemination and repeat inseminations to gain a sense of fertility patterns in a dairy herd.

HISTORY

An 819 (lactating and dry cows) Holstein dairy herd (28,386 lbs milk rolling herd average, 3.8% fat and 3.2% protein) had been monitored for production and reproductive performance for a number of years (Figure 1). Herd size and rolling herd average (RHA) had increased from 645 cows and 24,203 lb in May, 2006 to 806 cows and 29,280 lb by May, 2011 (Figure 1).

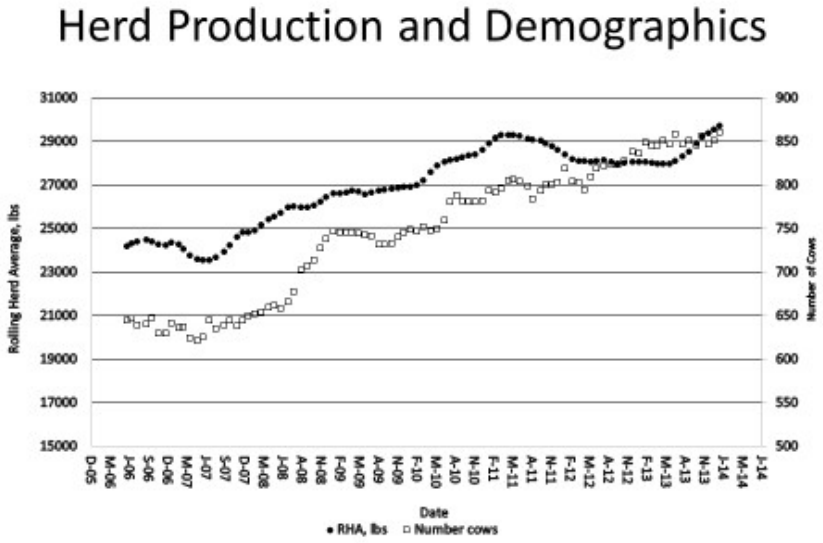

Figure 1. Herd demographics and production from May, 2006 to May, 2011. Rolling Herd Average, RHA, lb/cow/year, ●; Number of lactating and dry cows, Number cows, o.

Pregnancy rate (PR) had generally been below 20% in 2005 and 2006 but had increased in 2008 to 21% (Figure 2). This increase in PR was associated with an increase in first service CR (FSTCR) over this time period from 30% and lower to around 40%. Second service CR also improved in 2008 but was typically around 33% in 2008 and 2009.

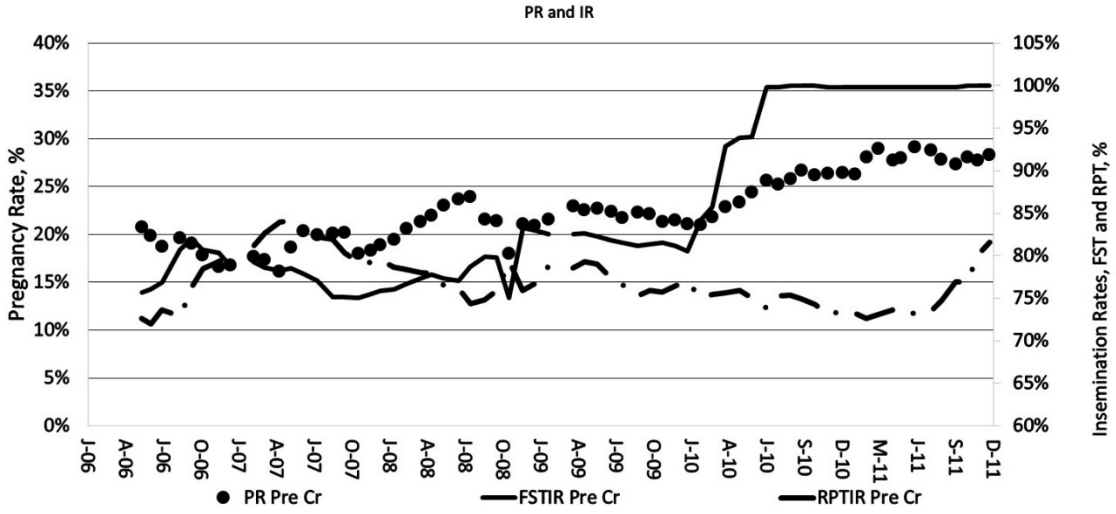

Figure 2. Pregnancy rate, first insemination rate, and repeat insemination rate prior to chromium supplementation from May, 2006 through December, 2011. Pregnancy rate, PR, pre supplementation with chromium, PR Pre Cr, ●; First insemination rate pre chromium supplementation, FSTIR Pre Cr ▬; Repeat insemination rate pre chromium supplementation, RPTIR Pre Cr, ▬ ● ▬.

In 2010 the farm management committed fully to a Pre-Synch OvSynch timed artificial insemination program (TAI) which increased first service insemination rate from just under 80% to 100% (Figure 2). Associated with this change was a change in voluntary waiting period from 57 days to 72 days. Coincident to the increase in FSTIR, PR increased to 28% and FSTCR began to increase in 2010 and reached a plateau of 45% in 2011. However, second service CR began to decline and dipped below 30% in 2010 and remained below 30% in 2011 (Figure 3). Repeat inseminations had been controlled using a post synchronization program since 2006 in combination with rectal palpation for pregnancy examination at 39 days post-insemination. This program had not changed during this time period.

Examining CR across 2, 3, and 4 services during 2010 and 2011 indicated that these rates were significantly lower than FSTCR and had declined from 2008 and 2009 values (Table 1, Conception rate at first service and second service, Figure 3). The binomial trend across service number from first to fourth service was significantly negative (Rosner, 2006). The difference in CR for first and second service (Figure 3) was seen to be diverging to a greater difference beginning in 2010. This suggested the herd was experiencing two different fertility populations: very fertile cows that conceived to first service and a group of cows with lower fertility at 2, 3, and 4 or more services. Segregating cows by transition problems, such as metritis, ketosis, displaced

abomasum, parity (first, second, third and greater), production, and by season of calving failed to yield any associated conditions with the lower fertility in cows which failed to conceive at first service. In addition, it was not apparent that cows that failed to conceive at first service were in thinner body condition or had lost more body condition than cows which conceived at first service.

Table 1. CR by service number for first, second, third and fourth service in December, 2011. Data represent cows with service number and if confirmed pregnant or unknown (either open or pregnancy status not yet confirmed) to that service number.

Service Number	Number Cows	Number Pregnant	Number Unknown	CR %
1	396	304	92	44.8
2	139	88	51	27.2
3	96	52	44	27.2
4	60	36	24	31.3
Total inseminated	770	510	260	35.9
Binomial trend[1]	slope -66.52	$\chi^2 = 15.04$	P<0.0001	

Example calculation: CR first service = 100 x (304/(770 – 92)) = 44.8

[1] Binomial trend for CR from first through fourth service calculated based on Rosner

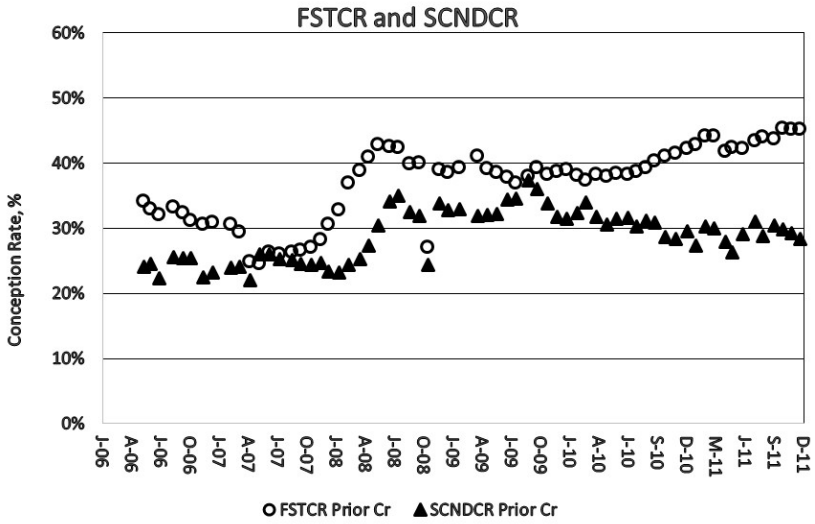

Figure 3. Conception rate at first service and second service from May, 2006 through December, 2011. First service conception rate prior to chromium supplementation, FSTCR Prior Cr, o; Second service conception rate prior to chromium supplementation, SCNDCR Prior Cr, ▲.

Pregnancy rate by days in milk reflected the change in CR with increasing service number (Figure 4). The slope for PR for first services was 38.5% whereas for second service it was 16.5% (Figure 4). Our goal is for PR to be greater than 20 to 25% for all days in milk.

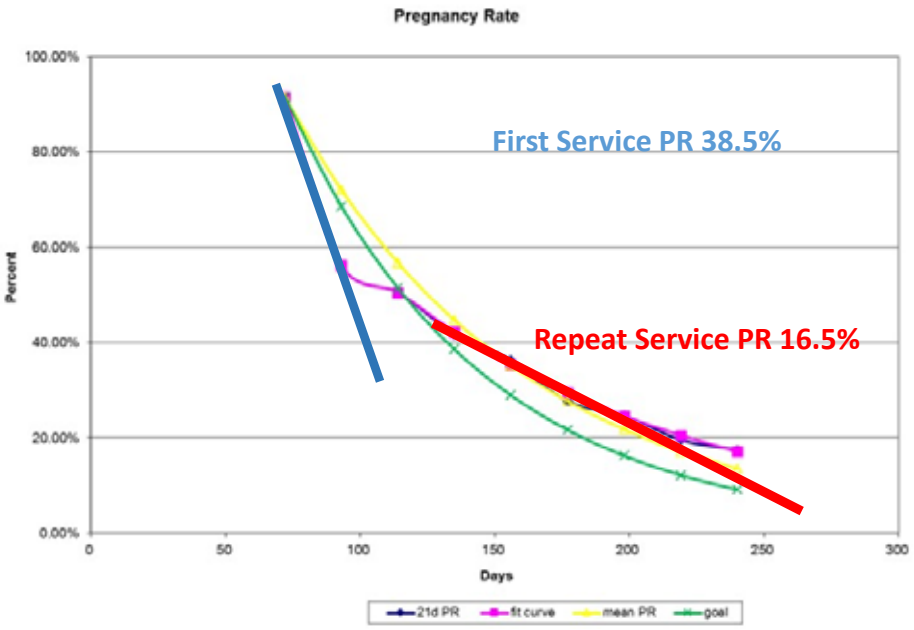

Figure 4. Pregnancy rate (PR) for the herd in December, 2011. Notice the change in PR for first service and for repeat services. The lower PR for repeat services reflects both a 42-d postsynchronization program and lower CR at 2, 3, 4 and later services.

In December, 2011 a discussion was undertaken to explore adding chromium (Cr) to the post-calving and high group rations. Literature suggested that Cr supplementation in the periparturient period reduced nonesterified fatty acid (NEFA) mobilization from body fat and increased tissue insulin, sensitivity, possibly improving glucose availability to peripheral tissues (Bryan et al., 2004; McNamara and Valdez, 2005). It was hypothesized that Cr may improve the energy status of cows and improve fertility of cows at 2nd, 3rd and 4th services (Rockwell and Allen, 2011). It was decided to include Cr propionate (0.4%) in a pre-mix to be included in lactating diets beginning in January, 2012. No other changes to the ration or reproductive management was made.

Animals, Facilities and Management

Cows were housed in modern 6-row, drive through free-stall barns with deep-bedded sand stalls. Alleys were cleaned with recirculated flush water when cows were milked. Ventilation fans were located over feed bunks and free-stalls with water sprinklers at the feed bunks. Barns were equipped for long day lighting on a cycle of 16-h light and 8-h darkness. Cows were fed once a day to 3 to 5% refusals with multiple feed “push-ups” throughout the day, and water was available free choice from troughs. Cows were

milked 3x per day in a double-12 parallel parlor. Milk volume per cow was automatically recorded at each milking with monthly DHIA testing for volume and milk components. For the year prior to the trial, monthly average days in milk (DIM) ranged from 169 to 201 DIM with a mean of 185 DIM across the year. Monthly mean milk production per cow ranged from 81.7 to 95.6 lbs/cow with an average production of 89.1 lb/cow across all months with a mean milk fat content of 3.4% and milk protein content of 3.0%.

There were a total of seven lactating groups: a post-fresh pen for cows 14 to 21 d post-calving, two mature high production groups for cows 14-21 DIM to 200 DIM, a first lactation group, two mid-lactation pens (first lactation and 2+ lactation groups), and a low production pen prior to dry-off. All feed ingredients were mixed daily and offered to each group. Ration ingredients and composition are presented in Table 2 and 3. Daily mean dry matter intakes were calculated for each pen. In January, 2012, Cr supplementation was initiated using KemTrace® brand Chromium Propionate 0.4% which was added at 1.72 lb/ton of a protein mix which was incorporated into the lactating TMR (Protein mix in Table 1, 2). This inclusion rate was calculated to provide 8 mg of Cr/head/day at a mean DMI of 55 lbs/head/day. Rations offered to the post-fresh, high, mid, and early first lactation and late first lactation groups are in Table 3. Rations offered to late and pre-dry off groups are not presented as these cows were not in breeding groups. Nutrient content of the TMR's was very similar over the last two years; there was no major difference in nutrient content.

Table 2. Ingredient composition and nutrient content of lactating protein mix (%, DM basis).

Ingredient	% DM	Nutrient	% DM
Amino Plus®[1]	50.08	CP	35.80
Distillers Dried Grains & Solubles	19.46	SP, % of CP	8.48
Limestone, ground	10.70	NDF	15.94
Blood meal, ring dried	5.02	ADF	6.89
SQ-810[1]	7.86	Lignin	1.63
Salt, white	3.89	Starch	2.54
Magnesium oxide	0.78	Sugar	9.46
MFP[2]	0.58	Ether extract	3.07
Vitamin ADE premix	0.41	Ash	31.74
Vitamin E-20	0.41	Ca	4.61
PSU #4[1]	0.33	P	0.51
Se Premix 0.06%	0.31	K	1.37
Rumensin 90	0.078	Mg	0.94
KemTRACE® Chromium 0.4%	0.086	S	0.50

[1]Amino Plus, AGP Inc. Omaha, NE; SQ-810; Sodium Sesquicarbonate, Arm and Hammer, Princeton, NJ; MFP, methionine supplement, Novus International, St. Charles, MO; PSU #4, trace mineral supplement, Sporting Valley Feeds, Manheim, PA.

Table 3. Ingredients and nutrient compositions of TMRs fed to lactating groups (%DM).

	Lactating Group				
Ingredient	Post-fresh	High 2+ Lactation	Mid 2+ Lactation	High 1st Lactation	Mid 1st Lactation
Corn Silage	34.29	36.80	37.95	37.99	42.49
Alfalfa Silage	13.49	12.58	15.81	13.60	17.16
Hay, mixed	4.66	---	---	----	----
Corn, fine	15.36	15.00	15.22	15.98	11.44
Protein Mix[1]	9.01	9.30	9.23	9.39	8.58
Topdress[2]	8.07	9.75	3.40	5.19	----
Canola Meal	4.65	4.86	4.92	5.01	5.53
Soybean meal, 48%	4.46	3.23	3.39	3.27	4.60
Wheat midds	3.54	6.88	8.39	7.85	8.99
Molasses	1.47	1.60	1.68	1.71	1.23
Energy Booster 100[3]	0.98	----	----	----	-----
Composition (% DM)					
CP	17.74	17.56	17.57	17.41	17.84
SP, % of CP	33.58	33.75	36.08	34.97	37.90
NDF	31.53	31.36	31.65	31.31	33.01
ADF	19.60	18.86	19.12	18.71	20.06
Starch	25.96	27.51	26.83	27.71	24.73
Sugar	4.55	4.36	4.46	4.47	4.24
Ether Extract	4.95	4.16	3.82	3.91	3.65
Ash	7.27	7.06	7.16	7.08	7.20
Ca	0.80	0.77	0.80	0.78	0.81
P	0.40	0.42	0.44	0.43	0.45
Mg	0.30	0.31	0.32	0.31	0.32
S	0.25	0.25	0.25	0.25	0.25
Vitamin A, KIU/lb	1.55	1.60	1.58	1.61	1.54
Vitamin D, KIU/lb	0.39	0.40	0.40	0.40	0.38
Vitamin E, IU/lb	11.60	11.97	11.88	12.09	11.53
DCAD[6]	26.20	24.80	24.30	24.00	24.70

[1] Protein Mix presented in Table 2.
[2] Contained (% DM): Soy Hulls, 42.42; Corn grain, 33.93; Soy bean meal, 48%, 11.88; Energy Booster 100, 5.94; Blood meal, ring dried, 4.24; SQ-810[4], 1.44; Smartamine M[5], 0.16; Composition (% DM): CP, 18.91; NDF, 32.50; Starch, 25.39; Sugar, 2.09; Ether extract, 9.33.
[3] Energy Booster 100; Milk Specialties Global Animal Nutrition, Eden Prairie, MN
[4] SQ-810, Sodium Sesquicarbonate, Arm and Hammer, Princeton, NJ
[5] Smartamine M, rumen-protected methionine; Adesso, Alpharetta, GA
[6] DCAD = dietary cation-anion difference, mEq/100 g DM; (Na + K) – (Cl + S)

Reproductive Management

All cows were enrolled in a Pre-Synch OvSynch program (Pursley et al. 1997). The program was as follows: cows were given prostaglandin F-2α (PGF, 25 mg IM) at 39 to 45 d which was repeated at 53 to 59 d post-calving; cows were then given GnRH (10 ug) at 64 to 70 d post-calving followed by PGF at 70 to 76 d postcalving. A second GnRH injection was given 72 to 78 d post-calving and cows were artificially inseminated on appointment (TAI) at 12 hours after the second GnRH injection, 73 to 79 d post-calving. The mean days to first breeding was 74 days on this program. This first service program had been implemented in 2010, as evident in Figure 2 with first insemination rates of 100% of cows inseminated within 21 d of VWP.

Estrus detection was employed for repeat insemination, but not aggressively. Only 24% of repeat inseminations occurred based on observed estrous. The majority of repeat inseminations were managed with a post-synchronization program (76%) which was combined with weekly veterinary visits for pregnancy diagnosis. When cows were 32 to 38 d post-insemination they were given a GnRH injection (10 ug) and were assigned for pregnancy examination at 39 to 45 d post-insemination. If cows were diagnosed as not pregnant they were given a PGF injection, followed by GnRH on day 41 to 47 post-insemination and then TAI on day 42 to 48 days post-insemination. The post-synchronization program was initiated in 2006.

Historical Trends

As seen in figure 2, pregnancy rate (PR) improved in 2007 and 2008 with the implementation of the post-synchronization program from approximately a mean rate of 18% in 2006 to a mean rate of 22% to 23% in 2007 through 2009. Part of the improvement in PR over this time period was an improvement in first service conception rate (FSTCR, Figure 2) from 25% to 32% in 2006 and 2007 to around 40% in 2008 through 2010. A major improvement in PR occurred in 2010 through 2011 with the application of the Pre-synch Ov-Synch program on all cows beginning in January of 2010 (Figure 2). It can be seen in Figure 2 that first insemination intensity (FSTIR) increased to 100% in 2010, with a resulting increase in PR (Figure 1) and an increase in FSTCR (Figure 2) to approximately 45%.

Over this time period from 2009 through 2011 PR and FSTCR had improved, but second service conception rate (SCNDCR, Figure 2) actually declined from 32 to 33% to just below 30% over this same period. Binomial trend analysis of the pattern of CR from FSTCR through second, third and fourth services indicated there was a significant negative trend in CR across these services (Rosner, 2006). Conception rates for first lactation cows were greater than for second and third lactation cows, but the same negative trend was apparent from first through fourth service in each of the age groups.

Application of Chromium Supplementation

Cows in the post-fresh and high production groups were body condition scored in January, 2012 with initiation of the chromium feeding. It was expected that it would take at least four months to observe any apparent trends in fertility. Cows would need to calve, be consuming the chromium supplement and move into the breeding groups, which began at 70 days postcalving. Monthly RepMon analysis was done for the herd and data compiled. Cows pregnant previously to January 2012 create a lag in the data and mitigate any improvement but are removed as they calve and enter a new lactation.

In Figure 5 it can be seen that as data progressed in 2012, second service CR increased to 38% by the third quarter of 2012. First service CR was not significantly changed over 2012. Conception rate to third and fourth services also increased during this period, increasing CR to all services (data not shown). Pregnancy rate increased to 32% with the improvement in CR to repeat services. Since 2012 the farm has continued to feed Cr and PR remains above 30% and second service and repeat service CR remains in the high 30%, with FSTCR around 45%. The improvement in fertility with the chromium supplementation has continued.

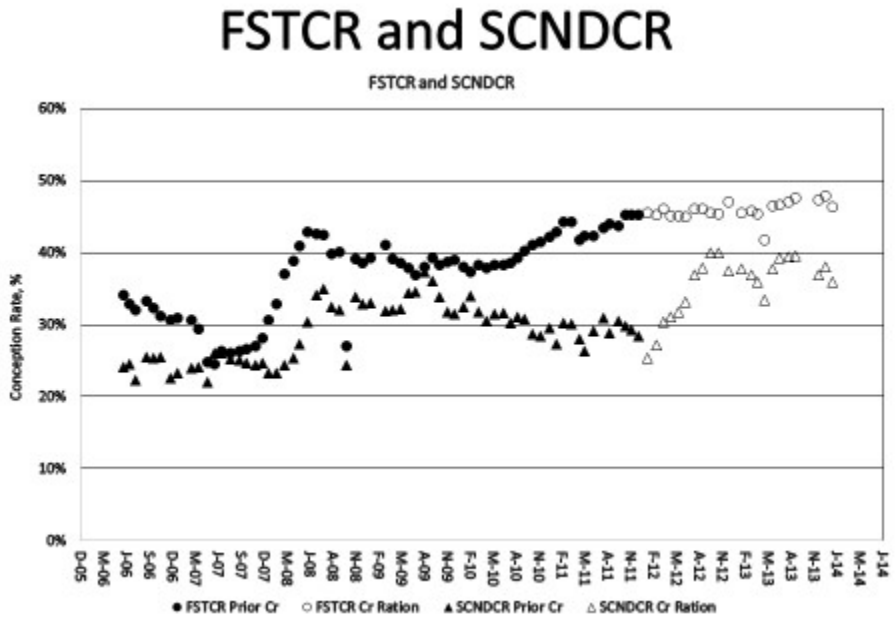

Figure 5. First service conception rate prior to Chromium supplementation (●) and after chromium added to herd TMR beginning in January, 2012 (o). Second service conception rate prior to chromium supplementation (▲) and after chromium supplementation began in December, 2012 (△). Data continue until July, 2014.

Table 3 presents data for CR by service number and month from November, 2011 through October, 2012, encompassing the main period of the trial. Binomial trend analysis was used to examine the trend in CR across monthly means over this period (Rosner, 2006). First service CR did not significantly change over this period (X^2 =

0.928, P<0.335, Table 3), although the coefficient for trend was positive. Conception rate for second, third, and fourth services all significantly increased over this period (Table 3), indicating an improvement in fertility for cows inseminated at these services.

Table 3. Binomial trends[1] across service number (first to fourth) from November, 2011 through October, 2012

	Service Number CR, %			
Month	First	Second	Third	Fourth
Mean Num.	749	334	182	107
Nov. 2011	45.2	29.3	25.5	30.4
Dec. 2011	45.5	28.4	28.7	32.5
Jan. 2012	40.8	23.5	24.3	29.6
Feb. 2012	45.3	27.1	31.9	33.3
Mar. 2012	41.0	26.9	26.3	28.8
Apr. 2012	45.2	30.8	27.9	33.1
May 2012	41.5	28.4	27.0	28.5
Jun. 2012	44.7	33.5	31.4	40.5
Jul. 2012	45.7	37.2	31.4	40.5
Aug. 2012	46.0	37.9	35.5	43.1
Sep. 2012	45.0	40.3	34.8	42.9
Oct. 2012	45.3	39.9	34.0	37.9
Trend	increasing	increasing	increasing	increasing
X^2	0.928	41.36	8.65	10.91
P<	0.335	0.0001	0.003	0.0009

[1] Rosner, 2006

The improvement in repeat service CR is apparent in comparing herd PR curves from December, 2011 and December, 2012 (Figure 6). Pregnancy rate for first service did not change, but the PR for repeat services improved to encompass the 25% PR curve (Figure 6, December, 2012). The initial decline in PR at first service is similar in both curves. The decline in the PR curve in December, 2012 is steeper and represents the improvement in CR at repeat services, as RPTIR was not different between the years. This increase in PR was associated with a gain of $66/cow/year with the same inputs in milk price, calf and cull cow value for December, 2011 and December, 2012 (RepMon calculation). At a cost of $0.04/cow/day for Cr, it would cost $8/cow to feed Cr through 200 days for a return of $56/cow.

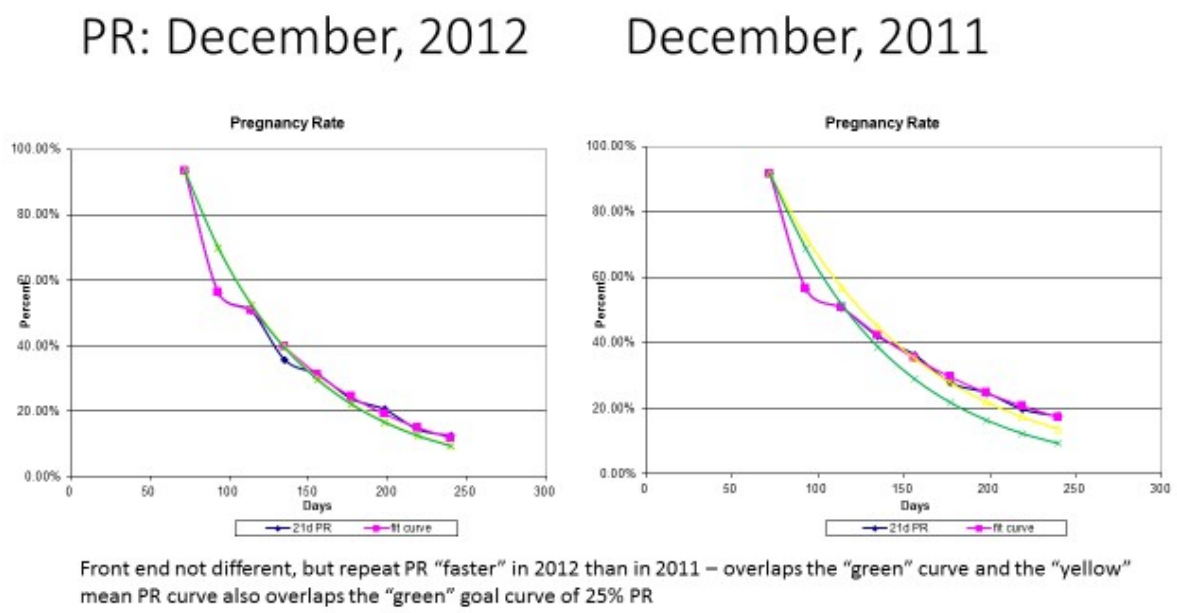

Figure 6. Pregnancy rate curves for December, 2011 (prior to chromium supplementation) and December 2012, after chromium supplementation began in January, 2012.

Body condition score was taken in the post-fresh and high groups in May, 2012. There was a significant improvement in the distribution of body condition between January and May. Over this time period, temperatures in the barn were largely within the thermal neutral zone of cattle (40 ºF to 70 ºF) and not often between 20 to 40 ºF (Figure 4). Thus, temperature likely had little influence on body condition between these dates, but other factors may have contributed to the improvement. Milk production over this time period was steady.

DISCUSSION

An issue with this type of field observation is that the improvement in fertility after the chromium supplementation began may be related to some other unidentified factors. However in this herd we had good, solid data for years prior to the feeding period with good data on transition health and reproductive management. There was no change in postpartum problems nor was there a significant change in milk production per day or in the age distribution of the herd over this time period. The same people were inseminating cows and managing reproduction as over the previous several years. Thus, we concluded the change in CR was probably related to the Cr supplementation.

A second issue with collecting monthly summaries from herd reproductive data is that pregnant cows at each service number remain in summary calculations until they re-calve, a period which would take approximately 8 months. This is typically referred to as “momentum” in DHIA data. Each month a proportion of pregnant cows re-calve and are removed from the summary data and replaced with “new” current confirmed pregnancies in the current lactation. Thus, trends may change very little until about eight

months post-implementation of a new program by which time all cows initially in the data will have calved and be replaced entirely by cows on the new program. This is apparent in the data in Table 3, where trends are positive across month but show almost “step” improvements from July, 2012 onwards for second, third and fourth inseminations. Although overall CR to repeat inseminations was lower than for FSTCR, the magnitude of the difference was less than that in 2011 and in 2010. Furthermore, trends across monthly CR data by service number in 2010 and 2011 were not significantly different (data not shown), thus the improving trend in monthly means for CR for second, third and fourth services from 2011 to 2012 was thought most likely due to the chromium supplementation.

An additional concern was that the trend to lower CR in repeat services, which began in 2009, may have been due to poorly synchronized ovulation at repeat inseminations on the post-synch program. To address this concern we examined CR for cows at second insemination between 18 to 24 days versus 36 to 48 days following first insemination in 2010 and 2011 inseminations. Cows inseminated between 18 to 24 days following first insemination were inseminated based on observed estrus whereas cows inseminated between 36 to 48 days in this herd were almost entirely from a TAI following a post-synch program. There was no difference in second service CR for these groups of cows, therefore CR at repeat services was not differentiated between observed estrus inseminations versus TAI services. Conception rate at observed estrus and TAI for second service inseminations were equally low in 2010 and 2011.

Chromium has been associated with increases in DMI in cows in early lactation with improvement in milk production and metabolic status (Hayirli et al., 2001, Nikkhah et al., 2010). Metabolic status has been observed as a reduction in serum NEFA concentrations, suggesting less fat mobilization in early lactation and improvement in energy balance. Both a reduction in NEFA serum concentration and an improvement in energy balance would improve CR. The interaction of negative energy balance and reproduction has long been recognized (Butler and Smith, 1989). Although NEFA were not measured, the improvement in body condition would suggest less fat mobilization and an improvement in energy balance. The fact FSTCR were not altered, but second, third and fourth service rates were improved, it suggests that these cows were the cows with improved energy status.

Improved reproduction with Cr supplementation has been reported by Bryan et al. (2004), Lavin-Garza et al. (2007), Rockwell and Allen (2011) and Soltan, 2010. The improvement in reproduction may occur via several mechanisms. Burton et al. observed that Cr improved immune status which may reduce the effects of subacute metritis on CR. We feel this is unlikely in this herd as metritis was not a significant problem. Secondly, Cr has been linked with increases in DMI in the postpartum period (Hayirli et al., 2001, Nikkhah et al. 2010, Yang et al., 1996) reducing NEFA (McNamara and Valez, 2005) and improving glucose utilization (Sumner et al., 2007). All these effects may benefit energy balance and improve fertility. It is possible the repeat breeder cattle in this herd experience more negative energy balance than cows that conceived to first insemination. This was slightly more than half the cows in the herd. In addition, second

and older lactation cows, the higher milk producers in the herd, had lower FSTCR than first lactation cows (Dec. 2011, Parity 3+, 41.3%, Parity 2, 41.3%, Parity 1, 51.2%, P<0.04), suggesting more energy stress as a role in reduced in FSTCR. After a year feeding the Cr, FSTCR was not significantly changed in these age groups (Dec., 2012, Parity 3+, 43.2%, Parity 2, 39.0%, and Parity 1, 51.1%, P<0.04), however CR to second service had increased in all lactation groups from 2011 to 2012 (First Parity, 35.9 to 42.1, Second Parity, 18.8 to 43.3, Third+ Parity, 26.6 to 31.5%, respectively). It was felt that Cr may have improved energy status in cows that failed to conceive at FSTCR and was reflected in the improved fertility at 2, 3, and 4th services.

Results of this case study should be interpreted cautiously. Many factors influence CR and chromium may be given credit for some other change within the herd population. As of this writing CR to all services has continued to slightly improve as has milk production. Certainly it is gratifying to see milk production and reproduction improve together. The cost of chromium supplementation was about six cents per cow per day in 2012. Thus, situations of bimodal fertility, as this herd experienced, may benefit from Cr supplementation. First, one should explore associations of effects of postpartum disease within the two populations. If CR is low to all services, then other factors should be explored such as poor semen handling or poor timing of insemination relative to ovulation.

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