BOOK VALUES FOR FORAGES IN MINERAL NUTRITION -- WHEN DO THEY MATTER?

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INTRODUCTION

Often, dairy rations are balanced either assuming the TM in forages are zero or using standard reference values, e.g. NRC (2001). While trace mineral (TM) concentrations of forages are often lower than the TM requirements of most domestic livestock, they are not negligible. Endogenous minerals in forages are highly available upon digestion, while minerals arising from soil contamination are poorly available. If forage TM concentrations are not considered in the feed formulation process, it is not likely that the targeted dietary concentrations will be met. There is a greater risk of excess minerals in the ration than deficiency due to the skewed distributions in forage TM concentrations or if the forage minerals are set to zero. This risk is of concern especially with Cu, where liver accumulation occurs when dairy cattle consume diets with 20 mg/kg Cu (Balemi et al., 2010) and may be detrimental to animal health and performance (Weiss & Faulkner, 2015). Using TM concentrations for forages and other basal feed ingredients will reduce the risk of mineral imbalances, will improve the efficacy of TM supplementation, and can reduce TM excretion into the environment via manure. This presentation focuses on the contribution of geographical location to the TM variation, and demonstrates how variation in forage TM concentrations affects ration TM concentrations under different supplementation strategies.

TM VARIATION IN CALIFORNIA FORAGES

Data from Cumberland Valley Analytical Services for forages from the 2009 to 2014 growing seasons with concentrations of major nutrients as well as mineral concentrations were used. Data were statistically filtered to remove misidentified feeds and outliers based on macronutrient concentrations using the procedures outlined in Yoder et al., 2014. After this step, the national corn silage, legume hay, and small grain silage data sets contained 20654, 8856, and 5047 observations, respectively. With regards to California forages, there were 3848, 1355, and 2258 samples of corn silage, legume hay, and small grain silage, respectively.

As expected, TM concentrations for corn silage, legume hay, and small grain silage displayed skewed distributions (data not shown). TM values were log normalized before analysis of variance and mapping of geographical variation (Knapp et al., 2015a,b).

Geographical location and total ash content were the largest sources of variation in forage TM concentrations ($p<0.0001$). Ranges in TM concentrations of forages from the most variable regions of the U.S. were 4 to 10 times greater than the more consistent forages grown in other regions (Knapp et al., 2015a). Growing season was often a non-significant ($p>0.20$) contribution to total TM variation, while the interaction between location and season was significant ($p<0.05$). This suggests that a portion of the variation attributed to geographical area is dependent on weather conditions during the growing season, e.g. dust carried by winds, or harvesting and storage practices that differ between regions.

For California forages, the mean Cu and Zn concentrations (Table 1) were not significantly different from national averages used by NRC (2001). However, there is a substantial amount of variation as seen in the range between the 5th and 95th percentiles (Table 1). The two- to four-fold differences between the 5th and 95th percentiles suggest that sampling and testing will be valuable in formulating for more precise trace mineral levels in dairy rations.

Table 1. Forage copper and zinc concentrations in California, given as mean, 5th and 95th percentiles calculated from log normalized data compared to NRC 2001 values. Obs = number of observations for each forage.

Cu	NRC 2001	mean	5th perc	95th perc	obs
Corn silage	6	5.9	4.0	9.4	3848
Small grain silage	9	7.4	4.0	16.1	1355
Legume hay	7-9	10.5	7.3	14.4	2258

Zn	NRC 2001	mean	5th perc	95th perc	obs
Corn silage	24	27.9	18.7	43.4	3848
Small grain silage	24	29.8	17.3	56.7	1355
Legume hay	27-37	26.6	18.3	37.5	2258

Soil contamination can contribute to higher TM concentrations, especially Mn and Fe (Figures 1 & 2). Titanium (Ti) concentration in forages is considered by agronomists to be the gold standard in determining soil contamination of forages. However, Ti is not measured in routine nutrient analysis of feed ingredients. Soil contamination of forages reduces the concentration of organic nutrients, and soil Fe can decrease the absorption and utilization of dietary copper and perhaps other minerals (NRC, 2001; references within Hansen & Spears, 2009 and Spears, 2013). In the past, Fe from soil contamination has been assumed to be non-reactive and not interfere with absorption of other trace minerals (TM). However, *in vitro* studies have shown that soil Fe solubility and bioavailability can be increased during ensiling (Hansen & Spears, 2009).

Figure 1. Geographical variation in soil contamination as depicted in total ash concentrations in corn silage. Areas represent individual mailing centers, not counties.

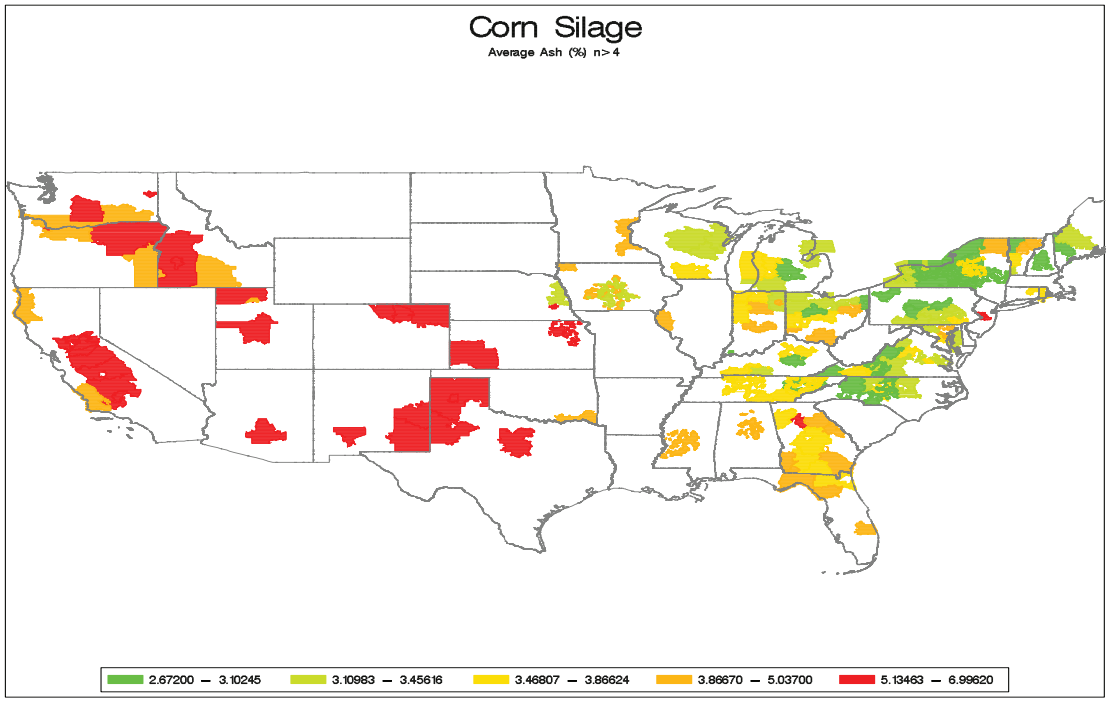
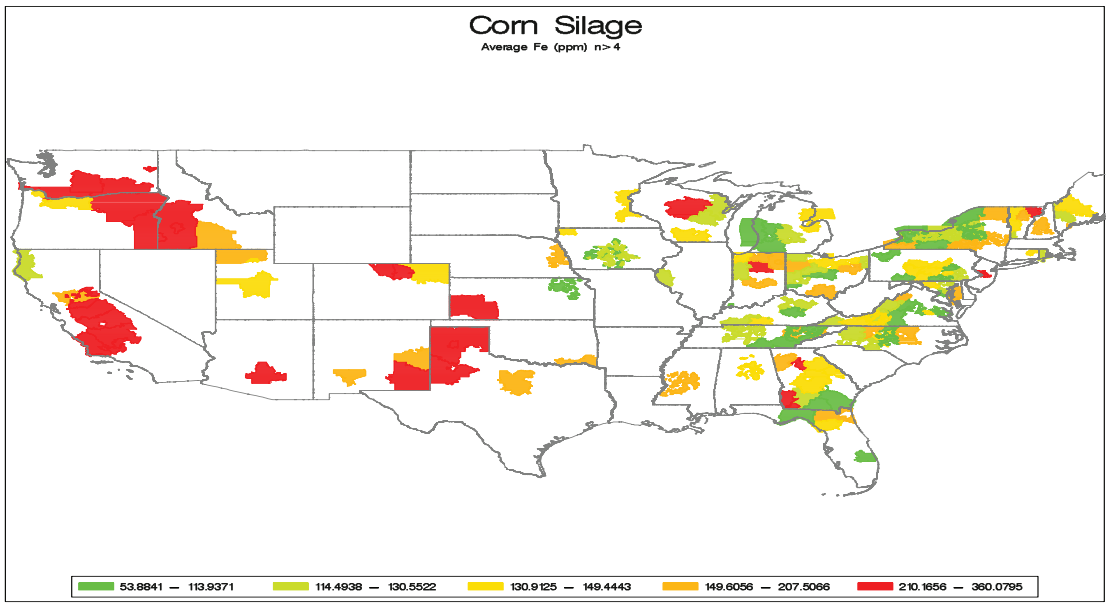
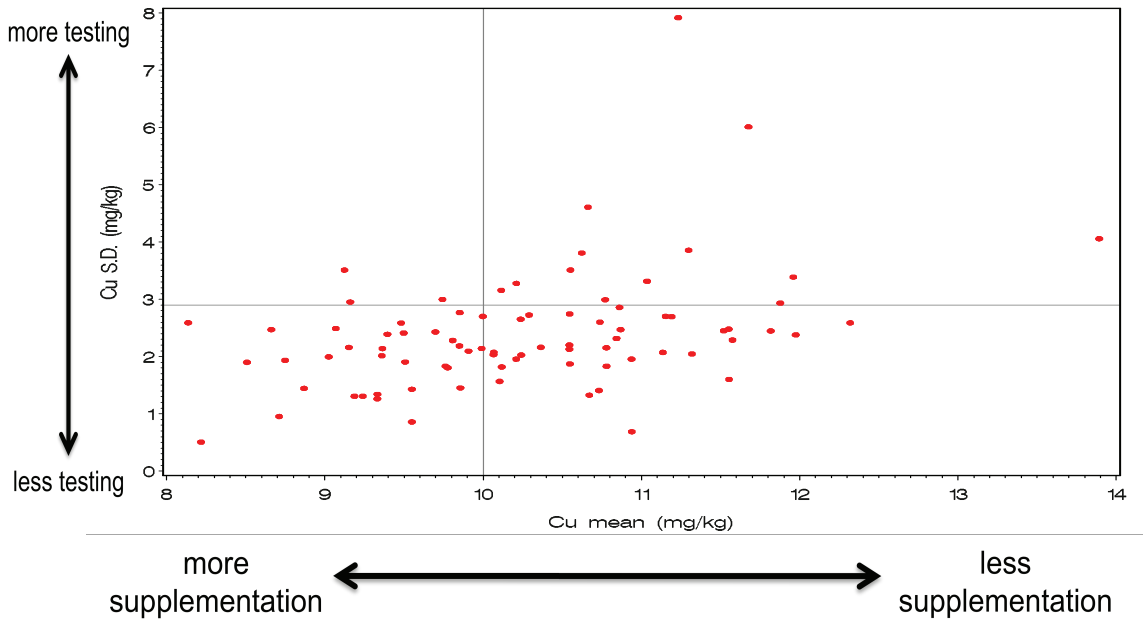


Figure 2. Geographical variation in corn silage Fe concentrations is a reflection of total ash concentrations and soil contamination. Areas represent individual mailing centers.



Soil contamination in forages was estimated using a modification of the residual ash (RA) calculation from Cary et al. (1986). Few corn silage samples showed more than 4% soil contamination, but 10 to 33.5% of legume hay and small grain silages, respectively, had levels greater than 4%. These levels of soil contamination are associated with Fe concentrations greater than 800 mg/kg with the most extreme samples exceeding 2000 mg/kg (Figure 2). Both total ash and Fe concentration are highly correlated with the level of soil contamination estimated by residual ash (R^2 ranging from 0.47 to 0.75; Knapp et al., 2015b).

Figure 3. Recommendations for testing and supplementation will vary according to the observed variation in TM concentrations. Example given is in legume hay, median Cu = 10.0, S.D. = 2.9 mg/kg. Each dot represents an individual mailing center.



RATION FORMULATION AND TM SUPPLEMENTATION

Nutritionists want to know how to best manage the variation in forages to reduce variation in the finished rations. They also want to know how to supplement under conditions of varying TM concentrations in ingredients. The first step is to know how much variation is occurring. This requires appropriate sampling and testing. Frequency of sampling will be determined by how much variation there is in forages and how often forage ingredients are changed in the ration (Figure 3), with more variation requiring more sampling and analysis. Amount of TM supplementation will depend on the median TM concentrations in the forages (Figure 3).

This data on forage variation when combined with data on TM concentrations in grains and protein meals allows us to predict TM concentrations in rations. Mixing feeds together always reduces nutrient variation compared to the variation in individual ingredients, and using more variable ingredients at lower inclusion rates can also reduce variation. Most dairy rations in the U.S. do require supplementation with Cu, Zn, and Mn to reduce the incidence of deficiencies (Table 2). Supplementing in the range of 11-14 for Cu, 30-50 for Zn, and 40-60 for Mn (mg/kg) should nearly eliminate the possibility of any individual rations being deficient (Table 2).

Table 2. Using feed mixing to reduce TM variation in finished rations. Predicted TM concentrations in total mixed rations with 0, 1x, or 1.5 supplementation of basal ingredients. Dietary levels (mg/kg) of 11, 52, 40, and 17 were set as minimums for Cu, Zn, Mn, and Fe, respectively (NRC, 2001).

Supplementation Level	Zero	1x	1.5 x	S.D.
Cu ave	6.0	17.0	23.0	1.9
Cu < 11 mg/kg % failures	99.58	0.05	0	
Cu > 30 mg/kg % failures	0	0	0.01	
Zn ave	33.2	85.2	111.2	8.5
Zn < 52 mg/kg % failures	98.75	0	0	
Mn ave	35.1	75.1	95.1	10.0
Mn < 40 mg/kg % failures	69.02	0.01	0	
Fe ave	204	221	230	86.2
Fe < 17 mg/kg % failures	0.06	0.01	0	

Basal ingredients: corn silage, legume hay, flaked corn, dried distillers' grains, corn gluten feed, soybean meal
1x supplementation (mg/kg): 11 Cu, 52 Zn, 40 Mn, 17 Fe added
1.5 x supplementation (mg/kg): 17 Cu, 78 Zn, 60 Mn, 25.5 Fe added

Knowing the basal TM concentrations in forages is key to accurate and precise supplementation! Higher levels of copper supplementation should be avoided to reduce long-term Cu accumulation in liver and potential chronic Cu toxicity (Weiss and Faulkner, 2015). Obviously, excess supplementation of other minerals should be avoided to reduce feed costs, and also to reduce excretion into the environment.

CONCLUSIONS

Geographical location and total ash content are significant sources of variation in TM concentrations in commonly used dairy forages. There are areas in the U.S. that have consistently low TM concentrations, while forages in other areas have high concentrations with high variation. Soil contamination is higher in regions of the western U.S., including California.

Soil contamination may contribute to variation and can be attributed to weather patterns, soil types, and harvesting and storage practices. Variation in TM concentrations can be reduced with standard feed mixing protocols, but requires knowledge of concentrations for accurate and precise formulation of dietary TM levels. These results support sampling and analysis of forages and formulation of dairy rations for TM based on analytical results rather than reference values.

ACKNOWLEDGEMENTS

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Optimizing the trace mineral status of pre- and post-weaned beef calves[1]

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Introduction

Trace minerals are known to be essential for the support and maintenance of immunity in cattle. Beef cattle production systems typically rely on a cow/calf grazing system culminating at weaning when calves are permanently separated from their dams and moved onto other phases of production, such as backgrounding or feedlot. The trace mineral status of weaned calves is dependent, therefore, on the contribution of milk, forage, and supplement. Unfortunately, warm season forages are commonly deficient in essential trace minerals and milk is a poor source of the two most commonly deficient trace minerals – Cu and Se. This situation, therefore, results in a reliance upon supplements to ensure adequate trace mineral status of calves. During the grazing season, most mineral supplements are blended with salt and formulated to address regionally-specific targeted intakes by the cow. Very little focus is placed on calf intake, which is largely unknown or inadequate. These conditions commonly result in marginal to deficient trace mineral status of weaned calves. Management opportunities aimed at improving the trace mineral status of calves prior to weaning hold merit relative to optimizing post-weaning health and productivity. This article will examine recent efforts to utilize pre-weaning supplements in a limit-fed creep feeding system to improve the mineral status of pre- and post-weaned beef calves.

Limit-Fed Creep Feeding

Traditionally, creep feeding is associated with the practice of providing grain-based supplements to pre-weaned calves on pasture in unlimited amounts. When fed over a 3 month period prior to weaning, calves often consume very small amounts initially, but work up to amounts exceeding 5 kg daily by the time of weaning. The practice of unlimited creep-feeding has been associated with decreased feed efficiency (Stricker et al., 1979; Faulkner et al., 1994) attributed to less ruminal and total tract NDF digestibility leading to decreased forage utilization. These negative responses could be avoided if creep feeding supplements are limit-fed (Cremin et al., 1991). Limit-fed creep-feed supplements up to 1.0 kg/d increased pre-weaning ADG of calves (Lusby and Wettemann, 1986; Faulkner et al., 1994) and improved concentrate intake in the feedlot (Faulkner et al., 1994). Collectively, these studies imply that limit-creep feeding may be

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a logical management system to deliver supplemental trace minerals to calves without the negative factors associated with traditional unlimited creep feeding.

Trace Mineral Nutrition and Stress

There is very little evidence that marginal trace mineral deficiencies among pre-weaned beef calves impact performance. In normal, low-stress, pasture-based production systems, calves may be marginal or deficient in essential trace minerals and still display adequate growth and final body weight at weaning. The problem occurs after weaning, when limited reserves of trace minerals are excreted during stress, resulting in a trace mineral-deficient calf. These deficiencies then accompany the calf into the most stressful events they will encounter throughout their productive life. It is these deficiencies that that have the greatest impact on immune competence, disease outcome, and overall productivity. These stress events are numerous and typically unavoidable as they are associated with normal beef production management systems. Weaning and transport, which are likely the most stressful events in a calves' life, are also often combined with other stressful events such as vaccination, commingling, and changes in housing diet. These physiological reactions are considered normal, but must be controlled through a negative feedback mechanism orchestrated by cortisol. The trace mineral status of calves (namely Cu and Se) impacts a calves' ability to respond normally through the initiation and subsequent shut down of these inflammatory process. Existing research has illustrated alterations in beef calf physiology and nutrition due to weaning, transportation, and feedlot entry (Loerch and Fluharty, 1999). These alterations are closely related to the activation of the acute phase response (Arthington et al., 2005, 2008), which releases proinflammatory cytokines associated with impaired nutrient metabolism and animal growth (Johnson, 1997). Also, these stressors will impact the trace mineral status of calves (NRC, 1996) by liberating tissue stores of Cu, Zn, and Se for the support of immune function, particularly in newly received feeder calves (Duff and Galyean, 2007).

Mineral-Fortified Limit-Fed Creep Feeding

We have had a long-term interest in nutritional management applications that will optimize the trace mineral status in beef calves prior to weaning. It is out central aim to optimize the trace mineral nutrition of calves, prior to weaning, to help to ensure adequate trace mineral status following recovery from the stress of weaning. One area of investigation is the use of "limit-fed" creep supplements. The concept of "limit-fed" is essential in this application. As discussed earlier, many studies have confirmed that the efficiency of added gain among creep-fed calves is poor, in fact, the poorest of all phases of the beef production system. Therefore, we sought to use limited creep feeding as a system for delivering trace minerals to pre-weaned calves. In our first studies, we discovered that calves had a strong aversion to consumption of mineral-fortified creep feed, which did not exist in calves consuming the same supplement without mineral fortification (Moriel and Arthington, 2013). We hypothesized that the

sulfate sources of minerals, particularly Cu and Zn, were disassociating in the calves' mouths, causing a taste aversion, such as a person might experience with a metallic taste experience. This hypothesis is supported by the highly soluble nature of Cu- and Zn sulfate. Visual observation of the calves' reactions as they attempted to consume the supplements also supported our hypothesis.

Discussions with academic and industry colleagues raised the question regarding the potential for differences in voluntary intake among calves provided trace mineral-concentrated supplements containing hydroxy- vs. traditional sources (sulfate or organic) of Cu, Zn, and Mn. Hydroxy trace minerals are a relatively new technology available to the feed industry. These specific crystalline inorganic mineral sources are formed by covalent bonds within a crystalline matrix. The covalent bond structure differs from the ionic bonds present in common sulfate-based minerals and is more similar to the covalent bonds present in organic trace mineral sources. Whereas organic trace minerals are covalently bound to a carbon-containing ligand, hydroxy trace minerals are covalently bound to an OH group. One of the most functional characteristics of hydroxy trace minerals is their lack of solubility at neutral pH ranges, such as the mouth or rumen of healthy cattle. Dissolution of the metal occurs at lower pH values, which are common in the lower gastrointestinal tract. Additional to these functional characteristics, hydroxy trace minerals are also highly concentrated allowing for greater flexibility with formulation space within a mineral-concentrated, limit-fed supplement. For example, a mineral supplement containing 4,000 mg/kg Zn would require only 0.73% of the formulation space for hydroxy Zn inclusion (IntelliBond [Micronutrients Inc.]; 55% Zn), but would require 2.7% of the formulation space for organic Zn inclusion (i.e. Bioplex [Alltech] Zn; 15% Zn).

These attributes provided the rationale for our next study aimed at evaluating the preferential intake of three experimental supplements, each containing the same base ingredient formulation, but differing by source of Cu, Zn, and Mn. This was achieved in 4 individual studies involving 8 pens of early-weaned calves (2 calves/pen) with an average age of 120 days and an average body weight of 115 kg. Each pen was provided free-choice access to concentrate and grass hay. On each study day at 1000 h, all feed was withdrawn from the pens and calves were offered three different mineral fortified supplements, for a 4-hour period. The supplements were provided in three separate feeding containers. The supplements differed by the source of Cu, Zn, and Mn, which were hydroxy- (IntelliBond; Micronutrients, Inc.), organic-, and sulfate-sources. The supplements were created using a base mixture containing 52, 46, and 2% cottonseed meal, ground corn, and salt fortified with 2,000, 750, and 3,000 mg/kg of only Zn (Experiment 1), only Cu (Experiment 2), and only Mn (Experiment 3), respectively. The last evaluation (Experiment 4) contained the same base supplement mixture fortified with a mixture of Zn, Cu, and Mn. Preferential intake was measured over 7- (Experiments 1, 2, and 3) and 14-d (Experiment 4) evaluation periods. Results are expressed as preferential intake as a % of the total amount of supplement consumed. These results revealed a lesser preferential intake of supplements fortified with sulfate or organic sources of Cu, Zn and Mn compared to supplements fortified with hydroxy sources of these elements (Figure 1). When all three trace minerals were

combined together, calves almost exclusively selected the supplement fortified with the mixture of hydroxy Cu, Zn, and Mn (Figure 1).

Effect of Source of Cu, Zn, and Mn on Performance of Pre- and Post-weaned Calves Offered Limit-Fed Creep Supplements

This initial information created the rationale for a second 2-year study aimed at evaluating the effects of source of Cu, Zn, and Mn blended into limit-fed creep feeding supplements and offered to beef calves for approximately 3 months prior to weaning.

Research Methods

Pre-weaned calves (Brahman x British; n = 20 and 24 in year 1 and 2, respectively) were hand-fed supplements within 6 m^2 cow-exclusion areas for 84-days prior to weaning (2 calves/pasture; 10 and 12 pastures in year 1 and 2, respectively). In year 1, calves were sorted by sex (10 heifer and 10 steer calves) and assigned to pastures providing 1 heifer and 1 steer calf/pasture. In year 2, 24 heifer calves were stratified by initial BW and randomly assigned to pastures. Pastures consisted of established bahiagrass (*Paspalum notatum*) with free-choice water and supplemental white salt with no added minerals throughout the experiment. Three supplements were formulated to contain either no additional Cu, Zn, or Mn (**control**) or 775, 2,250, and 3,000 mg/kg Cu, Zn, and Mn from two sources (**hydroxy and sulfate**). Treatments were randomly assigned to pastures (n = 4, 3, and 3 and 4, 4, and 4 pastures for control, hydroxy, and sulfate treatments in year 1 and 2, respectively). Supplements were fed 3 times weekly (Monday, Wednesday, and Friday) in amounts to ensure a maximum target intake (as-fed basis) of 114 g/calf daily. Ingredient composition of supplements are shown in Table 1. Hand plucked pasture samples during the pre-weaning phase were collected in June of each year. Pre-weaning supplement samples were collected from three random bags/treatment at the start of each year and pooled for analysis of nutrient content.

Consumption of limit-fed creep was calculated on each feeding day by subtracting the amount of refusal DM from offer and total DM consumption calculated on weekly basis. In each year, calf body weight was measured at the beginning (day 0) and end (day 84; time of weaning) of the experiment. In year 1, there were no differences in BW gain between heifers and steers, thus values were not adjusted for calf sex. In year 2, only heifers were enrolled. At weaning, all heifers were retained for a 16-day evaluation (1/pasture in year 1 and all heifers in year 2; n = 10 and 24, respectively). Immediately following cow and calf separation calves were transferred to fully-covered, individual pens (10 m^2). Over a 16-day period, heifers were provided free-choice access to ground grass hay and a grain-based concentrate. Individual dry matter intake (DMI) was calculated daily by subtracting the DM of the daily refusal from the DM of the daily offer of both hay and concentrate, which were offered free-choice in separate individual feed bunks. Final heifer body weight was determined on day 17 following a 16-hour period of water and feed withdraw. Heifer ADG was calculated from

the body weight change from initial weaning weight and shrunk body weight on day 17 after weaning.

Plasms cortisol and the acute phase protein response (via ceruloplasmin and haptoglobin) was assessed on day 0, 2, 5, 9, and 16. Liver biopsies were collected at the time of weaning from all heifers and analyzed for trace mineral concentration using inductively coupled plasma-atomic emission spectroscopy techniques.

The limit-fed creep supplements used in these experiments were manufactured by a commercial company. Ingredient formulation and nutrient specifications of the final products are provided in Tables 1 and 2, respectively. A single manufacturing run was made for each experiment.

Results (Pre-weaning)

Complete consumption of the limit-fed creep would result in a total of 9.6 kg of supplement intake/calf for the 84-day pre-weaning supplementation period (i.e. 114 g/day x 84 days). Irrespective of mineral fortification, there was greater ($P < 0.001$) supplement intake in year 2 vs. 1 (9.1 and 5.8 kg, respectively; SEM = 0.36). Averaged over both years, calves provided hydroxy sources of Cu, Zn, and Mn had a 25% greater ($P = 0.01$) total supplement intake compared to calves consuming sulfate-sources of Cu, Zn, and Mn (8.2 vs. 6.5 kg; SEM = 0.43; Table 3). Mineral fortification of limit-fed creep supplements did not impact (P = 0.32) pre-weaning calf body weight gain; however, calves provided supplements fortified with hydroxy sources of Cu, Zn, and Mn tended (P = 0.12) to have greater body weight gain compared to calves consuming supplements fortified with sulfate sources of the same elements. Calves provided mineral-fortified supplements had greater (P = 0.003) liver concentrations of Co and Se, and tended (P < 0.07) to have greater concentrations of Cu, compared to calves consuming supplements without mineral fortification (Table 4).

Results (Post-weaning)

Irrespective of source, calves provided mineral-fortified vs. unfortified supplements had greater ($P \leq 0.05$) peak concentrations of ceruloplasmin and haptoglobin following weaning (Figure 2; Inset A).

Calves provided mineral-fortified creep supplements had less body weight gain during the 16-day post-weaning evaluation period compared to calves provided supplements without mineral-fortification (Figure 2; Inset B). This response was mostly attributed to the treatment containing sulfate sources of Cu, Zn, and Mn. Calves consuming these supplements had less body weight gain compared with calves consuming supplements fortified with hydroxy sources of these elements (Figure 2; Inset B).

Voluntary forage and grain DMI was determined separately. Data were analyzed in 5, 3-day intervals. Data from the final day (day 16) was not included since heifers did

not have a full 24 hour time period for measure of DMI. There was a treatment x interval x year interaction for voluntary intake of grain (P < 0.001), but not hay (*P* = 0.28). This interaction occurred due to a much greater voluntary grain intake among calves receiving mineral-fortified supplements in year 2 vs. 1 (average grain DMI = 0.22 vs. 1.07% body weight for year 1 and 2, respectively; SEM = 0.195). Averaged over both years, voluntary grain DMI was less (*P* < 0.001) for calves consuming pre-weaning supplements with mineral fortification, regardless of source, compared to calves consuming supplements without fortification (Figure 3). This difference resulted in greater (*P* < 0.001) overall DMI among calves consuming pre-weaning supplements without mineral fortification and likely helps explain, at least partially, the greater post-weaning body weight gain among these calves when compared to those consuming mineral-fortified, pre-weaning supplements. Voluntary hay DMI intake did not differ (*P* = 0.69) among treatments when averaged over both years (0.75% body weidht daily; SEM = 0.055).

Summary

The results of this most recent study implicate that that hydroxy sources of Cu, Zn, and Mn can be effectively used in mineral-concentrated, limit-fed, pre-weaning supplement formulations for beef calves. By replacing sulfate, and likely organic sources of these elements, the previously reported intake aversion is no longer an impeding condition. The use of hydroxy sources of Cu, Zn, and Mn in supplementation systems aimed at improving the trace mineral status of pre- and post-weaned beef calves holds merit in modern beef management systems.

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Tables and Figures

Table 1. Ingredient composition of limit-fed supplements.

Item[1]	Control	Hydroxy	Sulfate
	---------- % as-fed basis ----------		
Soybean meal	73.75	73.75	73.75
Dehydrated alfalfa meal	10.00	10.00	10.00
Dried molasses	5.00	5.00	5.00
Wheat middlings	6.40	5.09	4.87
Ca carbonate	2.50	2.50	2.50
NaCl	1.25	1.25	1.25
Liquid fat	1.00	1.00	1.00
Zn sulfate	0	0	0.63
Hydroxy Zn	0	0.41	0
Mn oxide	0	0	0.50
Hydroxy Mn	0	0.68	0
Cu sulfate	0	0	0.31
Hydroxy Cu	0	0.13	0
Ca propionate	0.10	0.10	0.10
Se selenite	0	0.08	0.08
EDDI	0	0.01	0.01
Co carbonate	0	0.002	0.002
Total	100	100	100

[1]Supplements were provided in cow exclusion areas 3 times weekly (Mondays, Wednesdays and Fridays) in amounts to ensure a maximum target intake (as-fed basis) of 114 g/calf daily.

Table 2. Formulated nutrient specifications of limit-fed supplements (DM basis).

Item	Control	Hydroxy and Sulfate
CP, %	38.4	38.2
Ca, %	1.30	1.33
P, %	0.56	0.55
Na, %	0.521	0.521
Cu, mg/kg	17	775
Mn, mg/kg	39	3,000
Zn, mg/kg	53	2,250
Co, mg/kg[2]	0.1	9.3
Se, mg/kg[2]	0	8.0

Table 3. Pre-weaning calf BW and overall creep intake.[1]

	Treatment				Contrast[2]	
Item	Control	Hydroxy	Sulfate	SEM[4]	1	2
	---------- kg ----------					
Initial BW[3]	130	137	134	4.5	0.56	0.30
Final BW[3]	213	229	217	5.9	0.15	0.15
BW Gain	83	90	83	3.4	0.12	0.32
Creep Intake[4]	7.6	8.2	6.5	0.43	0.01	0.72

[1]Values are least square means.
[2]Contrast 1 = hydroxy vs. sulfate; Contrast 2 = control vs. hydroxy and sulfate.
[3]Initial and final BW (d 84; weaning) were collected without feed or water withdrawal.
[4]Total creep intake over 84 d. Calves were provided 114 g/d.

Table 4. Liver mineral concentrations of calves receiving limit-fed creep-feeding supplements with or without trace mineral fortification.[1]

	Treatment				Contrast[2]	
Item	Control	Hydroxy	Sulfate	SEM[4]	1	2
	---------- mg/kg (DM basis) ----------					
Co	0.09	0.28	0.17	0.032	0.02	0.003
Cu	129	163	209	25.7	0.22	0.07
Fe	247	259	296	25.3	0.31	0.34
Mn	9.9	9.4	12.5	1.62	0.83	0.17
Mo	3.04	2.97	3.29	0.352	0.88	0.49
Se	0.25	0.51	0.62	0.075	0.32	0.003
Zn	161	161	158	7.8	0.75	0.84

[1]Values are least square means. Liver biopsy samples were collected on d 9, relative to receiving feedlot entry. N = 34 total samples (10 and 24 for years 1 and 2, respectively).
[2]Contrast 1 = hydroxy vs. sulfate; Contrast 2 = control vs. hydroxy and sulfate.

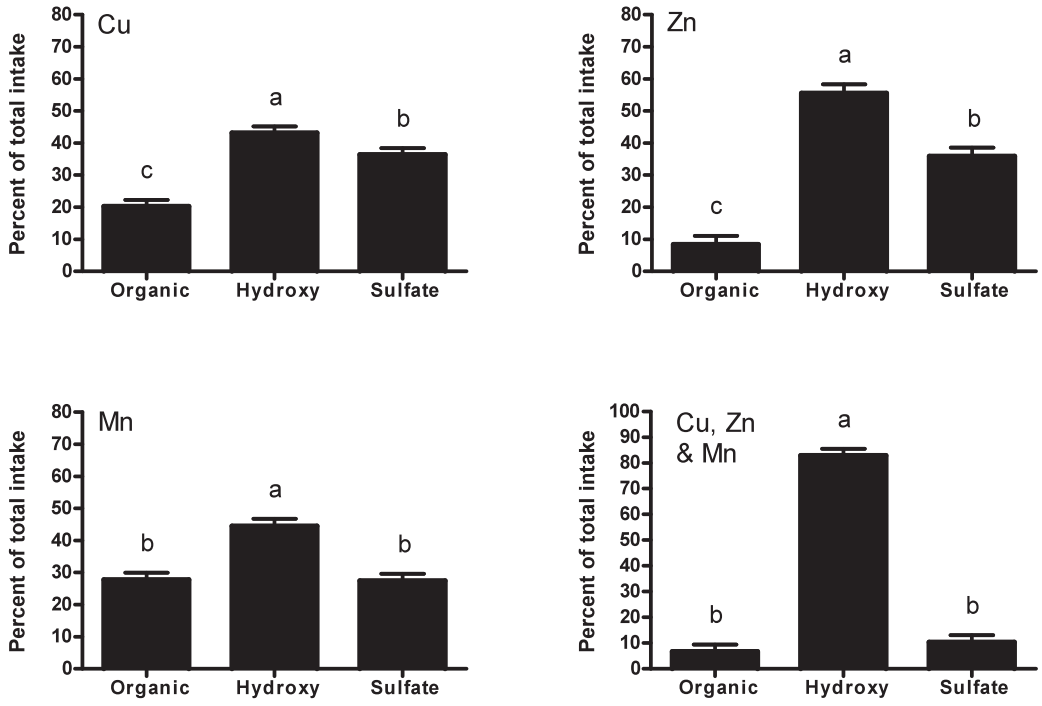

Figure 1. Preferential intake of mineral concentrated supplements when offered simultaneously to young calves. The sum of means of each inset figure sums to 100%.

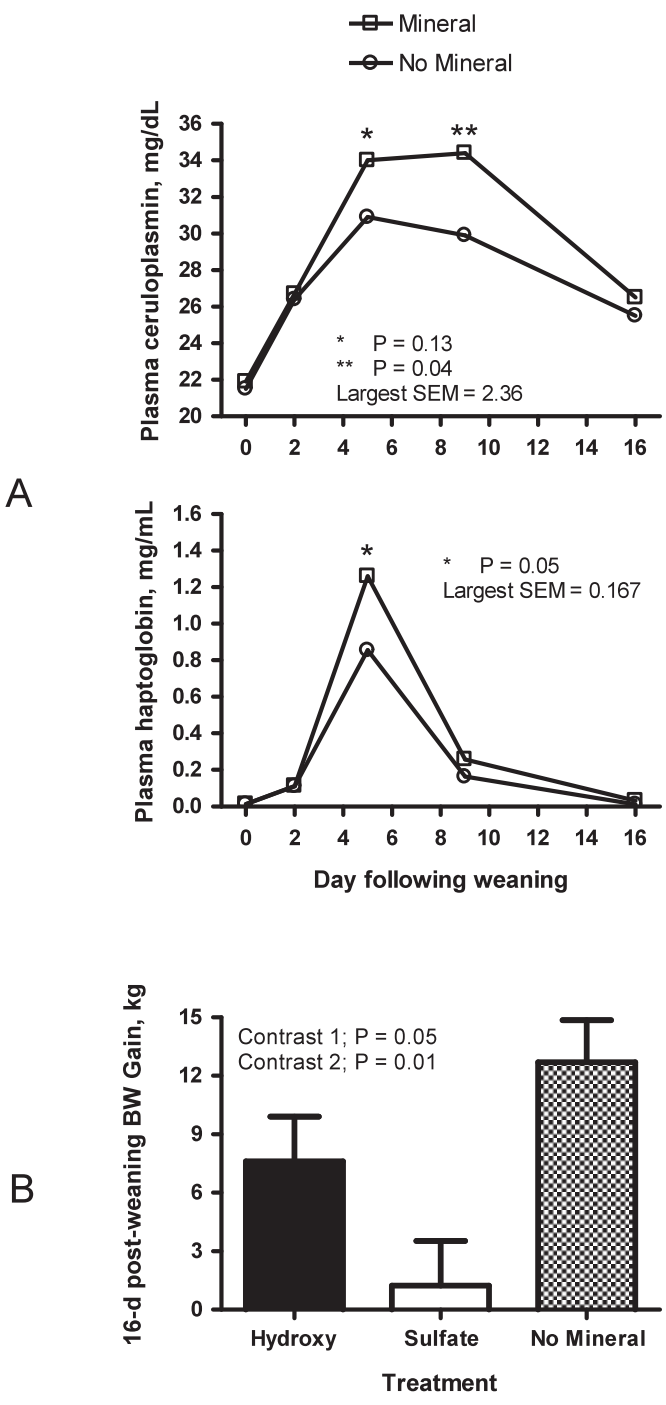

Figure 2. Plasma concentrations of ceruloplasmin and haptoglobin in post-weaned calves provided limit-creep supplements with and without mineral fortification for 18 wk

prior to weaning (Inset A) and BW gain of calves during a 16 d post-weaning evaluation (Inset B). Contrast 1 = hydroxy vs. sulfate; Contrast 2 = control vs. hydroxy and sulfate.

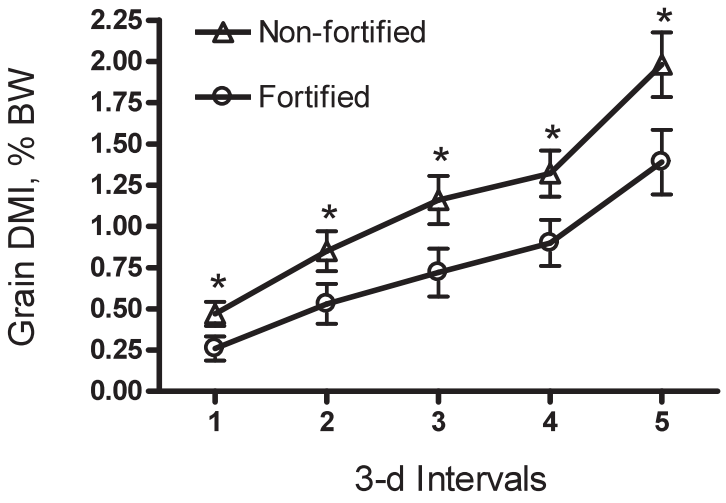

Figure 3. Voluntary grain DMI of post-weaned calves provided limit-creep supplements with and without mineral fortification for 18 wk prior to weaning. * = Means differ at each sampling day; $P < 0.05$.

Reassessing Trace Mineral Requirements and Supply in Dairy Calves

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INTRODUCTION

Young growing dairy calves require the commonly identified trace minerals similar to other species. In most cases, requirements were established many years ago with cattle much different from today. More importantly, requirements during the milk-fed period were almost always determined in calves fed limited amounts of milk that only allowed low rates of growth. In recent years, more and more calves have been fed higher rates of milk replacer or milk, which with the advancement in genetic indices for milk production raises the question of whether currently specified requirements are accurate. If requirements for current genotypes of dairy calves growing at higher rates early in life have changed, then they might be able to be satisfied either by increasing total mineral concentrations in milk solids or by using sources of trace minerals with higher bioavailability (organic or hydroxy forms rather than typical sulfate sources).

The purpose of this article is to provide a summary of recent research and current status of efforts to revisit trace mineral requirements. Most of the previous literature has been well summarized by the National Research Council (NRC, 2001). Discussion here will center primarily on copper, manganese, and zinc, and in particular will consider how more bioavailable sources may impact calf growth and health.

GENERAL ASPECTS OF TRACE MINERALS FOR DAIRY CALVES

Copper

Copper serves many functions in calves, and inadequate intakes can impact growth and health. Copper is a constituent of many enzymes, such as cytochrome c oxidase and lysyl oxidase. With inadequate copper, lower activity of cytochrome c oxidase can impair respiratory burst function in neutrophils and so decrease innate immune protection. Ceruloplasmin participates in copper transport and incorporation of iron in the storage protein ferritin. Copper also participates in hemoglobin synthesis; copper deficiency therefore can lead to anemia. Lysyl oxidase is a key enzyme for collagen crosslinking, which if decreased can lead to reduced integrity and elasticity of connective tissue. Inadequate copper can lead to improper growth, interfere with wound healing, and weaken blood vessels.

Another critical function of copper is as a component of copper-zinc superoxide dismutase, which exerts antioxidant activity in the cytosol of cells. Ceruloplasmin also has antioxidant activity, so copper deficiency leads to greater damage to cells of the immune system. Dairy calves growing more rapidly might be more sensitive to copper adequacy because with greater metabolic activity, more reactive

oxygen species are released. Immune protection also might be compromised. Evidence indicates that the requirement of copper to maintain normal immune function is greater than that to prevent classical deficiency signs (NRC, 2001).

The copper content of growing tissue is relatively constant, with the exception of liver which is the primary site of storage of excess copper. Daily requirements for copper therefore increase with greater body mass but less with growth rate. The requirement is predicted by NRC (2001) to be met when 10 mg/kg of dietary DM is provided in both milk replacer and starter or grower. The absorption coefficient for copper in milk-fed calves before weaning was set at 60% (NRC, 2001). As rumen development proceeds, however, the absorption efficiency for copper decreases dramatically; absorption of dietary copper is only 1 to 5% in functioning ruminants. This reduction occurs because of the three-way interactions among copper, sulfur, and molybdenum in the rumen. Sulfur can be converted to sulfide, which can combine with molybdenum to form tetrathiomolybdate that in turn forms an insoluble complex with copper, thereby decreasing its absorption. Although the requirement for copper relative to other components of intake decreases with age, the similar recommended concentrations in the diet between milk replacer and early dry feeds reflects these widely different absorption coefficients.

Bioavailable sources of copper might help maintain adequate copper for the young rapidly growing calf. High iron interferes with copper absorption, especially in the presence of high sulfur. In areas that have high iron content and/or high sulfur content in water, bioavailable forms may improve copper supply to the animals. Protection of copper from the effects of thiomolybdate in the rumen is another purported function of bioavailable copper sources, although direct evidence for this effect has not been published.

Manganese

Manganese is important for growth and skeletal formation in calves, and also can impact immune function. Manganese functions both as an activator ion for a variety of enzyme classes that catalyze hydrolase, kinase, decarboxylase, and transferase reactions, and as an integral component of the metalloenzymes pyruvate carboxylase and manganese superoxide dismutase. Pyruvate carboxylase is an important regulatory enzyme for lipid and carbohydrate metabolism, and its low activity may result in lower fat accumulation in young animals if manganese is deficient. Manganese superoxide dismutase is found primarily in the mitochondria. Inadequate activity due to shortage of manganese can result in more oxidative damage to highly active cells.

A major function of manganese relative to growth is its involvement in function of galactotransferases and glycosyltransferases. These enzymes are needed for formation of the mucopolysaccharides and glycoproteins that make up the organic ground substances in cartilage and bone formation.

The absorption of manganese is very low, <1% of dietary intake. Manganese homeostasis is regulated via its excretion by the liver into bile. Quantitative data for absorption coefficients are limiting in calves. The manganese content of calf carcasses is about 2.5 mg/kg of

carcass DM (Suttle, 1979) and so daily requirements increase in proportion to the growth rate. The NRC (2001) stated that manganese requirements should be met with milk replacer or starter/grower contents of 40 mg/kg diet.

Like copper, high concentrations of iron may decrease manganese availability. In addition, high concentrations of dietary calcium, potassium, or phosphorus can decrease absorption of manganese. For these reasons, again, bioavailable sources of manganese might be beneficial to young calves that are growing rapidly.

Zinc

Zinc is the trace mineral with the most ubiquitous distribution of functions in the calf, just as in other mammals. Cousins et al. (2006) estimated that at least one zinc-requiring protein is needed for almost every signaling and metabolic pathway in all mammalian species.

Zinc is a component of numerous metalloenzymes including copper-zinc superoxide dismutase, carbonic anhydrase, carboxypeptidases, alkaline phosphatase, and RNA polymerase, giving zinc an important role in metabolism of carbohydrates, lipids, and proteins. Hundreds of enzymes contain "zinc finger" binding motifs that are involved in such processes as gene transcription, translation, mRNA trafficking, cytoskeletal organization, epithelial development, cell adhesion, protein folding, and chromatin remodeling (Laity et al., 2001). In addition, zinc is a component of thymosin, which regulates cell-mediated immunity. It is easy to see that inadequate zinc supply could have wide-reaching negative impacts on early calf growth and health.

The role of zinc in antioxidant and immune functions may be particularly important for young calves. The importance of copper-zinc superoxide dismutase was discussed earlier. The high rates of cellular turnover in the immune system require zinc for the proliferation, differentiation, and apoptosis of these cells (Haase et al., 2006). Through thymosin and other mechanisms, the thymus and T-cell function are impacted by even small changes in zinc availability (McDowell, 2003). The ability to respond appropriately to a pathogen may be impacted by zinc insufficiency.

Milk-fed calves absorb about 50% of dietary zinc, but efficiency of zinc absorption decreases to about 30% in ruminating calves. In the milk-fed preruminant, sources of phytate such as soybean proteins can cut absorption by more than half. Other dietary components that may decrease zinc absorption include iron, copper, and calcium. The NRC (2001) recommended dietary concentrations of zinc in both milk replacer and starter or grower feeds of 40 mg/kg as adequate to meet the requirements. Given the importance of zinc and the potential for diet or water-derived antagonistic substances, the potential of bioavailable sources of zinc to improve utilization in calves at high growth rates is a justifiable hypothesis.

EFFECT OF BIOAVAILABLE TRACE MINERALS ON CALF GROWTH AND HEALTH

Relatively little recent research has investigated trace mineral adequacy as affected by differing growth rates in calves. Nonnecke et al. (2010) fed calves a 30% protein, 20% fat milk replacer in differing

amounts to obtain no growth (actual mean = 0.11 kg/d), low growth (mean = 0.58 kg/d), or high growth (mean = 1.16 kg/d). Zinc concentrations in serum did not differ significantly by growth rate. However, serum copper concentrations were higher for the high growth group than the other two groups. The contrasting results probably reflects the fact that zinc is deposited at a relatively constant amount in body tissue so that its use is greater with greater growth, whereas copper is primarily accumulated in liver and is less responsive to growth so that higher intakes of milk replacer led to a greater surplus of copper.

Our research group has conducted two experiments with bioavailable trace minerals in young calves. Osorio et al. (2012) compared calves growing at a "conventional" rate with those growing at an "intensified" rate. Male calves were purchased during the first week of life and transported to the research facility. Calves were fed either the low plane of nutrition (568 g/d of a 22/20 milk replacer) or high plane of nutrition (810 g/d during wk 1 and 1,136 g of powder/d during wk 2 through 6; milk replacer contained 28% protein and 20% fat). Each of the milk replacers was supplemented either with sulfate forms or organic forms (Zinpro Performance Minerals, Eden Prairie, MN) of zinc, manganese, copper, and iron. The starter and grower concentrates fed with each of the high or low planes of nutrition contained either sulfate forms or organic forms (Zinpro) of zinc, manganese, copper, and cobalt. Calves were weaned at the end of wk 6 (low plane) or wk 7 (high plane), and remained on experiment through 20 wk of age.

Average daily gains and gain:feed were higher for the high plane of nutrition through weaning as expected, and differences were maintained through 20 wk. The group fed the high plane of nutrition plus organic trace minerals had the greatest growth in withers height, hip height, and body length, and the greatest overall gain:feed of all treatments. That group also had the lowest mortality and electrolyte use. There were few differences between the organic and sulfate trace minerals when fed with the low plane of nutrition.

In a more recent experiment (LaPierre et al., 2015), we compared sulfate forms with hydroxy forms (Intellibond, Micronutrients) of copper, manganese, and zinc. Each of the forms was fed in milk replacer only, in starter only, or in both milk replacer and starter. Male calves were fed milk replacer (28% protein, 20% fat) at a rate of 500 g/d of powder for the first 2 d after arrival, then fed at 700 g/d of powder for the remainder of wk 1, and 900 g/d of powder between wk 2 and 6. Feeding was reduced to once daily at a rate of 450 g/d of powder during wk 7, and calves were weaned at d 49. The same starter was fed to all calves and contained 25.9% protein (DM basis) with either sulfate or hydroxy forms of the trace minerals.

Calves fed hydroxy trace minerals in milk replacer had greater ($P < 0.05$) starter intakes (1.20 vs 1.08 kg/d) than those fed sulfate minerals in milk replacer. Mineral form in starter did not affect starter intake. This result was contrary to our hypothesis that the sulfates would be less palatable than the hydroxy forms (based on preliminary research and field experience by others). Final body weights and average daily gain did not differ significantly but followed the same pattern as intakes. Mean withers height tended ($P = 0.06$) to be greater for milk replacer containing hydroxy minerals than

sulfate minerals (87.2 vs. 86.4 cm), but mean withers height was lower ($P < 0.05$) when starter contained hydroxy minerals than when it contained sulfate forms (86.3 vs. 87.3 cm), without interaction.

Including the hydroxy trace minerals in milk replacer had interesting effects on health. Calves fed the hydroxy minerals in milk replacer had a significant reduction in risk of being medicated (odds ratio = 0.53) compared with calves fed sulfate forms in milk replacer, and a significant reduction in likelihood of incurring scours during wk 1 to 3 (odds ratio = 0.56). In addition, calves fed the hydroxy milk replacer plus sulfate starter had a reduction in number of days medicated (0.44 d) compared with the other three treatments (average 1.23 d). Similarly, the same treatment group (hydroxy milk replacer plus sulfate starter) had fewer days scouring (1.0) than the other three treatments (2.1 d). Of interest is that the type of mineral in starter had little impact on the health measures, perhaps because most of the health events were associated with scouring during the milk feeding period.

SUMMARY AND CONCLUSIONS

Most of what we know about trace mineral nutrition in dairy calves was obtained many years ago and at lower milk feeding rates than many farms are employing today. There is a basis behind predictions that rapidly growing calves might benefit from more bioavailable forms (organic or hydroxy) of copper, manganese, and zinc compared with their sulfate forms. From our experiments to date, it appears that calves may benefit from inclusion of bioavailable forms of trace minerals in milk replacer but perhaps not in starter. Benefits were observed both in increased growth and in reduction of early health problems. Expanding and refining these effects seems to be a worthy area of additional exploration.

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Strategies to improve reproduction in high producing dairy cows: Focus on estrus and activity monitors

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Take home messages

- More information from activity monitors can be useful
  - Intensity of estrus is closely associated with fertility
  - Artificial insemination (AI) and embryo transfer can be affected by expression of estrus and its intensity
- Reproductive programs with strong reliance on estrous detection are highly efficient
  - Combination with timed AI is still necessary
  - Expect more variability among farms
  - Watch closely for differences between estrus vs. timed AI fertility

Next Steps

- Refine estrus-based reproduction programs
  - Voluntary waiting period, types of protocols, selective synchronization
- Improve knowledge related with estrous detection, behaviour and ovulation timing
  - Standing, lying and rumination data
  - Different sensors, analyses of multiple sensors
- Genetic selection
  - Collection of correct phenotype for genomics
  - Individual variation and association with body condition, parity and milk production

Introduction on estrous behaviour

Estrus represents the period of sexual receptivity, which is followed by ovulation. Estrous expression is characterized by a wide variety of behaviours, some of which are more specific than others. Because ovulation in cattle occurs around 30 h after onset of signs of estrus (Roelofs et al., 2005), ability to recognize behaviours and detect estrus is critical for determination of AI time. Estrous detection is an essential component of reproductive programs, but concerns about low detection rates are not recent (e.g. Helmer and Britt, 1985).

Major behaviour

Estradiol is the hormone that induces estrus behaviour: In cattle, estradiol 17-β is the hormone responsible for induction of estrus behaviour and ovulation. Studies with ovariectomized and intact cows showed that injection of various dosages of estradiol induced expression of behaviour at similar levels (Allrich, 1994). This led to a conclusion that the effect of estradiol on estrus behaviour occurs in an "all or none" fashion: estrus behaviour is induced once estradiol reaches a set concentration (threshold) in the systemic circulation. Increasing estradiol concentration above this threshold, which was not quantified, would not improve expression of estrus behaviours (Allrich, 1994).

The primary sign of estrus in cows is to stand still when mounted by another female (Roelofs et al., 2010), also described as an immobilization reflex (Albright and Arave, 1997). Research from the 70's and 80's performed with continuous visual observation reported a greater frequency of standing to be mounted than that observed nowadays, which also lasted longer (Figure 1 a, b, c). Although it seems evident that display of the standing to be mounted behaviour has declined, this behaviour should not be considered the sole method to evaluate the intensity of estrus. Estrus behaviour varies with number of animals in estrus, flooring surfaces and group sizes (Albright and Arave, 1997). Mounting activity for instance is significantly greater in outdoor or dirt-floored spaces (Britt et al., 1986), with around 80% of the mounting activity observed at the dry lot pen (Pennington et al., 1985).

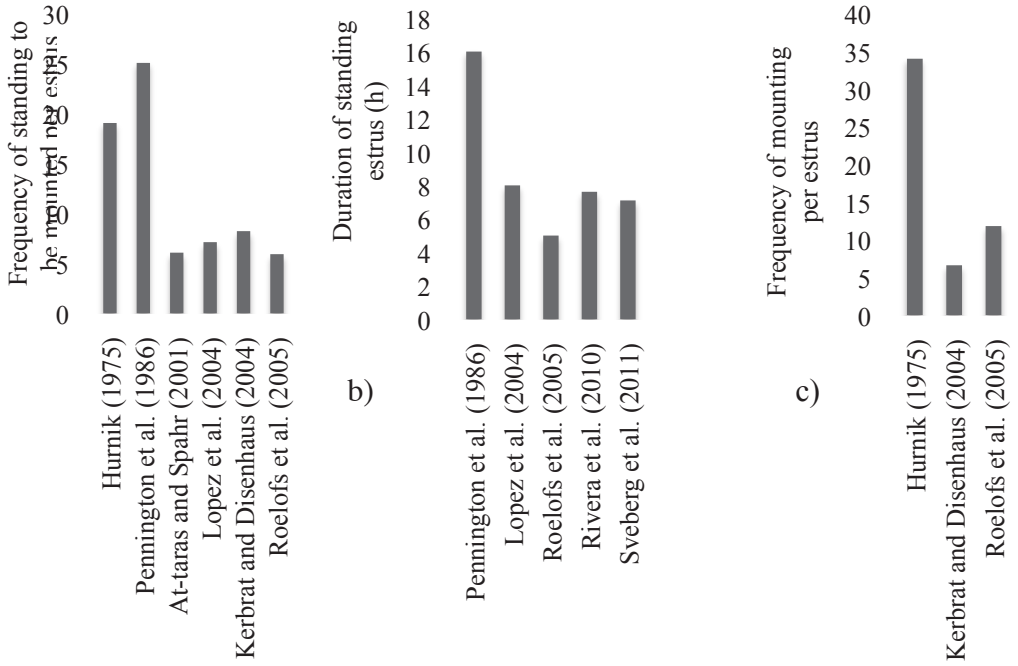

Figure 1. a) Frequency of standing to be mounted per estrus, b) duration of standing estrus (h), and c) frequency of mounting per estrus according to reports from 1975 to 2011

Mounting activity during the past 30 years reveals changes in behavioural manifestations of estrus, most likely due to resources we provide or restrain from the cows. It is clear that modern dairy cows in the standard dairy farming setup do not stand to be mounted frequently enough to for estrus to be appropriately detected by visual observation. Alternatives should be considered: 1) provide cows with an environment that stimulates mounting activity, 2) improve/ and develop methods for detection of mounts, 3) research detection methods for alternative behaviours. The last two alternatives have been addressed more extensively in the past 10 years, respectively with use of electronic mount detectors and automated activity monitors (AAM).

Timing of physiological events related to estrus

The intervals between the events occurring from luteolysis and ovulation are timely regulated (Figure 2). Variation in these intervals could be one of the causes of low conception rates (Saumande and Humblot, 2005). Assuming an interval of 28 h from estrus onset to ovulation and 25 h from LH surge to ovulation (Bloch et al., 2006), estradiol

peak, GnRH surge, and LH surge occur in a time frame of only 3 h. By the time behavioural estrus ends, ovulation is the only event left to complete the cycle. Onset of high activity (measured by an AAM) occurred 29 h before ovulation (Valenza et al., 2012). A farm using AAM would breed cows around 7 to 12 h after high activity onset (Neves and LeBlanc, 2015), leaving an interval of around 10 h from AI to ovulation. The large variation reported for ovulation timing relative to estrus onset could be a cause of low conception rates (Valenza et al., 2012). Large variation in duration of estrus and timing of peak activity within this period could also contribute to variability in ovulation timing. Using a different sensor, Stevenson et al. (2014) reported similar estrus onset to ovulation interval (26 h), but with lower variation. Interestingly, while Valenza et al. (2012) evaluated estrus after hormonal treatments, Stevenson et al. (2014) studied spontaneous estrus. Estrus synchronization could be expected to reduce variability of timing between endocrine and physiological events. Stevenson et al. (2014) also reported similar timing from estrus onset to ovulation when onset of estrus was determined by onset of high activity or first stand to be mounted (24.6 ± 0.7 h vs. 26.4 ± 0.7 h, respectively).

The LH surge stops aromatase activity and terminates the synthesis of estradiol by the follicle (Forde et al., 2011). Accordingly, a reduction of estradiol concentrations to 50% of peak concentration by 5 h post-LH surge has been reported (Chenault et al., 1975). By 14 h post-LH surge, estradiol is already at basal levels (2 pg/mL; Chenault et al., 1975), and below a set threshold of 2 SD above baseline (Aungier et al., 2015). Relatively to behavioural estrus, it has been observed that estradiol concentration peaks at time of maximal behavioural expression (Van Eerdenburg et al., 1996), but then reduces to 60% of peak values by 6 h after maximal behavioural expression (Lyimo et al., 2000). It can be extrapolated that the last hours of behavioural estrus occur under lowering concentrations of circulating estradiol. However, it is unclear why there is no linear correlation between intensity of estrus and estradiol concentrations (Aungier et al., 2015; Madureira et al., 2013; Silper et al., 2015c).

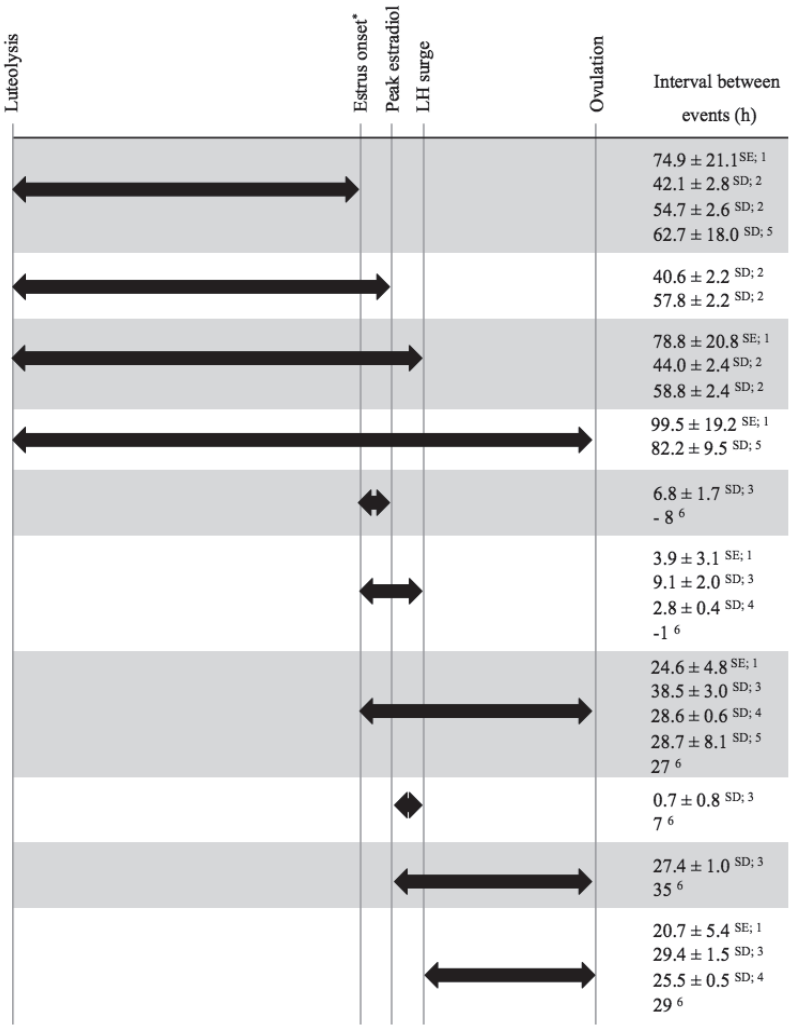

Figure 2. Time interval (h ± SE or SD) between events occurring from luteolysis to ovulation in dairy heifers and lactating cows. *Estrus onset was determined by [visual observation of standing estrus ([1]Chenault et al., 1975; [4]Bloch et al., 2006; [3]Saumande and Humblot, 2005)], [electronic mount detectors ([2]Stevenson et al., 1998)], or [increased physical activity ([5]Valenza et al., 2012; [6]Aungier et al., 2015)]. [2]Stevenson et al., 1998: early luteolysis (d 6 to 9); late luteolysis (d 14 to 15).

Estradiol and progesterone role

The rise in circulating estradiol concentration up-regulates progesterone receptor expression in the endometrium by stabilizing estradiol receptor α (ERα) mRNA (Ing and Ott, 1999). The constant release of progesterone for a relatively long period (10-14 d) during diestrus induces a decrease the gene expression of both the receptors (Spencer and Bazer, 1995). Overall, the hormone

and receptor dynamics from both progesterone and estradiol are essential for an ideal endometrium environment and a successful pregnancy.

The circulating concentration of estradiol is not linearly related to estrous behaviour and it is likely that other factors contribute to variation in estrous behaviour. Even though induction of estrous behaviours by injection of estradiol at different dosages resulted in similar behavioural expression of estrus (Allrich, 1994), it cannot be assumed that the amount of estradiol acting in the hypothalamus was the same among cows receiving the same dosage. Individual behavioural variation, metabolic rate, endogenous steroids, neuronal development and expression of estradiol receptors are some of the potential sources of variation in the expression of estrus in addition to circulating concentrations of estradiol. Furthermore, it is possible that the lowest tested dosage of estradiol was already enough to induce a satisfactory degree of behavioural expression.

In the bovine, only distribution of ERα has been studied. ERα are expressed in the same hypothalamic areas already reported for other species, namely the MPA and VMH, during the luteal phase (van Eerdenburg et al., 2000). During estrus and metestrus, however, ERα expression was noted only in the arcuate nucleus. According to the authors, the absence of expression of ERα around estrus and in ovariectomized cows excludes the possibility of receptor self down-regulation and might indicate why estrous expression is reduced in dairy cows. Absence of staining for ERα was also related to the possibility that receptors were occupied by the ligand, but this was not considered a strong hypothesis. Collectively, van Eerdenburg et al. (2000) concluded that estradiol and ERα are key factors in synchronization of physiological and behavioural reproductive events. Control of estrous behaviours is then dependent on expression of ERα in MPA and VMH, as well as concentrations of estradiol high enough to elicit sexual behavioural reflexes.

The role of progesterone in priming the bovine brain for estradiol actions is evidenced by the absence or low expression of estrus with the first postpartum ovulation or the pubertal estrus, events which are not preceded by a luteal phase. Greater expression of estrus with timed AI protocols that include progesterone inserts (CIDR) give evidence for the role of progesterone as a primer for estradiol induction of estrus behaviour (Rhodes et al.,

2002). It has been hypothesized that, at each cycle, estradiol concentrations during estrus would induce a refractory state in the brain that requires luteal phase peak progesterone concentrations to be reverted (Allrich, 1994; Woelders et al., 2014). Estradiol and progesterone have been shown to induce estrous behaviour in ovariectomized non-lactating cows (Vailes et al., 1992). However, the authors noted that intensity of behavioural estrus is more complex than only the estradiol and progesterone relationship. The induction of a profile of high progesterone and estradiol reduces more the standing than the mounting behaviour, compared with cows under a milieu of estradiol only (Vailes et al., 1992). Estradiol likely does not regulate estrus expression alone (Roelofs et al., 2010), but it is at least required as a trigger. Even if higher estradiol concentration does not improve estrous expression, it is likely to improve reproductive tract function (Allrich, 1994).

Proestrus and estrus on reproductive tissues

During estrus, elevated estradiol concentrations increase epithelial cell height and ciliation in the fimbria (Murray, 1996) and ampulla (Murray, 1995). In addition to this, estradiol induces maturation of oviductal secretory organelles and stimulates production and release of granules from the non-ciliated epithelial cells until day 3 after fertilization (Murray, 1995). An estradiol-dependent glycoprotein is secreted from the oviduct of both cows (King and Killian, 1994) and sows (Buhi and Alvarez, 2003) at estrus and during early gestation. Estradiol administration, either in vitro or in vivo, leads to the synthesis and secretion the estradiol-dependent glycoprotein by non-ciliated cells of the ovine ampulla at estrus and on days 1.5, 2, and 3 after fertilization (Murray, 1995; Murray, 1996).

Proestrus

The concentration of estradiol during proestrus is also important to the lifespan of the corpus luteum (CL; Mann and Lamming, 2000; Kieborz-Loos et al., 2003). It was demonstrated that sub-optimal concentrations of estradiol before ovulation can alter the normal cyclic expression of progesterone and ERα (Mann and Lamming, 2000; Robinson et al., 2001), resulting in elevated gene expression of oxytocin receptors and eventually a premature release of prostaglandin F2α (PG). In addition, other functions of preovulatory estradiol are to increase insulin-like growth factor-1 mRNA in the uterus (Robinson et al., 2000) and

oviduct (Pushpakumara et al., 2002) during fertilization and early embryo development.

Ovariectomized ewes have been extensively used as models to study the effect of estradiol on pregnancy establishment. Ewes that did not receive exogenous estradiol, similar to those obtained upon estrus, failed to deliver a normal embryo after 21 days of gestation induced by synchronous embryo transfer (Miller and Moore, 1976). Eliminating estradiol lead to a decrease in the rate of uterine protein synthesis, the ratio of total RNA to total DNA and also uterine weight reduction in comparison with those animals that received adequate amounts of exogenous estradiol (Moore and Miller, 1976).

The deleterious effects on pregnancy rate of a reduced proestrus duration can still be observed when embryo transfer is used instead of AI (Mussard et al., 2003). Atkins et al. (2013) observed an increase in pregnancy maintenance from day 7 to 27 of gestation induced by increased serum estradiol concentration on day 0 and progesterone concentration on day 7 of the recipient cow. In the same studies, it was observed that concentrations of estradiol of the donor cows played a critical role in fertilization rates whereas the estradiol concentration of the embryo recipient cow at the time of ovulation was key to maintain the pregnancy (Atkins et al., 2013). In a retrospective study, the authors categorized the embryo donor and embryo recipient cows each into two groups of low estradiol (< 8.4 pg/mL) or high estradiol (≥ 8.4 pg/mL) based on blood samples collected at induced ovulation (Jinks et al., 2013). Their conclusions showed that ovulation of small dominant follicles induced by GnRH was associated with reduced serum estradiol concentrations, fertilization rate (donor cows), and pregnancy establishment (recipient cows). Furthermore, ECP supplementation during the preovulatory period has had a positive effect on pregnancy rate of cows with smaller dominant follicles. Other indirect effects of manipulating the preovulatory estradiol include the alteration of gene expression of oxytocin receptors and cyclooxygenase-2 in the uterine endometrium on day 5 of the estrus cycle (Bridges et al., 2005). During the development and refining of estradiol-progesterone based protocols, it became clear that anticipating the PG injection by one day (Pereira et al., 2013) or extending the length of the protocol (Pereira et al., 2014) can have positive effects on fertility by

increasing the number of animals in estrus before AI and consequently increasing P/AI and decreasing pregnancy loss.

Estrus

The most recent studies have shown that not only proestrus length or estradiol levels during this estrous phase affects reproductive tissues, but the actual display of estrous behaviour seems to have a profound effect on fertility (Madureira et al., 2015a, Madureira et al., 2015b). Most of the data currently available in dairy cows on the effect of proestrus and estradiol pertains the manipulation of the timing of luteolysis and ovulation induction, therefore modifying the proestrus only. Studies that modified follicular dominance length (Cerri et al., 2009), concentrations of progesterone during diestrus (Cerri et al., 2011), proestrus length and estradiol exposure (Mussard et al., 2003; Bridges et al., 2005) and production parameters (e.g. lactation and age; Sartori et al., 2002) have described these effects on fertilization, embryo quality and uterine environment, as well as reduction in pregnancy losses during the late embryonic development (Ribeiro et al., 2012). However, in spite of marked effects related with the afore mentioned modifications of the estrous cycle, not much emphasis has been placed on the isolated or additive analyses of the effect of expression of estrus (within a variety of different treatments) on reproductive tissues. The effect of estrus on fertility will be discussed in the last chapter of this manuscript, but it is clear that estrus has an important positive impact on fertility. Moreover, this effect also seems to be associated with the intensity of estrus, which collectively leads us to questions regarding the detailed physiological mechanisms associated with this improvement in fertility associated with estrus.

In order to answer some of these questions, we aimed to investigate the association of estrous expression at the time of AI with the expression of critical genes in the endometrium, CL and embryo during pre-implantation period (Davoodi et al., 2016). In addition, the difference in estrous expression was evaluated for reproductive parameters such as CL volume, conceptus size, concentration of P4 in plasma, and follicle diameter. Evidence from this study supports our hypothesis that estrous expression positively influences the expression of target genes important for embryo survivability. Cows that expressed estrous behaviour near AI had a significant improvement in the profile of endometrium gene expression critical for

suppressing the local maternal immune system and likely improving adhesion between endometrium epithelial cells and conceptus, as well as partly inhibiting the mRNA machinery for PG synthesis (Figure 3). Genes related to immune system and adhesion group in the endometrium were also significantly affected by concentration of progesterone in plasma on day 7. Results from the gene analysis of the CL (Figure 4) also confirmed down-regulation of cellular pathways associated with apoptosis and PG synthesis which favours CL maintenance and secretion of progesterone, both key to sustain pregnancy (Davoodi et al., 2016). Moreover, cows that displayed estrus yielded longer conceptuses, which can be associated with better chances of survival. The effects of expression of estrus seems to interact with progesterone concentration on d 7 of the estrous cycle in a way that positively influences endometrium receptivity and embryo development. This study could not verify length of dominance or progesterone levels during the growth of the preovulatory follicle. The specific causes that lead to the presence or absence of estrous expression are unknown based on the data collected in this study (Davoodi et al., 2016) and warrant further investigations. The expression of estrus can indicate the state of sensitivity of the hypothalamus to estradiol and perhaps the best timing for the optimal function of all other reproductive tissues related with the survivability of the early embryo.

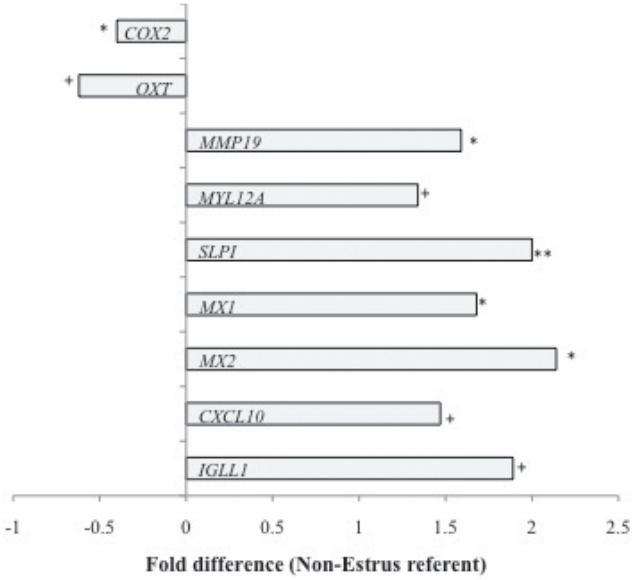

Figure 3. Effect of estrous expression on endometrium gene expression. Significant fold difference based on Non-Estrus expression as referent has been shown for genes with significant pattern of expression in endometrium tissue.

For this graph, the asterisks (*), (**), (***) and (+) refer to $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ and $P \leq 0.10$, respectively.

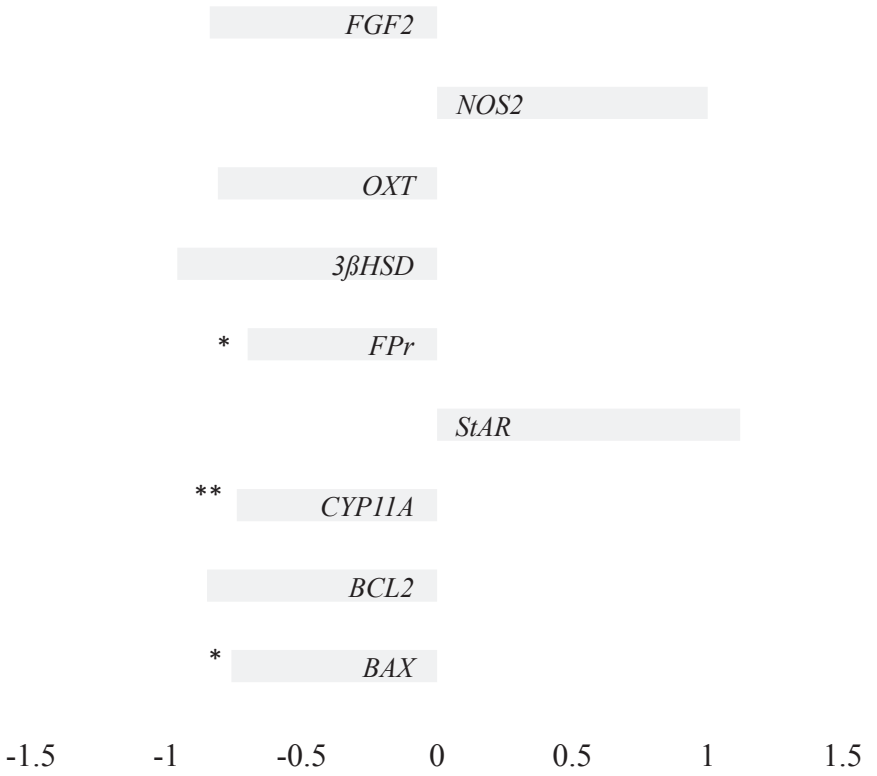

Figure 4. Effect of estrous expression on corpus luteum genes involved in steroidogenesis, angiogenesis and apoptosis. Significant fold difference based on Non-Estrus expression referent has been shown for genes with significant pattern of expression in corpus luteum tissue. For this graph, the asterisks (*) and (**) refer to $P \leq 0.05$ and $P \leq 0.01$, respectively.

Production parameters and expression of estrus

The detection of estrus in confined dairy cows became a greater challenge as milk production increased. Previous studies that took into account only mounting behaviours as a measure of intensity and duration of estrus have consistently recorded a decrease in this behaviour as milk production increased (Lopez et al., 2004; Rivera et al., 2010). A major question still unanswered is that if mounting behaviour can be used as a gold standard for estrous expression (i.e. intensity and duration),

considering the challenges faced by dairy cows in free-stall barns and concrete flooring for an activity that leads to significant physical stress on foot and legs. The estrous detection rate in a recent survey (Denis-Robichaud et al., 2015) has been reported to be below 50%, but the proportion of cows truly bred upon estrous detection is still unclear as this data is confounded by timed AI use. This extensive failure to submit cows for artificial insemination (AI) has a major impact in the pregnancy rate of Canadian herds, but also indicates a unique window of opportunity to improve fertility.

Parity, milk production and body condition

A large field study (Lopez-Gatius et al., 2005) described that the two main factors affecting activity increase were lactation number and milk production, whereas the degree of activity increase was positively correlated with fertility after AI. The later was not clearly stated by the author, but was later corroborated by recent studies (Madureira et al., 2015a). Milk production, for example, seems to affect the overall sensitivity of pedometers or activity monitors to detect true events of estrous behaviours (Holman et al., 2011). However, none of the studies above measured more detailed reproductive physiological events associated with natural estrous behaviours and the level of activity of AAM systems associated with those events. In addition, just recently more robust studies using adequate number of observations of estrus and cows have been published for more reliable conclusions.

A recent study by our group identified several risk factors associated with the intensity of estrous expression. In our study, multiparous cows expressed lower peak activity and duration of episodes of estrus than primiparous (Madureira et al., 2015a). López-Gatius et al. (2005) found that for each additional parity number, walking activity at estrus was reduced by 21.4%. On the contrary, Walker et al. (1996) described that duration of estrus was nearly 50% shorter for primiparous than for multiparous lactating dairy cows. Our study does not support findings from recent studies that reported no association between parity and physical activity at estrus (Arney et al., 1994; Løvendahl and Chagunda 2010; VeerKamp et al., 2000). Methodological differences may explain variation among different studies on the association between parity and physical activity, such as frequency of data transmission from sensors to software, or different

breeds of cows. Moreover, the detailed information about different AAM systems reading correlations will be key to properly use automated behaviour data with physiological parameters. In a simple analysis by our group comparing a neck vs. a leg-mounted AAM, correlation between the peak intensity of estrus episodes of both systems was acceptable, but not at a level that justifies a seamless translation of the data from one system to the other (Madureira et al., 2015; Silper et al., 2015c). Different AAM systems will capture different movements and different algorithms and software filter the background data in specific manners, therefore, influencing measurements of baseline levels and relative increases in activity during estrus.

Greater milk production has been negatively correlated with standing to be mounted at estrus (Lopez et al., 2004; Rivera et al., 2010). The decrease in concentrations of estradiol, possibly caused by increased hepatic blood flow and steroid clearance (Sangsritavong et al., 2002; Vasconcelos et al., 2003), is a possible cause for decreased estrus-related behaviour, most notably the standing to be mounted behaviour. Madureira et al. (2015a) also found greater peak intensity and duration only for animals in the lowest quartile of milk production, but not among the other milk production categories. We could assume that the data partially agree with previous research (Lopez et al., 2004; Rivera et al., 2010), however, it seems that mounting activity is more affected than overall physical activity measured by AAM systems. Recent studies from our group (Silper et al., 2015a; Madureira et al., 2015a) found that heifers and cows with lower baseline levels of activity tend to have greater relative activity increase, but not necessarily greater absolute increases in step counts during estrus. In spite of the results discussed above, peak intensity during estrus was still weakly associated with milk production, emphasizing the influence of factors such as BCS and parity, and probably other factors such as group size, health status, and lameness (Van Vliet and Van Eerdenburg, 1996; López-Gatius et al., 2005; Morris et al., 2009).

Some have found negative effects of milk production on conception rates (López-Gatius et al., 2005; Valenza et al., 2012), whereas others did not (López-Gatius et al., 2006; Madureira et al., 2015a). The ability of individual cows to cope with high milk yield and current management practices are important in determining if a negative effect

of lactation on overall fertility is more or less likely to occur. It is difficult to establish this relationship because cows with low milk production might be sick from diseases that will also affect the reproductive tract, while high producing cows are often times the healthiest ones (Santos et al., 2009). As described by Bello et al. (2012), milk production and reproduction are not linked by a "universal homogeneous relationship".

Body condition score was the major factor associated with physical activity at estrus and P/AI (Madureira et al., 2015a). This study supported conclusions by Løvendahl and Chagunda (2010), who observed that in the first 5 months after calving, low, early postpartum BCS had a negative correlation with estrous activity. Further support is provided by Aungier et al. (2012), who reported that a 0.25 increase in BCS was significantly correlated with an increase in physical activity prior to ovulation. Cows that lost less than 100 kg of BW from 2 weeks pre-calving to 5 weeks post-calving had greater intensity of estrus in the first two estrus episodes post-partum (Burnett et al., 2015). The specific mechanism by which a temporary state of negative energy balance reduces estrogen-dependent estrus behaviour is unclear.

Ovarian follicle dynamics

Ovulation of larger follicles by lactating cows could be a result of extended follicular dominance or prolonged proestrus, which originate from lower progesterone concentrations, lower estradiol concentrations, and longer time interval for induction of GnRH and LH surges. Follicle diameter and estradiol concentration in plasma have been reported to be negatively correlated in cows (Saumande and Humblot, 2005), or to not correlate at all in heifers and cows (Aungier et al., 2015; Madureira et al., 2015a; Silper et al., 2015c). Larger follicles are more likely to delay or fail to ovulate, and oocytes are less likely to be fertilized. Greater incidence of reproductive abnormalities (e.g. ovulation failure, multiple ovulations, ovarian cysts) in lactating cows might originate from lower circulating estradiol in the preovulatory period (Sartori et al., 2004).

The correlation between the preovulatory follicle diameter and plasma estradiol is weak (Silper et al., 2015c; $r = 0.17$) and is in agreement with values reported elsewhere (Glencross et al., 1981; Cook et al., 1986; Sartori et al., 2004; Walker et al., 2008). Although reports have found that a larger follicle is associated

with greater concentration of estradiol in plasma (Cerri et al., 2004), it is clear from the current experiment that parity, BCS and ultimately milk production are the factors with the greatest impact on circulating concentrations of estradiol. Cows classified as having high activity had similar preovulatory follicle diameter, but slightly greater concentration of estradiol in plasma than cows classified as low activity (Madureira et al., 2015a). In spite of the differences in estradiol concentrations found when cows were divided in categories by estrous activity, the peak intensity measured by different AAM systems was only weakly correlated with concentration of estradiol in plasma, demonstrating a greater than expected variation. A recent study by Aungier et al. (2015) observed no correlation between activity clusters measured by AAM and FSH, LH and estradiol profiles. However, a greater peak concentration of estradiol in plasma was in fact associated with standing and estrus-related behaviours.

The ovulation of preovulatory follicles with similar diameter would suggest little change in concentrations of progesterone after AI. Data from Madureira et al. (2015) suggests that concentrations of progesterone 10 d after AI was greater in cows displaying high intensity estrus at AI. The faster increase in progesterone early in the cycle could result in increased early embryonic development (Mann and Lamming, 2001; Bisinotto et al., 2010) possibly due to changes in the endometrium receptor profile (Lonergan, 2011). This could represent, therefore, a possible cause for the increased P/AI found in animals with greater peak activity at estrus.

Detection of estrus and relative intensity: Consequences to fertility

Some estrous detection methods are visual observation, tail chalk, pressure patches, pedometers and sensors (Caraviello et al., 2006). Visual observation of estrus has high labour demands and, normally, low efficiency (At-Taras and Spahr, 2001). Timed AI following hormonal manipulation of the estrous cycle has been used as an alternative for achievement of reproductive goals with reduced necessity of estrous detection (Pursley et al., 1995). This implies better overall pregnancy rates because of increased rate of submission to AI. No major improvement in conception rates have been observed with timed AI (Santos et al., 2009), although more recent ovulation synchronization protocols that includes an intensive pre-synchronization (Double-ovsynch; Souza et al., 2008) and double injections of PG

before AI to ensure complete luteolysis (Wiltbank et al., 2015) may yield around 50% conception rate at the first post-partum AI.

There are plenty of systems available for dairy farmers, but further exploration of the AAM is necessary. Some of these systems have resources such as adaptable thresholds per farm or groups of cows, but these do not seem to be explored or extensively used. For example, adjustments could be made according to season of the year or level of milk production. These examples of possible adjustments also illustrate a challenge to the allied dairy industry related with sensors in general. There is a learning curve on how to use these systems. Even the simplest of AAM will probably require some time and patience from herd personnel in order to learn and extract the most from sensors and respective software.

Detection of estrus and activity monitors

Automated systems currently can be different (e.g. step counts, acceleration of movement, rumination time/frequency, lying time/bouts) regarding their output or variable to be analyzed. Some examples are ALPRO (DeLaval; Sweden), SmartDairy Activity (Boumatic, USA), AfiTag (Afimilk, Israel), CowAlert (IceRobotics, UK) and HR Tag (SCR Engineers, Israel). These AAM proved to be efficient at detecting estrus. Using Heatime, Valenza et al. (2012) detected 71% of the preovulatory phases, but missed 13% of the recorded ovulations. Similarly, with the same sensors, Aungier et al. (2012) reported 72% of the preovulatory follicular phases identified correctly, but 32% of false-positives. It is possible that some of these false positives did not occur because the cut point used to determine high progesterone status (false-positive estrus) was extremely low (progesterone > 0.6 ng/mL). It is agreed that progesterone in milk of 3 ng/mL or higher indicates presence of an active CL. Moreover, a study from Denmark (Løvendahl and Chagunda, 2010) using activity tags also showed a 74.6% detection rate and 1.3% daily error rate when using the most efficient algorithm calculated by the authors. The study demonstrates the great potential of this technology to solve the estrous detection problem in commercial dairy herds.

Rumination is another parameter that can be used for automated detection of estrus. Changes in feeding behaviour, which are in accordance with increased physical activity and restlessness characteristics of estrus, result in decreased rumination time during estrus. Pahl et al.

(2015) demonstrated reduction of feeding time and rumination time at d -1 and d 0 relative to AI. Reduction of time spent at each visit to the feed bunk could be another indicator of restlessness.

There has been little research on the use of lying and standing behaviour for estrus detection. Rutten et al. (2013) reviewed 48 papers but only two reported lying and standing information (de Mol et al., 2009; Brehme et al., 2008). Recently, our group has analyzed lying and standing information in relation to the estrous period in more detail (Silper et al., 2015b; Silper et al., 2015d). Results from these studies indicates a large potential to improve the accuracy of the detection of estrus, as well as the use of quantitative information (e.g. proportional changes on lying behaviours on the day of estrus in relation to the day before and after) from these monitors to assist farm-level decision-making regarding breeding. To our knowledge there was only one not so recent paper (Brehme et al., 2008) describing the absence of lying time over long periods (16 h) during estrus. However, this paper does not provide detailed information about measurements or factors that affect lying time. One AAM system (AfiTag, Afimilk) uses steps, lying time and an index of restlessness in its estrous detection algorithm, but literature regarding its efficiency and measurements of estrous expression is still unclear. Given the variability reported by many and the low levels of estrus expression in general, it seems that combining measurements within one system is potentially a better alternative for reduction of false negatives. A combination of activity and lying behaviour data from IceTags (IceRobotics) significantly reduced error rate (false alerts) and increased probability of estrus detection (Jónsson et al., 2011). Peralta et al. (2005) also suggest combinations of systems are the best alternative to enhance detection and conception rates during period of heat stress. The use of more than one measurement within the same sensor can also enhance specificity and reduce false positives (Firk et al., 2002).

Expression of estrus and fertility: Reproduction programs

A survey of Canadian dairy herds has also shown that programs based on estrus detected by AAM have similar reproductive performance to timed AI (Neves and LeBlanc, 2015; Denis-Robichaud et al., 2015). A few studies, normally large surveys, have been able to draw a picture of the state of reproductive programs in North America. Caraviello et al. (2006) showed that over half of all dairy

farms in North America used timed AI programs. In Canada, a recent large survey indicated a strong use of timed AI programs, but visual detection remains the management system mostly used by farmers (Denis-Robichaud 2015). This number, however, is highly dependent on region. For example, the area of Quebec, which concentrates a large number of tie-stall farms with small number of cows, tends to use less reproduction programs and other technologies.

In this survey (Denis-Robichaud et al., 2015), we reported the results from 772 survey answers, which represents 6% of the total number of dairy farms in Canada. The average herd size was 84 lactating cows (median = 60; interquartile range = 40-95 cows/herd), and herds were located in all Canadian provinces. Lactating cows were housed in tie-stall (55%) and free-stall barns (45%). Automated activity monitoring systems were used in 28% of the participating herds (4% of the tie-stall, but 59% of the free-stall herds) and were consulted for high activity alerts at least twice daily by almost all (92%) users. Interestingly, 21% of the participants never confirmed heat by visual observation before insemination, while 26% always did. Results from this survey highlight the variability in reproduction management among Canadian dairy herds. Knowledge of producers' attitudes toward different management practices should help optimize the development and implementation of reproduction management tools.

Reproductive programs with intensive use of timed AI protocols are still the gold standard regarding improvements in pregnancy rates. Recent field trials have been comparing different "degrees" of combination of timed AI and AI upon estrous detection using AAM. Conception risk (30% vs. 31%) and days to pregnancy (137 and 122 d to pregnancy) were not different among cows bred by timed AI or following estrus detection by Heatime (Neves et al., 2012). Other recent studies have experimented with different combinations of use between AAM and timed AI programs (Valenza et al., 2012; Stevenson et al., 2014; Fricke et al., 2014; Burnett et al., 2014) and overall results indicated that it is possible to achieve similar pregnancy rates in more estrous detection-intensive programs. Collectively, these large field trials aimed to modify several factors that are key to the response of the dairy's reproduction program, particularly in the first AI. For instance, the voluntary waiting period varied from 50 to 100 DIM depending of the treatment. The use of pre-synchronization protocols that could either focus on

induced estrus (PG based) or cyclicity and ovulation synchrony (GnRH based) were tested. All the studies demonstrated that the combination of methods (timed AI and AAM) is perhaps the best reproduction program as it maintains high rates of conception while submitting a large number of animals to AI. In this case timed AI protocols are still necessary as a safe guard for a proportion of animals that would not be bred upon estrus up to 100 DIM. The question of when to intervene with timed AI protocols is probably an area that could still gain valuable information from future research. It is very likely that the adoption of AAM systems as part of a large reproduction program will vary largely from farm to farm. Work from Neves et al. (2012) and Burnett et al. (2014) demonstrated a large variation by farm in the adoption of timed AI and AI upon AAM alerts within the same treatment. Another advantage of the combination of the timed AI and AAM is probably the reduction in the use of pharmacological interventions. However, it is yet to be demonstrated how these programs would behave under sites exposed to intense heat stress, as temperature tends to have a major impact on the detection of estrus and intensity.

Expression of estrus and fertility: Display and intensity near AI

In the current study, some major risk factors related with peak intensity and duration of estrus events were assessed. Even though new technologies capture physical activity using sensors and algorithms for data processing that are significantly different than those used in recent past, it was interesting to observe a lack of, or relatively weak correlation between measurements of estrus expression and milk production and preovulatory follicle diameter. In a series of recent studies using different AAM systems, farms, timing of studies and geographical location, it became consistent the observation of substantial increases in P/AI from events of estrus of high peak activity (Madureira et al., 2015a; Madureira et al., 2015b; Burnett et al., 2014) and large decreases in lying time at the day of estrus (Silper et al., 2015d). Improvement in fertility was somewhat expected from cows with greater intensity of estrous expression; however, this was commonly associated with improvements in BCS, lower milk yield, primiparous animals and even health status. In fact, we have observed greater peak intensity and duration as BCS increased as well as in primiparous cows, but greater P/AI still occurred in spite of those and other

risk factors known to affect conception rates. It is possible that information already available in herd management software used on commercial dairy farms could be used to calibrate AAM to take into account present phenotypical conditions of the cow. The use of peak intensity and duration measurements could assist in the prediction of fertility and improve decision-making in reproductive programs using activity monitors. Moreover, there is potential to use AAM systems as an objective and accurate tool to select animals of superior estrous expression, although this topic still warrants further research.

Cows with high peak intensity had approximately 12 to 14 percentage units greater P/AI than cows with low peak intensity, which represents 35% improvement in fertility (Madureira et al., 2015a; Madureira et al., 2015b). Previously, Lopez-Gatius et al. (2005) reported an improvement of 1.001-fold for every unit of relative increase in walking activity.

It was previously mentioned that preovulatory follicle diameter was not different between peak intensity categories, but that does not imply that proestrus or dominance length was similar as there was no control of follicular emergence in recent studies. Therefore, proestrus and dominance length (Bleach et al., 2004; Cerri et al., 2009) cannot be ruled out as possible causes related to the reduced fertility observed. Another possible factor influencing P/AI is the ovulation rate from cows with different peak intensity at estrus. Madureira et al. (2015b) observed a greater failure of ovulation rate of cows that displayed estrus with a relative increase in peak intensity from 80 to 100%, the lowest relative increase possible after crossing the threshold from the AAM used. While this observation is certainly important to explain our observations, it is limited to cows expressing very low peak intensity during estrus as the threshold dividing high and low peak intensity categories was over 300% relative increase in the current study. It is important to note that one of mentioned studies used ECP to induce estrus and ovulation, therefore bringing circulating estradiol to high concentrations. In spite of this, the peak intensity measured by a pedometer system still significantly affected P/AI results (Madureira et al., 2015b).

The display of estrus at AI (Pereira et al., 2014) has been associated with a reduction in pregnancy losses. Furthermore, Pereira et al. (2015) also reported that

animals that display estrus at AI had decreased pregnancy losses regardless of the diameter of the preovulatory follicle. The study by Pereira et al. (2015) was a large field trial and one of the first studies to describe the immense impact of estrous expression on the reduction of pregnancy losses. Moreover, this study showed that this effect is true for both AI and embryo transfer based programs, indicating a possible major modification of the uterine environment as the cause for the improved fertility. This practical result from Pereira et al. (2015) corroborates our data from beef cows that showed an extensive modulation of gene expression of key transcripts related with the immune system and adhesion molecules (Davoodi et al., 2016). Collectively, it seems that the expression of estrus has important positive effects in the maintenance of gestation (decrease in pregnancy losses between 32 and 60 of gestation).

In a study conducted by Bisinotto et al. (2015), the authors aimed to modify concentrations of progesterone during the growth of the preovulatory follicle comparing the first with the second follicular wave. Major results described the key importance and how exogenous progesterone (2 intravaginal devices) is able to "rescue" a preovulatory follicle of the first follicular wave to yield optimal fertility. An interesting finding from this study related to estrus is that animals that ovulated follicles from the first follicular wave growing under low concentrations of progesterone in plasma (worst possible scenario in this study), but that expressed estrus at AI, had P/AI similar to the other treatments.

A potential explanation to correlate intensity of estrus and P/AI, that has not been extensively studied, is that cows could have greater than expected individual variations in the ability to express estrogen receptors in the endometrium and, perhaps more importantly, in the hypothalamus. This would in turn generate cows that are more likely to translate circulating concentrations of estradiol into estrus-related behaviours, and later into a more adequate uterine environment for embryo development.

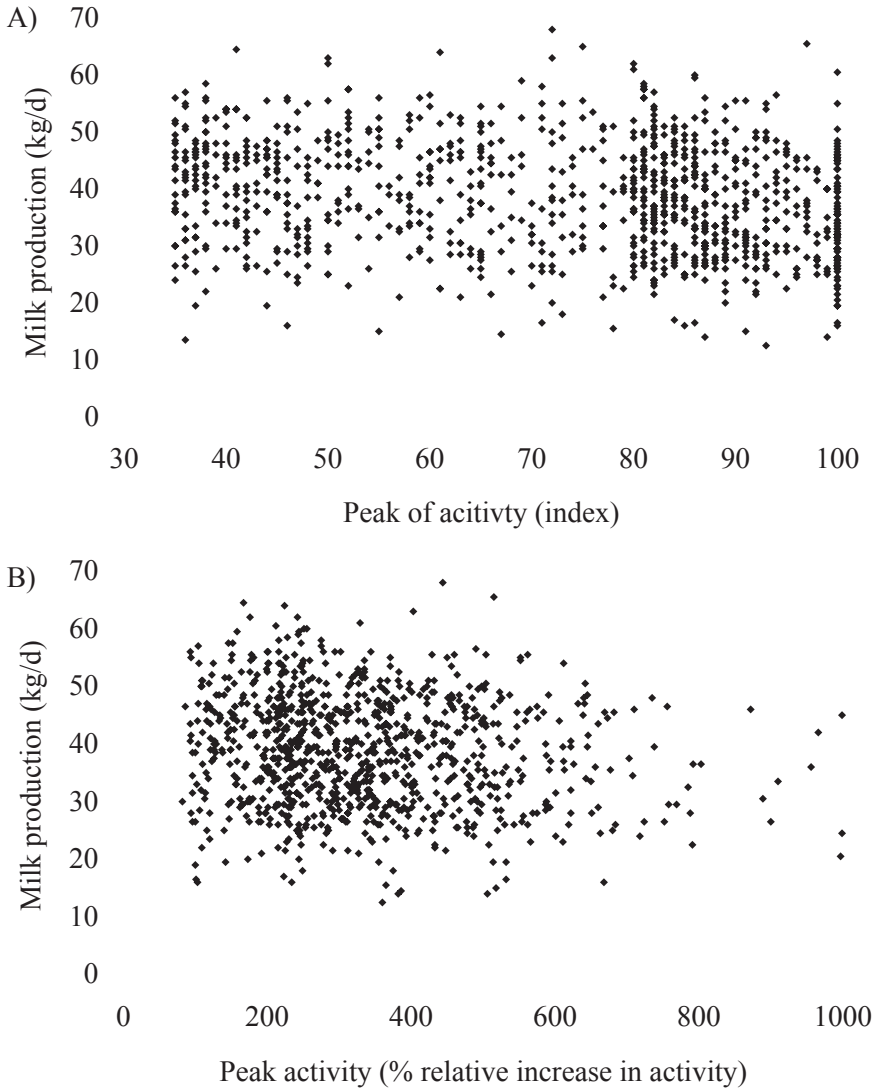

Figure 5. Correlations between milk production at the day of AI and, A) peak of activity (index value) measured by a collar-mounted sensor ($r = 0.20$, $P < 0.01$), and B) percent relative increase in activity measured by a leg-mounted sensor ($r = 0.05$, $P < 0.01$).

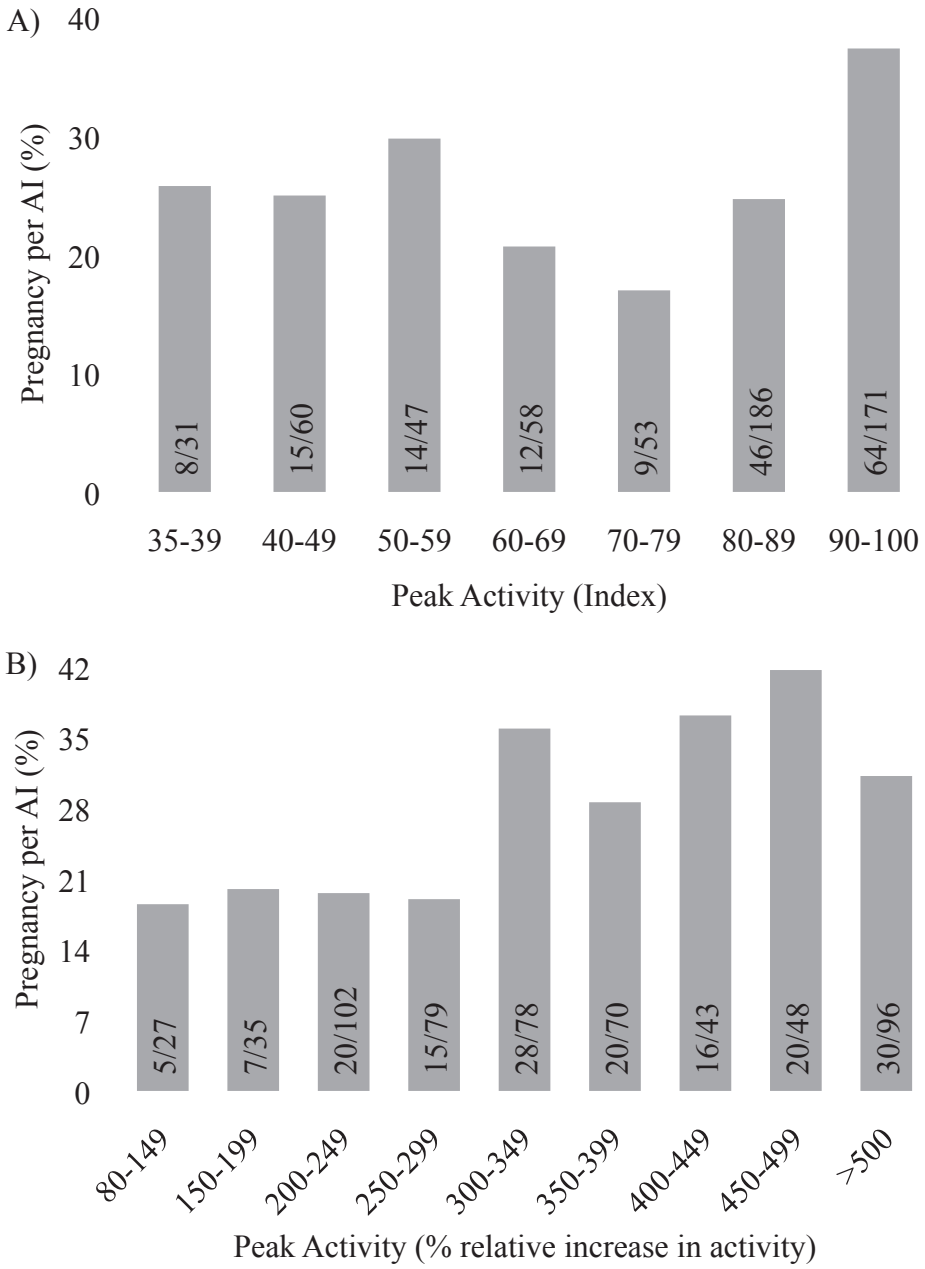

Figure 6. Distribution of pregnancy per AI (%) according to peak activity during estrus detected by A) a collar-mounted sensor and B) a leg-mounted sensor.

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Factors affecting the efficacy of trace mineral supplements.

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Introduction

The nutritional value of copper sources depends on their bioavailabilities and interactions with other feed components. The bioavailability of a mineral source depends on its chemical properties including solubility, valence, and affinity for other food components such as tannins, fiber, phytates and oxalate (Starcher 1969; Ao et al. 2009). It is very difficult to predict the bioavailability of a copper source based on any single property, including solubility. For example, tribasic copper chloride (TBCC), which is a hydroxy trace mineral, has a lower solubility than cupric sulfate pentahydrate (CS5) at neutral pH (Pang and Applegate 2006) but has a higher bioavailability (Miles et al. 1998; Luo et al. 2005; Lu et al. 2010). The solubility of copper compounds in various solvents, in vitro, is not a good index of bioavailability in vivo (Aoyagi 1993). Cupric oxide (CuO) and cuprous chloride (CuCl) are only about 50% as soluble as cupric sulfate pentahydrate (CS5) in dilute acids but CuO is completely unavailable, whereas CuCl has greater bioavailability than CS5 (Baker 1999). The mechanisms underlying these disparities have received very little investigation but may be due to the location within the digestive tract where a mineral source becomes ionized and subjected to interactions with antagonists versus exposure to copper transporters on enterocytes. Perhaps if a copper source is highly soluble, it may be rendered unavailable by antagonists in the crop, stomach, and gizzard prior to entering the small intestine where copper is absorbed in chicks (Starcher 1969).

The bioavailability of many minerals are also affected by the metabolic state of an animal. Situations that increase the quantitative needs for a mineral often result in increased bioavailability. For example, high rates of growth or egg production markedly increase calcium absorption and bioavailability. In a previous study (Koh et al. 1996), the requirement for copper was increased during an acute phase response to inflammation. However, the impact of metabolic state on copper bioavailability has received little attention.

We designed experiments to examine the bioavailability of Tribasic Copper Chloride (TBCC; IntelliBond C, Micronutrients) and CS5 in a purified diet where antagonist levels are low and in a complex diet containing typical antagonists such as tannins, phytates, and fiber. Additionally, we examined bioavailability in chicks challenged with the inflammatory compound LPS, which increases the

use of copper for production of acute phase proteins and antioxidant enzymes. Although liver copper concentrations have historically been used to determine copper sufficiency, the enzyme lysl oxidase is more sensitive to copper deficiency than most other enzymes (Rucker et al. 1999). This enzyme is enriched in tendon so we used both liver and tendon to calculate copper bioavailability. We also Examined the effect of dietary copper source and level on the concentration of copper in the lumen and epithelium of the intestine throughout its length.

Material and Methods

Three experiments using one day old male broiler chicks (Cobb 500 x Cobb 500) were conducted Petersime brooder batteries (Petersime Incubator Co., Gettysburg, OH), which had raised wire floors with pine wood shavings below. Chicks were provided ad libitum access to water and food. All metal (feeders, waterers, raised wire floors) in contact with the birds was stainless steel. Water was deionized and had less than 0.01 ppm Cu. In the first two experiments, broiler chicks were fed the basal semi-purified diet that contained a deficient copper level for 7 days starting on the day of hatch to deplete their copper stores. Then, the 9 experimental diets were each fed to 4 pens of 4 chicks/pen for 10 days. Three chicks, selected at random, were removed from each pen and bled. The remaining chick was injected with lipopolysaccharide (LPS) from *S. typhimurium* (1 mg/kg BW in PBS injected intra-abdominally) in order to initiate an acute phase response. After 16 hrs the LPS-injected chicks were bled and tissues collected.

Bioavailability of copper was determined by the common-intercept multiple linear regression (slope-ratio) method (Aoyagi and Baker 1993; Littell et al. 1995).

The third experiment was designed to determine the chemical state of the copper sources along the intestines and was a 2 X 2 factorial with 2 levels of copper (10 ppm or 150 ppm added copper) and 2 copper sources ($CuSO_4$ pentahydrate and TBCC). Each of the 4 treatments was fed to 6 pens of 3 chicks per pen beginning on day 3. After two weeks, chicks were killed and luminal contents from six regions of their intestinal tract were collected into sterile 15 ml culture tubes and analyzed for total copper content. Intestinal regions examined were: middle of duodenum; middle of jejunum, first third of ileum (ileum-1), middle of ileum (ileum-2), last third of ileum (ileum-3), cecum. For soluble copper, samples were diluted 1:2 (duodenum, jejunum) or 1:4 (ileum, cecum) in deionized double distilled water (ddH_2O) and filtered through a 0.22 µm membrane to remove copper bound to digesta. The retentate

(RET) was further analyzed for extractable copper using the chelator EHPG.

Activity of ceruloplasmin in plasma was assayed using o-Diansidine dihydrochloride. Lysyl oxidase activity was approximated by measuring tendon copper concentrations as described by Rucker et al., (Rucker et al. 1999) using inductively coupled plasma atomic emission spectrometry. Tissue and intestinal luminal copper levels were determined by atomic absorption spectrophotometry

Data from the basal treatment and the copper supplemented treatments were regressed against analyzed copper levels to obtain linear dose response relationships and bioavailability was calculated from the ratio of the slopes obtained from the two copper sources (Littell et al. 1995). The data from each experiment were also analyzed separately as 2-way factorial designs for copper level and copper source after excluding the data from the basal diets fed without supplementation.

Results

Increasing dietary copper level significantly increased rate of weight gain and efficiency of gain for chicks fed the purified diet (Table 1) and the complex diet with copper (Table 2). Regression of copper level against rate of gain (Table 3) resulted in a significantly greater slope for TBCC compared to CuSO4, with a ratio of slopes of 1.15 with the purified diet and 1.17 with the complex diet.

Liver and Tendon Copper Increasing dietary copper level significantly increased liver and tendon copper concentrations in chicks fed the purified diet (Table 4) and tendon copper in chicks fed the complex(Table 5). Regression of analyzed copper level in complex diets against tendon copper concentration resulted in a significantly greater slope for TBCC compared to CuSO4 (Table 7), with a ratio of slopes of 1.18.

Acute Phase Response Both LPS and copper supplementation caused an increase in plasma ceruloplasmin activity in chicks fed a purified diet or the complex diet (Table 4). A significant interaction between LPS and copper source indicates that LPS increased ceruloplasmin to a greater extent in chicks fed TBCC than in chicks fed CS5 (Table 5). Additionally, increasing dietary copper resulted in a much greater increase in ceruloplasmin activity in LPS-injected chicks than in non-injected chicks (Table 6). In LPS injected chicks fed the complex diet, TBCC resulted in a greater slope for this relationship than CuSO4 (slope ratio of 1.26).

Copper concentrations in the intestinal lumen The total copper concentration in the intestinal contents increased posteriorly. This was likely due to the grater digestion and absorption of diet dry matter (e.g. starch, protein) than copper. The dry matter digestibility of a corn-soy diet in poultry is around 75%, whereas copper is absorbed at 30% or less. Thus copper concentrates as the digesta moves along the GI tract. Total copper levels were higher in ileum-1 of TBCC fed chicks than $CuSO_4$ fed chicks at 10 ppm. This may reflect absorption of $CuSO_4$ earlier in the GI tract than TBCC. This was not seen for chicks fed 150 ppm; likely because only a small per cent is absorbed when levels are surfeit. Water extractable copper levels were lower in the duodenum of TBCC fed chicks than $CuSO_4$ fed chicks at 150 ppm. This is likely because TBCC has low water solubility and not because of greater absorption. Interestingly, there were high levels of water extractable copper throughout the intestinal tract of TBCC fed birds. Two factors may explain this observation. First, much of the copper in the GI tract originates from excretion via the bile and this source likely has greater solubility than the TBCC originating from the diet. Second, TBCC solubilized in the low pH environment of the stomach is likely complexed by small molecules (e.g. amino acids, peptides) making this portion water extractable. The strong copper complexing agent EHPG extracted more copper from TBCC fed chicks than those fed $CuSO_4$ across all intestinal segments (P value for Copper Source = 0.01) and this difference was especially great in the upper intestines. It is likely that more copper in $CuSO_4$ fed chicks was absorbed in the upper GI and also removed by water extraction, leaving less copper behind for EHPG extraction. Unremovable copper was calculated and represents copper that is not extractable by EHPG or water. This pool of copper is likely not available for absorption or for microbicidal activity. The chicks fed 150 ppm TBCC had lower levels of unremovable copper compared to $CuSO_4$ fed chicks (P value for Copper Source by Level < 0.001).

Discussion

Broiler chickens responded to increasing copper supplementation with improved gain, efficiency of gain, and ceruloplasmin activity as well as higher concentrations of liver and tendon copper. Tendon copper levels in chicks are a useful proxy for the copper dependent enzyme, lysyl oxidase, which has a relatively low priority for copper and is the major copper pool in tendon (Rucker et al. 1999).

In both experiments, TBCC had a greater bioavailability than CS5 with significant ($P<0.05$) slope ratios between 1.18 for tendon copper, 1.26 for ceruloplasmin in LPS-challenged chicks and 1.31 for efficiency of gain. Many others have found greater weight gain or liver iron accumulation with TBCC compared to CS5 when high levels (> 100 mg/kg) were fed (Miles et al. 1998; Luo et al. 2005; Arias and Koutsos 2006; Lu et al. 2010), but this experiment is the first to demonstrate higher bioavailability at dietary levels below the nutritional requirement. It is interesting that the largest difference in bioavailability occurred during the acute phase response when copper needs are transiently increased for the production of acute phase proteins and the antioxidant enzyme super oxide dismutase (Koh et al. 1996; Song et al. 2011). It may be that the higher bioavailability of TBCC provided more copper for the functioning of the innate immune system (e.g macrophages) that responds to LPS and consequently triggered a more robust acute phase response. Alternately, the higher bioavailability of TBCC may have provided more copper that could be recruited to the liver for production of ceruloplasmin. The acute phase response to LPS markedly increased the dose response relationship between dietary copper level and ceruloplasmin. This might be directly related to an increased copper requirement during this metabolic state (Koh et al., 1975).

The bioavailability of copper in the complex diet was considerably lower than that in the purified diet as indicated by smaller increases in gain, efficiency, ceruloplasmin, and tendon copper with increasing doses of copper. This can be seen as lower slopes for the regression of dietary copper level versus each of these parameters for the complex diet compared to the purified diet (Tables 4 and 7). Tendon copper, gain and LPS-induced ceruloplasmin levels, there was not a significant difference in the bioavailability of the sources when the purified diet was fed but there were relatively large differences with the complex diet. The purified diet was lower in copper than the sorghum-soy diet and the magnitude of the growth responses to added copper were more robust, so it might be that the sorghum-soy basal diet was insufficiently low in copper to clearly test the hypothesis, except when the copper requirement was magnified by the induction of an acute phase response or with end points that have a low priority for copper such as tendon copper accumulation.

In conclusion, the dose response relationship between added copper and gain, efficiency, tendon copper or ceruloplasmin was steeper with the purified diet than the complex diet. TBCC had a bioavailability that ranged from 15 to 31% greater than CS5. The difference in bioavailability was more pronounced when antagonists were present in the diet if efficiency of gain, tendon copper or LPS-induced ceruloplasmin were used as the criteria for estimating bioavailability. This is likely because TBCC results in less

"unremovable" copper and more EHPG extractable copper in the intestines; this likely means that they have more ionizable copper available for absorption.

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Table 1. Effect of copper level and source on performance of chicks fed a purified diet, Experiment 1.

Added copper	Source	Gain (g/c/d)	Feed intake (g/c/d)	Efficiency (gain/feed)
0	-	33.1[a]	60.7	0.545[a]
1	CuSO4	34.9[ab]	60.8	0.574[ab]
3	CuSO4	39.4[bc]	57.6	0.684 [b]
4.5	CuSO4	38.6[bc]	59.1	0.653 [ab]
6	CuSO4	40.5[c]	59.0	0.686 [b]
1	TBCC	36.1	57.2	0.631
3	TBCC	38.0	59.5	0.639
4.5	TBCC	39.6	59.7	0.663
6	TBCC	41.7	60.9	0.685
	SEM	1.3	1.9	0.03
Probability				
	Level	<0.01	0.44	<0.01
	Source	0.09	0.14	0.08
	Lev x S	0.15	0.44	0.08

[a-c]Basal and CS5 treatments were analyzed by one-way ANOVA; (P<0.02) for gain and efficiency. Means not sharing a common superscript are significantly different (P<0.05).

Table 2. Effect of copper level and source on performance of chicks fed a complex (sorghum-soy) diet, Experiment 2.

Added copper	Source	Gain (g/c/d)	Feed intake (g/c/d)	Efficiency (gain/feed)
0	-	38.5[a]	62.1	0.620
1	CuSO4	39.5[a]	63.7	0.620
3	CuSO4	41.3[ab]	62.4	0.662
4.5	CuSO4	43.1[b]	64.7	0.666
6	CuSO4	42.8[ab]	64.6	0.663
1	TBCC	39.0	64.2	0.607
3	TBCC	40.9	63.4	0.645
4.5	TBCC	43.9	63.6	0.690
6	TBCC	43.4	64.1	0.677
	SEM	0.9	1.7	0.03
Probability				
	Level	<0.01	0.33	<0.01
	Source	0.11	0.16	0.17
	Lev x S	0.20	0.42	0.08

[a-b]In order to examine the copper requirement, basal and CS5 treatments were analyzed by one-way ANOVA; (P = 0.03) for gain. Means not sharing a common superscript are significantly different (P<0.05).

Table 3. Regression analysis of dietary copper versus chick performance and calculated ratios of slopes.

	Gain			Efficiency		
Source	slope	Intercept	r^2	slope	Intercept	r^2
	----------Purified diet----------					
$CuSO_4$	1.18	33.8	0.80	0.02	0.56	0.69
TBCC	1.36	33.9	0.87	0.02	0.58	0.70
Prob	0.04	0.46		0.26	0.15	
Ratio	1.15					
	----------Complex diet*----------					
$CuSO_4$	1.01	38.5	0.91	0.013	0.62	0.81
TBCC	1.18	38.1	0.84	0.017	0.61	0.74
Prob	0.03	0.19		0.07	0.33	
Ratio	1.17			1.31		

*For the complex diet the regression analysis used the 0, 1, 3, and 4.5 levels only. This is because the highest level of copper addition had no apparent impact on gain or efficiency and because the regression r^2 were greatly improved when this level was left out of the analysis.

Table 4. Effect of copper level and source on hematocrit, ceruloplasmin and tissue copper in chicks fed a purified diet, Experiment 1.

Added copper	Source	LPS	Hematocrit (g/c/d)	Ceruloplasmin (g/c/d)	Liver Cu (mg/kg)	Tendon Cu (nmol/g)
0	-	-	30.6	5.11	4.74	1.87[a]
0	-	+	31	7.64		
1	CuSO4	-	31.2	5.5	4.77	2.22[ab]
3	CuSO4	-	31.4	5.3	5.3	2.99[ab]
4.5	CuSO4	-	32.9	5.7	5.87	3.12[b]
6	CuSO4	-	31.6	5.64	5.17	4.99[c]
1	TBCC	-	31.3	6.13	4.82	1.90
3	TBCC	-	32.3	6.19	4.68	2.94
4.5	TBCC	-	31.5	5.08	5.23	3.96
6	TBCC	-	31.7	6.24	5.68	4.84
1	CuSO4	+	30.3	8.15		
3	CuSO4	+	31.1	9.63		
4.5	CuSO4	+	32.5	9.72		
6	CuSO4	+	31.9	10.15		
1	TBCC	+	31.3	8.85		
3	TBCC	+	31.9	10.0		
4.5	TBCC	+	32.0	9.26		
6	TBCC	+	32.1	10.66		
		SEM	0.3	0.21	0.19	0.29
			Probability			
	Level		0.08	0.05	0.03	<0.01
	Source		0.24	0.07	0.21	0.56
	LPS		0.88	<0.01		
	Lev x S		0.28	0.09	0.10	0.34
	Lev x LPS		0.47	0.02		
	LPS x S		0.15	0.70		

[a-c]In order to examine the copper requirement, basal and CS5 treatments were analyzed by one-way ANOVA; ($P<0.01$) for tendon copper. Means not sharing a common superscript are significantly different ($P<0.05$).

Table 5. Effect of copper level and source on hematocrit, tissue copper and ceruloplasmin in chicks fed a complex diet (days 7-17), Experiment 2.

Added copper	Source	LPS	Hematocrit (g/c/d)	Ceruloplasmin (g/c/d)	Liver Cu (ppm)	Tendon Cu (nmol/g)
0	-	-	31.1	7.04	6.25	3.93
0	-	+	30.3	8.22		
1	CuSO4	-	31.3	7.25	5.96	4.38
3	CuSO4	-	30.8	7.8	6.29	4.42
4.5	CuSO4	-	31.6	7.85	6.15	5.12
6	CuSO4	-	32.4	7.97	6.00	4.89
1	TBCC	-	29.4	7.43	6.36	4.51
3	TBCC	-	33.3	7.99	6.44	4.95
4.5	TBCC	-	33.1	7.85	6.17	5.16
6	TBCC	-	32.8	8.24	5.89	5.54
1	CuSO4	+	32	8.85		
3	CuSO4	+	32.6	9.19		
4.5	CuSO4	+	29.2	9.81		
6	CuSO4	+	32.7	9.19		
1	TBCC	+	32.9	8.12		
3	TBCC	+	31	9.12		
4.5	TBCC	+	33.1	9.91		
6	TBCC	+	31.9	9.96		
		SEM	0.2	0.44	0.23	0.35
			Probability			
	Level		0.08	0.06	0.45	0.02
	Source		0.76	0.78	0.77	0.11
	LPS		0.67	<0.01		
	Lev x S		0.80	0.13	0.16	0.26
	Lev x LPS		0.15	0.02		
	LPS x S		0.58	0.03		

Table 6. Regression analysis of dietary copper levels versus ceruloplasmin and tendon copper concentrations.

		Ceruloplasmin			Tendon Cu		
Source	LPS	slope	Intercept	r^2	slope	Intercept	r^2
		----------Purified diet----------					
$CuSO_4$	-	0.08[a]	5.2[a]	0.53	0.46	1.70	0.77
TBCC	-	0.06 [a]	5.5[a]	0.76	0.52	1.59	0.87
$CuSO_4$	+	0.43[b]	7.8[b]	0.92			
TBCC	+	0.40[b]	8.1[b]	0.76			
Prob		<0.01	<0.01		0.09	0.10	
		----------Complex diet*----------					
$CuSO_4$	-	0.19[a]	7.07[a]	0.84	0.22	3.98	0.75
TBCC	-	0.19[a]	7.17[a]	0.70	0.26	4.04	0.80
$CuSO_4$	+	0.32[b]	8.33[b]	0.85			
TBCC	+	0.40[c]	7.99[b]	0.83			
Prob		<0.01	0.02		0.04	0.60	
Slope ratio		1.26			1.18		

*Regression analysis used the 0, 1, 3, and 4.5 levels only; this is because the highest level of copper addition had no apparent impact on gain or efficiency and because the regression r^2 were greatly improved when this level was left out of the analysis.
[a-c]Means not sharing common superscripts are different (P<0.05).

Table 7. Effect of Copper level and source on water soluble copper levels in intestinal contents

	Copper Level and Source			
	10		150	
Region	$CuSO_4$	TBCC	$CuSO_4$	TBCC
Duodenum	14.8 ± 1.2	10.9 ± 1.4	96.1 ± 3.4	52.9 ± 4.5*
Jejunum	15.1 ± 0.9	14.0 ± 1.6	87.9 ± 3.2	83.4 ± 2.8
Ileum-1	9.0 ± 0.6	10.6 ± 0.8	95.3 ± 3.4	102.5 ± 4.4
Ileum-2	8.4 ± 1.0	8.8 ± 0.7	71.6 ± 4.6	78.7 ± 4.6
Ileum-3	9.1 ± 0.7	9.9 ± 1.5	61.0 ± 3.6	73.9 ± 3.1
Cecum	4.7 ± 0.8	5.7 ± 0.8	47.7 ± 2.8	51.9 ± 3.5

Data are expressed as mean ± SEM. Units of measure are mg copper/kg dry matter.
*Significantly different from $CuSO_4$ (P< 0.05) by paired T test.
Main effect P values: Tissue = 0.00; Level = 0.00; Source = 0.14
Source x Level x Tissue = 0.03

Table 8. Effect of Copper level and source on EHPG extractable copper levels in intestinal contents

Region	Copper Level and Source			
	10		150	
	$CuSO_4$	TBCC	$CuSO_4$	TBCC
Duodenum	3.7 ± 0.8	11.5 ± 1.7*	50 ± 4	86 ± 8*
Jejunum	7.2 ± 1.0	12.55 ± 1.1*	95 ± 5	131 ± 10*
Ileum-1	12.2 ± 1.5	17.91 ± 0.9	206 ± 15	241 ± 13
Ileum-2	12.3 ± 0.7	15.6 ± 1.8	246 ± 19	268 ± 21
Ileum-3	15.9 ± 1.4	16.1 ± 2.0	276 ± 18	317 ± 23
Cecum	14.4 ± 1.5	14.2 ± 1.3	292 ± 19	330 ± 20

Data are expressed as mean ± SEM. Units of measure are mg copper/kg dry matter.
*Significantly different from $CuSO_4$ ($P < 0.05$) by paired T test.
Main effect P values: Tissue = 0.00; Level = 0.00; Source = 0.01
Source x Level x Tissue = 0.00

Table 9. Effect of Copper level and source on unremovable[1] copper levels in intestinal contents

Region	Copper Level and Source			
	10		150	
	$CuSO_4$	TBCC	$CuSO_4$	TBCC
Duodenum	10.5 ± 1.0	10.6 ± 0.3	31 ± 3	49 ± 2*
Jejunum	11.8 ± 0.7	13.5 ± 0.7	67 ± 7	53 ± 4
Ileum-1	14.9 ± 0.4	16.4 ± 1.2	95 ± 7	58 ± 3*
Ileum-2	19.3 ± 1.2	17.6 ± 1.1	158 ± 10	115 ± 8*
Ileum-3	22.9 ± 0.9	19.0 ± 1.1	131 ± 9	82 ± 11*
Cecum	26.8 ± 1.6	24.1 ± 1.8	141 ± 8	89 ± 13*

Data are expressed as mean ± SEM. Units of measure are mg copper/kg dry matter.
*Significantly different from $CuSO_4$ ($P < 0.05$) by paired T test.
Main effect P values: Tissue = 0.00; Level = 0.00; Source = 0.02
Source x Level = 0.01; Source x Level x Tissue = 0.00
[1]Unremovable copper = total copper – (water soluble + EHPG extractable copper)

WINNER OF THE 2016 CANC ANIMAL NUTRITION SCHOLARSHIP

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Performance and net energy in high and low RFI beef cattle on a restricted intake

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ABSTRACT:

In order to determine how beef cattle with known residual feed intake (RFI) phenotypes would perform under restricted feeding, 36 weaned Angus cross beef calves (24 steers and 12 heifers) were selected from a group of 98 calves that had been previously phenotyped for RFI. High and Low RFI animals (24 steers and 12 heifers) were subjected to a 52-d feeding trial with intake limited to 1.5% of BW. Feed offered and refused were measured daily, body weights were taken at 14 day intervals, and ultrasound measures (longissimus muscle area and subcutaneous fat over the 12th-13th ribs) were taken at the beginning, middle and end of the trial. After 52 days of diet restriction, RFI groups had similar BW, ADG, DMI, RFI and gain:feed. Fat gain, protein gain, and estimated recovered energy (RE) were similar between groups, although High RFI cattle had 0.26 cm more subcutaneous rib fat than Low RFI ($p = 0.01$). High RFI cattle also had more rib fat at the start of restricted feed trial. RFI groups did not differ in estimated heat energy (HE) or maintenance requirement (NEm; $p > 0.10$). Heifers had lower HE than steers ($p < 0.001$). All cattle had lower ADG, RE, NEm and HE in response to limited feed. Overall HE was reduced from 0.26 Mcal/kg$^{0.75}$ on *ad libitum* feeding to 0.16 Mcal/kg$^{0.75}$ on the restricted level of intake. The difference in HE from *ad libitum* to restricted feeding was -47 and -28% in High and Low RFI cattle, respectively ($p < 0.001$). Estimated NEm requirement was reduced from 0.095 to 0.073 Mcal/kg$^{0.75}$ overall, with a difference of -39% and +14% in High and Low RFI cattle ($p < 0.001$). Estimated NEm requirement changed by -32% and +7% ($p = 0.004$) in heifers and steers, respectively. These results indicate that when limited, both High and Low RFI cattle lower their maintenance requirement and heat production to similar levels, although High RFI cattle had higher HE and NEm during *ad libitum* feeding. Furthermore, heifers may be better equipped to adapt maintenance requirement and heat production in response to limited feed.

Key words: beef cattle, efficiency, net energy, body composition, residual feed intake

Table 1. Performance and net energy of RFI groups in response to diet restriction.

	RFI		Sex			P value		
Trait	High	Low	H	S	SD	RFI	Sex	RFI x Sex
Initial BW, kg	454.4	440.2	443.2	456.2	49.2	0.42	0.31	0.80
Final BW, kg	507.2	487.9	487.3	507.8	57.0	0.35	0.32	0.73
ADG, kg/d	1.015	0.917	0.940	0.993	0.210	0.87	0.49	0.51
DMI, kg/d	5.98	6.10	5.50	6.57	0.463	0.49	< 0.001	0.92
Gain:feed	0.171	0.152	0.171	0.152	0.035	0.14	0.15	0.63
RFI, kg/d	-0.242	-0.121	-0.717	0.354	0.464	0.47	< 0.001	0.94
Ribeye area, cm^2	80.19	80.54	76.79	83.94	7.74	0.90	0.014	0.41
12th-13th rib fat, cm	1.26	0.97	1.18	1.05	0.212	0.001	0.099	0.069
Fat gain, kg/d	0.37	0.28	0.32	0.33	0.18	0.15	0.79	0.15
Protein gain, kg/d	0.12	0.12	0.12	0.13	0.04	0.92	0.60	0.59
Fat:protein in gain	3.33	2.23	2.91	2.65	2.34	0.08	0.66	0.10
RE, Mcal/d	4.16	3.32	3.64	3.84	1.60	0.13	0.73	0.16
RE, Mcal/kg$^{0.75}$	0.043	0.036	0.039	0.040	0.014	0.16	0.85	0.11
HE, Mcal/d	13.68	14.86	12.76	15.78	2.38	0.07	< 0.001	0.25
HE, Mcal/kg$^{0.75}$	0.147	0.163	0.141	0.169	0.028	0.13	0.007	0.37
NEm, Mcal/kg$^{0.75}$	0.065	0.081	0.063	0.082	0.03	0.10	0.063	0.20

Update on dietary fat in humans

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INTRODUCTION

There has been and continues to be an unexplainable desire by dieticians and other "health" professionals to associate or link dietary components, particularly animal food products, with human disease (coronary heart disease, cancer, etc.). However, that perceived link is becoming increasingly ambiguous and is especially true with regards to ruminant derived products. In fact, not only is the link between dietary beef/dairy and disease suspect, there are a variety of micro-components in these high quality foods that are actually potent disease-fighting molecules.

Relative Risks vs. Absolute Risks

There are a variety of issues that makes conducting controlled (meaning there is an intervention and a control group) human experiments difficult (especially nutrition trials) and this is particularly arduous when the end measurement may be a life threatening disease. The first is the number of subjects necessary to gain statistical confidence, as disease incidence is often so low that it requires thousands of people (and a long period of intervention) to be enrolled in the experiment. The second is compliance, as humans are notorious for not following experimental dietary guidelines. The third is moral/ethical as humans certainly can not (or at least shouldn't) be administered a harmful molecule (i.e. a carcinogen) and then placed on a dietary regimen to see which treatment prevented/protected against the disease (as is normally done in animal models). Consequently, human nutrition trials rely heavily on "question" based data. An example question may be similar to the following; how many servings of a foodstuff (i.e. brussel sprouts, hot dogs….) do you eat on a weekly basis and have you been diagnosed with a specific disease during a period of time (i.e. 5 years). The respondents are then usually stratified (i.e. quintiles, etc.) into the quantity of the consumed food(s) of interest.

The difference in disease incidence between groups/quintiles can then either be presented as a relative difference or absolute difference. If the disease frequency in one Group A is 1/100 and the other is 2/100 then the absolute difference is 1% but the relative difference is 50% (Table 1). The 50% relative difference is an impressive number and headline grabber (especially compared to 1%) but it can be misleading (oftentimes intentionally) to readers not familiar with the data set. For example, alternatively (and probably a more rational and fair method of reporting the data) it could be presented as 99% of Group A and 98% of Group B people did not develop the disease. Would you alter your lifestyle, change your diet, take prescription medication, etc.. to improve your chances of not getting a disease by 1%?

The studies linking dietary fat with human disease use statistics often times based on relative risks. When these "links" are evaluated on an absolute basis, it is clear the associations are incredibly weak at

best (Taubes, 2001). In addition to the relationship in some studies being extremely low, some experiments are now indicating no risk of dietary animal fats (see below).

Table 1. Comparison of Data Analysis Methods: Relative vs. Absolute Risks

Disease Risk		Absolute Difference	Relative Difference
Group A	Group B	A - B	B/A %
20 % (2/10)	10 % (1/10)	10 %	50 %
2 % (2/100)	1 % (1/1000)	1 %	50 %
0.2% (2/1000)	0.1 % (1/1000)	0.1 %	50 %

http://www.acponline.org/journals/ecp/janfeb00/primer.htm

History of Nutritional Guidelines

The hypothesis that dietary fat is somehow deleterious to humans is over 50 years old. Ruminant lipid tends to be more saturated than other animal fats and this is especially true when compared to some common vegetable oils. The saturated fat content is the lightening rod for nutritionists and others as it has historically been the component identified as connecting diet and disease (Keys and Grande, 1957). Despite lacking a traditional scientific relationship (for an excellent description on the history of the dietary fat link with health, see review by Taubes, 2001) and regardless of recent reports contradicting the dogma, the 2000 Dietary Guidelines for Americans is as follows: "choose a diet that is low in saturated fat and cholesterol and moderate in total fat". The American Heart Association suggests to "choose foods like vegetables, fruits, whole-grain products and fat-free or low-fat dairy products most often" and the American Cancer Society indicates that "limiting saturated fat may be particularly important to reduce risk for both cancer and heart disease. Choose lean meats and low-fat dairy products, and substitute vegetables oils (like canola and olive) for butter or lard".

THE WOMENS HEALTH INITIATIVE (WHI) DIETARY MODIFICATION TRIAL

Until 2006, the reports linking diet, and specifically animal products, with cancer and other health disorders were primarily from epidemiological trials (Rose et al., 1986; WHO, 2003). Many of our national dietary recommendations are based on these international epidemiological trials. However, a large number of comparison trials recently published (within the last decade) do not support the hypothesis that dietary fat, specifically animal fat, increases the risk for cancer (Table 2) and it is perplexing as to why these reports are ignored by the American healthcare community. Nevertheless, comparison trials are limited by a number of scientific variables and results obtained should be used as initial suggestions for further randomized controlled investigations.

Table 2. Recent reports on the effects of total dietary and saturated fat on the incidence and relative risk of differing types of cancers.

Observ.	Cancer	Total Fat Risk	Saturated Fat Risk	Reference
4,980	Breast	↔	↔	Hunter et al., 1996
Cohort	Colorectal	↔	↔	Howe et al., 1997
Cohort	Breast	↔	↔	Lee & Lin, 2000
Cohort	Breast	↔	↔	Zock, 2001
Cohort	Colorectal	↔	↔	Zock, 2001
Cohort	Prostate	↔	↔	Zock, 2001
3,482	Breast	NR	↓	Shin et al., 2002
Cohort	Colorectal	NR	↓	Cho et al., 2004
910	Breast	NR	↓	Wirfalt et al., 2005
48,835	Breast	↔	↔	Prentice et al., 2006
48,835	Colorectal	↔	↔	Beresford et al., 2006
1,123	Skin	↓	NR	Granger et al., 2006

↔: no relationship
↓: Decreased risk
NR: Not reported
Cohort: a review of multiple trials

The WHI trial was designed in the early 1990's as a randomized controlled study with the goal of definitively testing the effects of dietary fat and its specific components on a variety of human diseases. The trial included more than 160,000 women (50-79 years old) from 40 different centers across the country, lasted for approximately 8 years and cost more than $700 million dollars. The women were either assigned to a low fat diet (while simultaneously increasing vegetable and fruit intake) or advised to stay on their usual eating pattern. Women on the low-fat diet had saturated fat intakes that represented about 7% of their total energy intake. Results from the largest and most comprehensive study on dietary fat in American history indicate that there is NO relationship between either total dietary fat or saturated fat on the incidence/risk of colorectal (Beresford et al., 2006) or breast (Prentice et al., 2006) cancer or on cardiovascular disease (Howard et al., 2006).

It is unclear why there are so many inconsistencies in the epidemiological literature with regards to dietary fat, specifically fat from animal products, on human health. The fact that there are such large inconsistencies makes it especially confusing as to how the dietary fat dogma became entrenched in the medical community. Regardless, the recent WHI controlled experiment should (in addition to the latest reports in Table 1) assist in creating new and more accurate nutritional guidelines and provide strong evidence as to why milk and other ruminant food products should remain an important part of a balanced healthy diet.

It is important to note that many organizations appearing to be concerned with public health (Table 3) may actually front for animal

rights groups (i.e. People for the Ethical Treatment of Animals: PETA; Animal Liberation Front: ALF). They have unsuccessfully persuaded the general American public that consuming animal products is immoral and unethical, but convincing consumers that the products are unhealthy is an alternative means to an end (elimination of animal agriculture). An example is the Physicians Committee for Responsible Medicine (incidentally, less than 5% of its members are physicians; Newsweek, 2004), which advocates that a vegetarian diet reduces the risk of cancer and other health disorders as stated on their website: "vegetarian foods may help prevent cancer and even improve survival rates". These groups have done an excellent job of convincing the public and media that they are legitimate scientists and actual health care professionals with a genuine concern for the public health.

Table 3. "Health organizations" that recommend decreasing animal food product consumption

Organization	Website
Center for Food Safety	www.centerforfoodsafety.org
Center for Science in the Public Interest	www.cspinet.org
Physicians Committee for Responsible Medicine	www.pcrm.org

ANTICARCINOGENS IN RUMINANT FOOD PRODUCTS

Numerous studies have been conducted with various human cancer cell lines and animal models showing that milk components can prevent the development and progression of cancer (see review: Gill and Cross, 2000). Many of these components are in the milk fat fraction and include butyric and vaccenic acids, ether lipids, sphingomyelin, Vitamin A and carotene (Parodi 1997). An additional molecule receiving considerable attention and the one most extensively studied is conjugated linoleic acid (CLA). For a detailed description on CLA ability to prevent different types of cancer, see recent reviews (Belury, 2002; Ip et al., 2003)

CLA describes positional and geometric isomers of linoleic acid, with the double bonds being separated by a single methylene group. CLA are synthesized in the rumen through biohydrogenation of polyunsaturated fatty acids and therefore are found naturally in dairy products and ruminant meat (Bauman et al., 2001). The *cis*-9, *trans*-11 isomer is the most abundant CLA isomer found in ruminant products, though both *cis*-9, *trans*-11 and *trans*-10, *cis*-12 have shown anticarcinogenic properties (Ip et al., 2003).

Although there is a wealth of evidence demonstrating that synthetic, purified CLA isomers have anti-cancer properties, recent attention has turned to CLA effects when presented as it would be in a normal diet (at smaller concentrations and in combination with many other fatty acids). In a recent study, mice were fed CLA (*cis*-9, *trans* -11/*trans*-10, *cis*-12 mixture) in combination with either a vegetable oil blend, corn oil, or beef tallow. Data indicate that CLA was more effective at decreasing tumors when beef tallow was added to the diet (Hubbard et al., 2006). Additionally, fatty acids extracted from beef (<1% CLA content) had a greater anti-proliferative effect on cancer cells than a

synthetically enriched CLA diet (De La Torre et al., 2006). Collectively, these trials suggest CLA found naturally in ruminant-derived products may potentially be significant contributors to a healthy and cancer preventive diet.

Increasing the CLA content in ruminant products

CLA is an intermediate in rumen biohydogenation of linoleic acid (C18:2; Bauman et al., 2001), but it primarily derived via desaturation of vaccenic acid (*trans*-11 C18:1, also a product of rumen polyunsaturated fatty acid biohydrogenation) by the Δ^9-desaturase enzyme (Corl et al., 2001; Kay et al., 2004). Vaccenic acid is also an intermediate of linolenic acid (C18:3) biohydrogenation (Bauman et al., 2001) so including both fatty acids in the diet of ruminant animals has the potential to increase the rumen output of *trans*-11 C18:1 and thus enhance the content of *cis*-9, *trans*-11 CLA in food products.

The milk fat CLA content from TMR-fed cows can markedly be increased (i.e. ≥ 5-7 fold) by adding a variety of plant oils (i.e. sunflower, linseed etc.) to dairy rations. Altering the oils with TMR-fed cows can increase the CLA content so that it is equal to or greater than that found in pasture-fed cows (which typically have an enhanced CLA content, Kelly et al., 1998). For a detailed description on successful methods to enhance the CLA content in dairy products see a recent review (Lock and Bauman, 2004).

DAIRY CALCIUM AND WEIGHT LOSS

Milk is a rich source of a number of vitamins and minerals (potassium, chloride, sodium, calcium etc.) that are required in the human diet such as fat-soluble vitamins (A, D, E, and K), as well as the B vitamin family, specifically thiamin, riboflavin, B_6, and B_{12}. Recently, calcium intake, particularly from dairy sources, has been implicated in decreased incidence of obesity within the human population. Dietary calcium is crucial to the regulation of energy metabolism, in that it has been found to attenuate adipocyte lipid accretion during over consumption of energy-dense diets, as well as to increase lipolysis and preserve thermogenesis during caloric restriction, leading to accelerated weight loss (Zemel, 2003). It has been demonstrated that calcium supplementation, in rodent and human models, decreases visceral adiposity, a precursor to the metabolic syndrome (Zemel et al., 2004; Azadbakht et al., 2005; Liu et al., 2005). The proposed mechanism of action for the role of calcium in decreasing adiposity is that supplementation of calcium results in a reduced concentration of intracellular calcium, via suppression of 1,25-(OH_2)-D, which leads to a coordinated deactivation of fatty acid synthetase (Sun and Zemel, 2004) and an increase in lipolysis (Shi et al., 2001; Zemel, 2001). In addition, it might also increase uncoupling proteins and thus increase metabolic heat production (Shi et al., 2001) and this might be the mechanism by which dairy products help with weight loss even though these people are not necessarily on a lower calorie diet.

A number of studies have been conducted utilizing calcium to modulate obesity ranging from epidemiological and observational studies to those investigating the mechanism of action utilizing a transgenic obese mouse model. It has been demonstrated that a dairy source of calcium, rather than a synthetic supplemental source such as calcium carbonate,

has greater impacts on weight loss (Zemel et al., 2000, 2004). In a study conducted by Zemel and coworkers (2004), it was demonstrated that calcium supplementation in obese adults, particularly in the form of dairy products, significantly increased weight loss, decreased body fat percentage and reduced waist circumference. Furthermore, individuals consuming high calcium diets in the form of dairy products had a significant reduction (44%) in circulating insulin levels. In a study conducted by Liu and co-workers, (2005), it was determined that consuming dairy products in middle-aged and older women was associated with a decreased incidence of metabolic syndrome. Women consuming a high calcium diet (>1,500 mg/day) exhibited decreased waist circumference, BMI, hypertriglyceridemia, high blood pressure, and incidence of type 2 diabetes and increased HDL cholesterol.

Recent evidence demonstrates that calcium has an anti-obesity effect, particularly when it comes in the form of dairy products. Utilizing yogurt, or non-fat dry milk in studies, regardless of the subject (rodent or human), increased weight loss and decrease fat percentage to a greater extent than calcium from a synthetic source such as calcium carbonate. Milk is a rich source of many bioactive compounds which either act independently or synergistically with calcium to accelerate lipolysis and/or effect nutrient partitioning between adipose tissue and skeletal muscle. Therefore, supplementation of calcium, in the form of low-fat dairy products, attributes to increased weight loss.

SUMMARY

The link between dietary fat, and specifically fat derived from ruminant animals, with human disease is incredibly small at best and probably does not exist. Unfortunately, the hypothesis that animal food products are “unhealthy” has become dogma in popular culture (driven in part by organizations with ulterior and covert motives) and even people with little or no biological knowledge now affiliate ruminant food products with “heart attacks” and “cancer”. In stark contrast to the “doom and gloom” message we have consistently heard from the medical community and dieticians for the past four decades, there are a variety of micro-components in dairy and beef products that are strongly associated with prevention and treatment of disease (cancer, obesity, etc.). A coordinated and concerted effort by agricultural and biological scientists AND the animal agriculture industry is necessary to re-educate consumers about biology and nutrition.

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Milk yield genotype affects metabolism, endocrinology and immunology of the Holstein

Brian Crooker, Wanda Weber and Georgina Cousillas

Introduction

The dairy industry has made tremendous advances in the past 50 years. These advances have been based primarily on greater milk yield per cow which has resulted in two major accomplishments; 1) increased efficiency of production and 2) reduced the environmental impact per pound of milk produced (Capper et al., 2009). Despite these advances, the upper limit has not been reached as the rate of increase has not abated and a new World Record Holder was just announced; Bur-Wall Buckeye Gigi with a 365 d record of 74,650 lb milk, 2,126 pounds of fat and 2,142 pounds of protein.

Overemphasis on one or only a few traits can be detrimental to others and can decrease overall productive efficiency. The dairy industry recognized the need for additional emphasis on reproduction and other functional traits (Van Raden, 2004; Egger-Danner et al., 2015) and fertility of the Holstein has increased over the last 15 years (Council on Dairy Cattle Breeding, 2015). These are clear examples of using new information to continue to increase overall performance of the cow, something that the dairy industry has excelled at doing. Given the new and developing tools for molecular analyses, it is clear that additional advances will certainly occur as greater knowledge and understanding is obtained for the processes that regulate performance and how these regulatory components function within changing physiological conditions.

Unique Animal Model

A breeding project was initiated in 1964 by Dr. Charles Young at the University of Minnesota as part of a regional research effort. The original foundation Holstein cows were paired by genetic merit and designated to subsequently produce either unselected (static, low merit for milk yield) or contemporary (high merit for milk yield) Holsteins (Weber et al., 2007). Contemporary cows and their female descendants have been inseminated with semen from the highest PTA-milk sires (n=4) available each year. From 1964 to 1991, the unselected cows and their female descendants were bred with semen from sires (4 sires/yr in a 5-yr rotation; 20 total) that were breed average for predicted transmitting ability for milk (PTA-milk) in 1964. Since 1991, breeding the unselected cows and their female descendants has continued as before except the semen has been from sons of the original 20 bulls and the unselected cows. Coefficients of inbreeding are not allowed to exceed 6.25% (Weber et al., 2007). Genetic merit of the unselected cows has remained stable and they continue to represent the U.S. Holstein population of 1964. Genetic merit of the contemporary Holsteins has continued to increase in a manner similar to that of U.S. Holsteins. The 50-plus years of intensive selection has resulted in contemporary cows that produce more than 4,500 kg of additional milk per 305-d lactation than their static, unselected herdmates.

Comparison of unselected and contemporary Holsteins provides a unique and powerful opportunity to gain a greater understanding of key regulatory genes and gene networks that regulate animal performance and the molecular mechanisms responsible for the multiple alterations associated with increased milk yield and lactational efficiency. Technical advances in high-throughput “omics” tools provide opportunities for greater and more in-depth evaluation of genomic, transcriptomic, proteomic, and metabolomic differences between these genotypes on a whole animal and individual tissue basis. Identification of these genes and networks and

understanding how they are regulated will contribute to an improved understanding of the molecular controls of animal performance (lactation, reproduction, health, etc.) and will provide opportunities for continued improvement in animal efficiency and well-being (Egger-Danner et al., 2015).

Genomics

Selection practices have indeed truly altered the Holstein so it should be no surprise that the genome of the cow has been altered. We conducted a whole-genome singe nucleotide polymorphism (SNP) study of DNA collected from our unselected Holsteins, from Holstein bulls born between 1975 and 1985, and from contemporary Holsteins to identify and categorize genome regions affected by selection since 1964 (Sonstegard et al., 2009, 2011). Allele frequency differences were greatest between unselected and contemporary Holsteins and least between 2 groups of contemporary Holsteins. Selection practices before and after 1985 had intermediate allele frequency differences but direction of change was consistent in these populations. Differences between genotypes were consistent among the chromosomes and results indicate about 20% of the Holstein genome has been altered by selection. When summed over all chromosomes, 1131 large frequency differences (absolute difference > 0.3) for SNP alleles were detected between the unselected and contemporary Holstein but none were detected between the 2 contemporary Holstein populations. These large selection signatures identify the tremendous genetic difference between our unselected and contemporary Holsteins and highlight their unique value in efforts to assess the impact of selection and the impact of increased milk yield on animal performance and physiology.

Our SNP results were used in a genome-wide association study (GWAS) with predicted transmitting abilities for production, health, reproduction, and conformation traits of Holsteins (Cole et al. 2011). The SNPs were mapped to gene regions or to specific genes. Associations between these SNP regions and specific traits can be used to help identify genes and thus mechanisms that might contribute to a particular phenotype. Indeed the top 100 effects for each trait of our GWAS analysis explained 40 to 56% of the trait variation. Thus, our GWAS analyses provide a number of targets of relevant genes and chromosome regions for more in-depth analysis. These results can be integrated with those from metabolic and gene expression studies with unselected and contemporary Holsteins to provide a powerful resource for identifying genes responsible for the large phenotypic differences between these milk yield genotypes. These results can be used to establish the connectivity and hierarchy of genetic signaling responsible for regulating aspects of animal performance.

Phenotype

Like other living organisms, cows attempt to maintain a relatively stable internal environment (homeostasis) through a variety of homeostatic regulatory mechanisms (Bauman, 2000). When their physiological status changes at the onset of lactation, their internal environment must also change to meet alterations in the metabolic demand for nutrients. New set points are established and these changes are coordinated through another form of regulatory controls known as homeorhetic regulation. Changes in blood glucose during the transition period provide a good example of the interaction of homeostatic and homeorhetic regulation. Blood glucose concentrations increase after an animal eats but this triggers an increase in the release of insulin which stimulates movement of glucose from the blood into cells and helps decrease blood glucose concentrations to what they were before the animal ate. This maintenance of a stable blood glucose concentration is an example of homeostatic regulation. When a cow transitions from pregnancy to lactation, her metabolic needs change and more glucose is needed to support milk synthesis. This reduces blood glucose concentrations to a new set-point and homeorhetic mechanisms help adjust or regulate whole body glucose use to meet this

change in metabolic demand. Homeostatic (acute) and homeorhetic (chronic) mechanisms interact to maintain a relatively stable internal environment and smooth transitions from one physiological condition to another.

Body Size and Energy Balance

Mature postpartum body weight (640 ± 15 kg) did not differ between our two genotypes (Weber et al., 2007) but prepartum body condition score on a 5 point scoring system of the unselected Holsteins exceeded that of the contemporary Holsteins (4.25 vs. 3.25 units) and there was little change in this 1.0 unit difference through lactation (Crooker et al., 2001). These characteristics are consistent with the shorter, more beefy nature of the unselected Holsteins and the enhanced angularity and dairy character of contemporary Holsteins. When offered the same total mixed ration (composed primarily of corn silage, alfalfa, corn, and soybean), contemporary Holsteins consumed more feed (3 to 4 kg dry matter/d during the first 5 weeks of lactation) than unselected Holsteins (Crooker et al., 2001). The similar body weight, similar condition score, greater milk yield and greater intake of the contemporary Holsteins resulted in nearly identical energy balance profiles for both genotypes. Postpartum serum non-esterified fatty acid (NEFA) concentrations also were similar and support the lack of difference in energy balance between the genotypes.

Selection practices have altered how nutrients are partitioned among body functions and may have altered how this balance is achieved, but the similar energy profiles of unselected and contemporary Holsteins indicate the homeorhetic ability of the well-fed cow to adapt to the greater metabolic demand for nutrients for milk synthesis has not been compromised in a manner that is detrimental to maintenance of body tissue or energy reserves. This does not mean that metabolism of the cow has not been altered. It clearly has and it would be unrealistic to suspect it had not.

Metabolites, Hormones and Gene Expression

Serum concentrations of glucose and insulin are decreased postpartum in both genotypes and the reduction is greater in contemporary than in unselected Holstein. The postpartum reductions are expected due to the greater demand for glucose to produce lactose with the initiation of lactation. The greater reduction in contemporary Holsteins reflects the greater demand to produce more milk (more lactose). These differences represent examples of homeostasis and homeorhetic regulation driven by differences in the changing needs of these genotypes as they change their physiological status during the transition period.

The postpartum reductions in insulin (insulinemia) uncouple the connection between growth hormone (GH) and insulin-like growth factor-I (IGF-I). This occurs in part through a reduction in amount of growth hormone receptors in the liver. A reduction in growth hormone receptors reduces the signal to produce IGF-I. Postpartum expression of growth hormone receptors in the liver is reduced more in the contemporary than in the unselected Holstein (Crooker et al., 2001, Weber et al., 2014). Serum concentrations of IGF-1 are reduced to a similar extent in both genotypes but the reduction occurs for a longer duration (21 vs. 84 d) in the contemporary cow (Weber et al, 2007). This decreases the negative feedback of IGF-1 on GH secretion from the pituitary so that serum GH concentrations are greater in the contemporary cow throughout lactation. Our results indicate selection for increased milk yield or the greater demand for nutrients associated with the increased milk yield may have altered how the connection between GH and IGF-I is regulated during the transition period and lactation. More comprehensive analyses of hepatic and peripheral tissue gene expression are needed to identify the impact on increased milk yield on this connection. We are currently conducting whole transcriptome analyses of gene expression in liver, mammary, and adipose collected from these genotypes

during the periparturient period. Our primary goals in this study are to identify mechanisms that regulate functional alterations within these individual tissues and mechanisms that integrate or coordinate these alterations among the tissues for a smooth orchestrated change from pregnancy to lactation.

Prolactin is another protein hormone produced in the pituitary that is involved with a number of functions including lactation and reproduction. We have also demonstrated that hepatic expression of the prolactin receptor is greater in the contemporary than in the unselected Holstein which indicates it has a potentially greater importance in the contemporary cow (Carriquiry et al., 2004).

Reproduction

Our work has indicated that the postpartum delay in resumption of ovulation is greater in contemporary than in unselected Holsteins (Weber et al., 2003). This delay in ovulation has been associated with a reduction in hypothalamic sensitivity to estradiol and a subsequent reduction in luteinizing hormone (LH) which is needed to support follicular growth (Lucy et al. 1998). Our SNP analysis has identified polymorphisms that are at least spatially related to genes for that code for estrogen receptors (Sonstegard et al., 2015). Contemporary Holsteins also have less progesterone in their blood during the estrous cycle than their unselected herdmates (Lucy et al., 1998; Weber et al., 2003). Progesterone stimulates endometrial function and embryo development (Wiltbank et al., 2014) and inadequate amounts of progesterone are detrimental to reproductive function. A reduction of LH pulse frequency during formation of the corpus luteum could be responsible for a decrease in progesterone (Butler, 2014) but we have not detected an effect of milk yield genotype on LH pulse frequency (Lucy et al., 1998, 2001). This indicates that a reduced luteal response to LH and/or a reduced expression of key steroidogenic enzymes might have larger roles in the reduced progesterone concentrations in the contemporary Holstein.

Immunology

Selection intensity for health components has been insufficient and there is considerable concern that the contemporary cow is more susceptible to disease and metabolic disorders than her ancestors (Elsasser et al., 2012; Pritchard et al., 2013). As milk yield per cow increases, endocrine and metabolic adaptations occur to enable the cow to meet the metabolic demands of increased milk yield (Crooker et al., 2001) and these adaptations are known to impact the immune system (Elsasser et al., 2012; Pritchard et al., 2013). During early lactation, the cow mobilizes body tissue (fat, muscle, and bone) to help meet the metabolic demands of lactation. Among the many changes is an increased proportion of saturated long chain fatty acids in circulation and saturated fatty acids activate inflammatory pathways (Wong et al., 2009). Hepatic inflammation contributes to the development of fatty liver, hepatic steatosis, and altered hepatic metabolism (Mamedova et al., 2013). Estimates indicate 40 to 60% of high-producing contemporary dairy cows develop moderate to severe fatty liver during early lactation (Jorritsma et al., 2001; Starke et al., 2011) which reduces milk yield and productive efficiency. An improved understanding of how selection for milk yield has altered the immune system and its interactions with endocrine and metabolic components will contribute to efforts to improve cow health and enhance food safety and security.

To examine the premise that the contemporary Holstein is more prone to disease and metabolic disorders, we examined how lactating cows from unselected and contemporary genotypes responded when lipopolysaccharide (LPS) was injected into their blood stream (Cousillas et al., 2015). Bacterial cells like E. coli have LPS on their cell membrane and detection of LPS by receptors in the body triggers an acute reaction by the innate immune system to defend the

body against a bacterial invasion. Administering LPS provides a way to stimulate the innate immune system without actually causing a bacterial infection. Blood samples were collected before and after LPS administration and analyzed for components of the innate immune system. Neutrophils are the most abundant form of white blood cells, are an essential component of the innate immune system, and they migrate to the site of an infection. Neutrophils function in part by engulfing (phagocytizing) foreign bodies (like bacteria) and oxidizing them to render them harmless. Neutrophil activity is frequently assessed by measuring their phagocytic and oxidative capacity. Neutrophil oxidative burst was greater and phagocytic capacity tended to be greater in unselected than contemporary Holsteins (Cousillas et al., 2015). These results support the notion that contemporary cows have a less responsive innate immune system than their ancestors and additional efforts are underway to more closely examine these differences and the regulatory factors involved.

Summary

The past 50 years of selection has truly altered genetic and phenotypic characteristics of the Holstein cow. The unselected Holsteins represent a unique opportunity to examine the impacts of this selection on factors that regulate metabolic, endocrine, immunological function and other traits of the Holstein. New knowledge gained from these examinations should enhance the ability to refine selection practices to further enhance profitability and well-being of the contemporary Holstein. Given the new and developing tools for molecular analyses, it is clear that additional advances will certainly occur as greater knowledge and understanding is obtained for the processes that regulate performance and how these regulatory components function within changing physiological conditions.

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Dairy culling strategies: are you considering all consequences?

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Approximately 35% of cows are culled every year. DairyMetrics, a benchmarking system from Dairy Records Management Systems in Raleigh, NC, reported a current (March 2016) average annual cow cull rate of 38% for 4,938 herds on DHIA with more than 100 cows (DRMS, 2016). The variation was large, however: approximately 16% of the herds had annual cull rates lower than 28% and 16% had annual cull rates greater than 48%.

USDA-NASS reported for 2015 for the US 2.914 million dairy cows slaughtered under Federal Inspection when inventory of milking cows was 9.307 million dairy cows (Gould, 2016), a cull rate of 31%.

For herds in California, Frazer LLP (2015) reported average cull rates of 42%, 41%, and 40% for 2012, 2013, and 2014. Data for 2015 were not yet available. For the same 3 years, the cost of feed as percent of milk sales were 70%, 64% and 51%, which is primarily the result of much improved milk prices. Cull rates stayed fairly flat, despite an increase in average beef prices from $809, $844, to $1140 in 2014. The costs of purchased cows (heifers) were $1376, $1290 and $1764 in 2014. The increase in 2014 again is the result of much improved milk prices.

Looking at USDA-NASS statistics for 1996 to 2015 (Gould, 2016), we see that the correlation between the number of dairy cows slaughtered and the USDA milk-feed ratio is -0.70 (Figure 1): slaughter goes up when the milk-feed ratio goes down. The USDA Milk-feed ratio is the number of pounds of 16-percent protein mixed dairy feed equal in value to 1 pound of whole milk. The same USDA-NASS data show that the correlation between dairy cow slaughter and the beef price was 0.39.

Although the number of dairy cows slaughtered increases with worse milk-feed price ratios and better beef prices, the national cull rate is not very variable. Barring large cattle exports, and a fairly constant number of dairy cows at approximately 9.2 to 9.3 million (matching demand for milk), cow cull rates are the result of the number of heifers calving, which is the result of breeding decisions made some 3 years ago (for example, how much sexed semen is used).

On many farms, cow culling is dependent on the number of heifer calves raised on the farm. Reproductive programs allow for the creation of more heifer calves than are needed to replace culled cows. One question to answer, therefore, is how many heifers the farm needs. Decisions regarding how many heifers to create in the reproductive program, heifer sales and purchases, and cow culling are necessarily made under conditions of uncertainty because feed, milk, beef and heifer prices can change quickly and would affect the optimal set of decisions.

In this paper, we'll focus on four factors that are associated with cow culling: 1) the economically optimal stall stocking density, 2) genetic

progress in heifers, 3) income over feed cost at voluntary culling, and 4) ranking cows for culling decisions in practice.

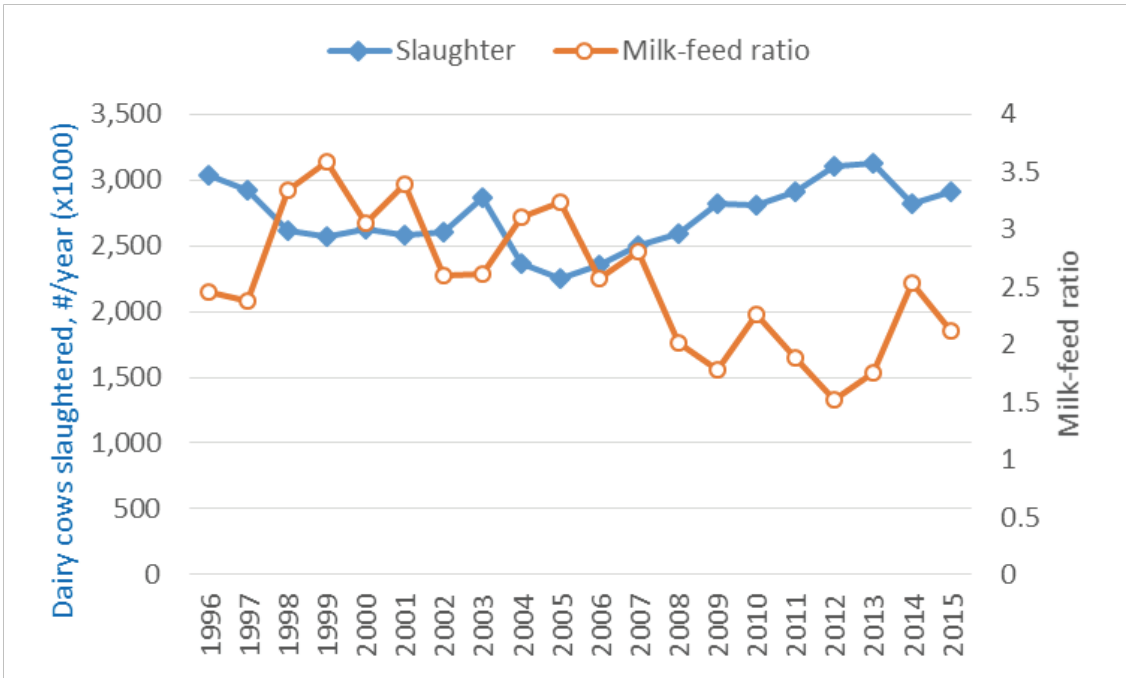

Figure 1. Dairy cows slaughtered under Federal inspection and the USDA milk-feed ratio for 1996 to 2014. The correlation is -0.7, suggesting a strong association between lower milk-feed ratios and increased dairy cow slaughter.

1) Stocking density

Stocking density is a quantitative measure of the area occupied by cows. One measure is the number of cows per stall in a pen. Adding another cow to the pen may reduce each cow's performance, such as lowering lying time or milk yield, but also adds the net revenue of the additional cow. The economic optimal stocking density is reached when the marginal return of the pen equals the marginal cost of the pen. At this stocking density, the profit per stall is maximized.

Some of our recent research has attempted to calculate economically optimal stocking densities for various milk and feed prices. When the economically optimal stocking density changes as a result of changes in feed or milk prices, the result is that cows should either be added or culled from the pen. Excerpts were taken from De Vries et al. (2015a,b) and De Vries et al. (2016).

Many studies exist that document the effects of (short term) stocking density on cow behavior but quantitative measures of stocking density on factors that directly affect cow cash flow (such as milk yield, fertility, lameness) are scarce. Lying time is reduced when stocking density >100% and starts to really be affected when stocking density >120%. Studies from Spain and New York both showed decreases of

approximately 1.1 lbs per day per 0.1 greater cows per stall in the range from 100% to 150% stocking density. Wisconsin data showed a loss of 0.01 conception rate per 0.1 greater cows per stall. The effect of stocking density on conception rate implies that the herd demographics change when stocking density is varied. Economic analyses of stocking density are hampered by a lack of good performance data, however.

We developed a spreadsheet of a herd budget that mimics the daily movement of cows through their lactations until they are culled. Examples of inputs are lactation curves, feed intakes, 21-day service rates, probabilities of conception, and involuntary culling risk. We chose our inputs based on plausible values for U.S. dairy herds during the last several years. The herd budget also calculates many statistics that follow from the chosen inputs, such as annual cull rate, average days open and herd milk production, as well as revenues, costs and profit per stall. In our analysis, stocking density affected milk production and reproduction. The effects linearly increased with stocking density greater than 100%. Milk production was reduced by 1.1, 1.7 or 2.2 lbs per day per cow in the pen, per 0.1 greater cows per stall. The 1.7 and 2.2 losses are slightly greater than the 1.1 lbs per day reported in the literature, but might include other not well quantified effects such as increased lameness or lower milk quality. Secondly, probability of conception was reduced by 0.1 per 0.1 greater cows per stall in all scenarios, as found by a Wisconsin study. A sensitivity analysis was carried out to reveal how the optimal stocking density depended on milk loss, milk prices, service rate and fixed versus variable costs. We varied stocking density of lactating cows from 100% to 150%.

Figure 2 shows that the level of milk loss has a large effect on the optimal stocking density and the gain in profitability. At a loss of 1.1 lbs per cow per day, the maximum profit per stall is at a stocking density greater than 150%. The profit per stall per year at 150% stocking density is $137 greater than at 100% stocking density. At a loss of 1.7 lbs per cow per day, the optimum stocking density is at 117% and the profit per stall per year is $27 greater than at 100% stocking density. At a loss of 2.2 lbs per cow per stall, the optimum stocking density is at 100%. Overstocking leads to losses.

We varied milk prices from $0.182 per lbs of milk to $0.227 per lbs ($0.205 was the default). Feed price was $0.16 per lbs of dry matter. Approximate USDA milk-feed ratios associated with these milk prices are 2.08, 2.34, and 2.60 at 55% dry matter in the ration in this case.

We used a milk loss of 1.7 lbs per cow per day. Higher milk prices increase the profitability of each additional cow and, therefore, encourage a greater stocking density. With a 22.7 cents per milk price, the optimal stocking density was 136% with a gain in profit of $139 per stall per year compared to 100% stocking density. The lower milk price of 18.2 cents reduced the optimal stocking density to 100%. At this milk price, overstocking was not profitable.

This scenario shows that less overstocking is economically better when milk prices are decreased. The same trends apply when feed costs are increased. Farmers may be tempted to overstock pens when milk income

over feed cost is reduced (milk-feed ratio decreases), perhaps to maintain cash flow from milk sales. But when the milk-feed ratio is lower, overstocking should decrease.

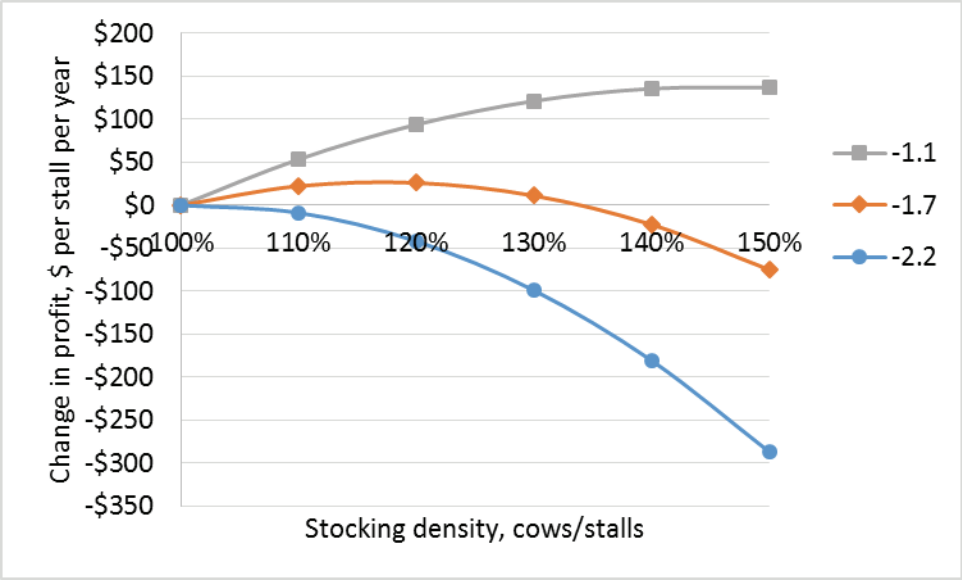

Figure 2. Profit per stall per year when stocking density is varied from 100 to 150% for three levels of milk loss (-1.1, -1.7 and -2.2 lbs per cow per day) per 10% greater stocking density.

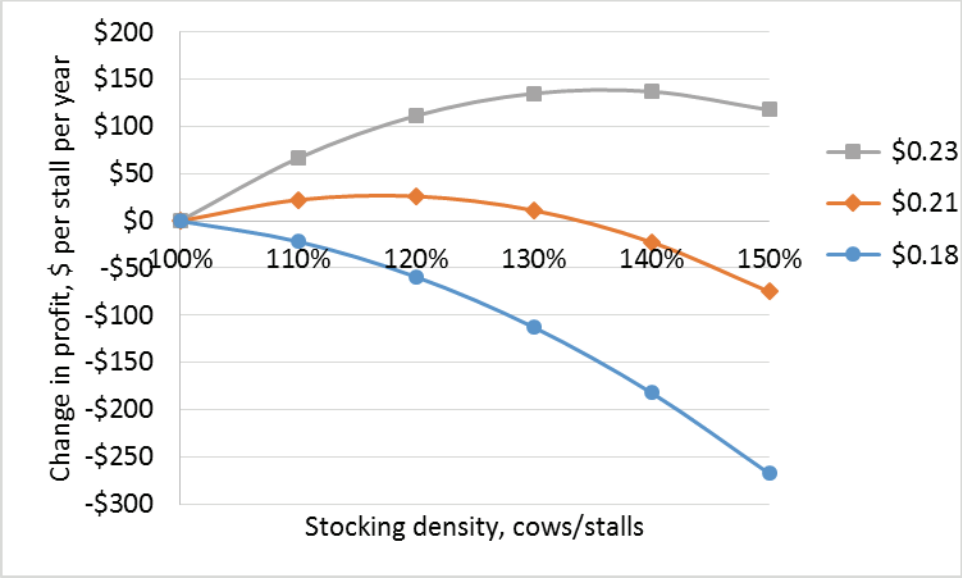

Figure 3. Profit per stall per year when stocking density is varied from 100% to 150% for three milk prices ($0.182, $0.205, and $0.227 per lbs of milk) per 10% greater stocking density. USDA milk-feed ratios associated with these milk prices are 2.08, 2.34, and 2.60.

From the limited scenarios shown, it is clear that the economically optimal stocking density is very sensitive to reasonable ranges in prices that affect the revenues and costs that vary with the number of cows. On the other hand, the marginal value around the optimal stocking density is low (a flat curve around the optimum, see Figures 2 and 3) which means that profitability per stall is not reduced much when the optimal stocking density is missed by 10% or 20%.

Again, the results shown should be interpreted with caution because the study revealed that the effect of overstocking on cow performance such as milk losses is not well documented. Other factors that should be considered too, such as cow welfare, are not included in the analysis.

2) Genetic progress

Genetic progress in dairy cattle overwhelmingly comes from breeding the herd to genetically superior service sires (excerpts taken from De Vries, 2015). Genetic lag is the difference in genetic merit between sires and dams (or more in general, between different populations). If an average cow is successfully mated with a genetically superior sire, it takes approximately 33 months before the heifer born from this mating calves for the first time and starts producing milk. A greater genetic lag implies a larger opportunity cost of missed production because genetic merit is not as high as it could be.

Genetic trends for various traits (milk, fat, protein, productive life, somatic cell score, daughter pregnancy rate, calving ease, and stillbirth) and for several dairy breeds in the US are available at https://www.cdcb.us/eval/summary/trend.cfm. Net Merit$ is a selection index, a combination of 12 genetic traits with weights that are the marginal economic value of improving each trait.

The average Net Merit$ has increased by approximately $700 from 2003 to 2014 for marketed Holstein sires (Figure 4). The annual increase is accelerating: from 2000 to 2004, the annual increase was $20 by year the sire entered AI production. From 2005 to 2009, it was $52. From 2010 to 2014, the increase was $86 per year. The latest acceleration is due to rapid adoption of genomic testing since 2009 which has reduced generation interval of sires dramatically and improved the rate of genetic gain. Mean sire age for Holstein male offspring born in 2012 was 2.7 years younger than males born in 2006, and 1.43 years younger for females.

The 2014 revision of the Net Merit index includes new fertility traits (heifer conception rate, cow conception rate, and a redefined daughter pregnancy rate), update genetic correlations, and updated marginal economic values. Using data from progeny-tested Holstein bulls born from 2002 through 2006, USDA-AGIL expects genetic progress in EBV (= 2 x PTA) from Net Merit (2014 revision) to be 122 kg milk per year (VanRaden and Cole, 2014). Expected genetic progresses for other traits are shown in the same publication. Combined, the increase in PTA for Net Merit is expected to be $75 per year. This means that the EBV of average animals is expected to increase by $149 per year. It also means that each year heifers will on average get better by up to $149 Net Merit because the genetic trend in their mothers is expected to be similar, although with their genetic level is lower (the genetic lag).

Because Net Merit is a lifetime value (about 3 years), we can expect that heifers born in 2015 are about $50 more profitable per lactation than heifers born in 2014 ($149 / 3). The increase in profitability calculated from the other USDA selection indices, such as the Cheese Merit, is similar.

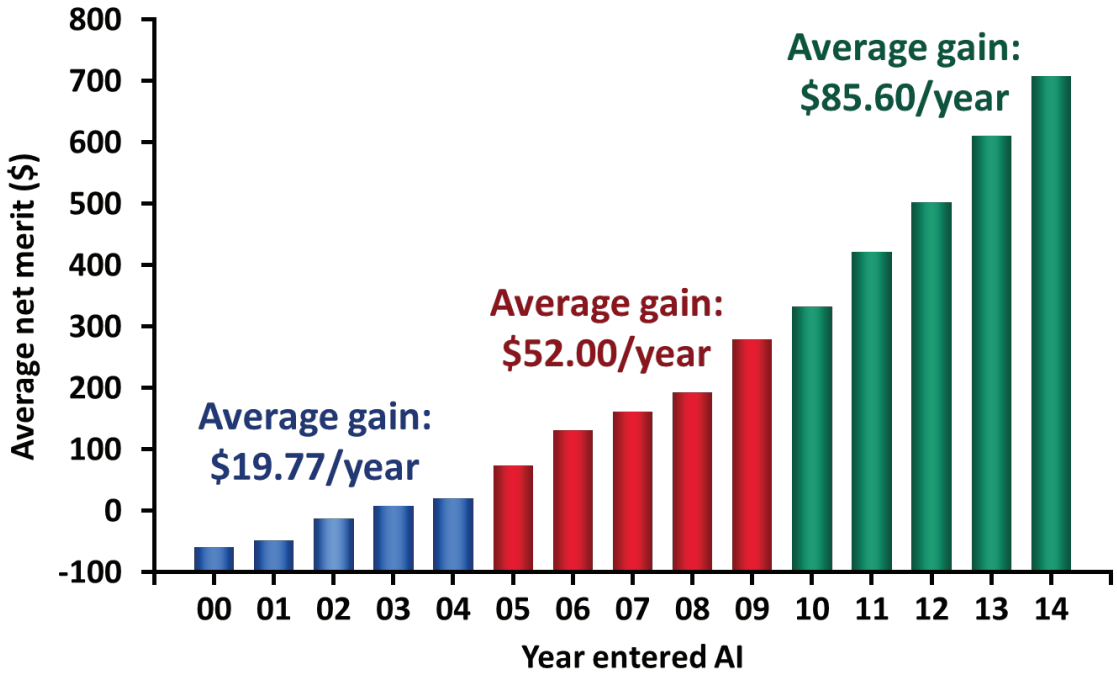

Figure 4. Average Net Merit for marketed Holstein sires by year entered AI. Source: G. Wiggans, 2014. http://aipl.arsusda.gov/publish/present.htm Accessed March 7, 2016.

Culling affects the genetic lag in the herd. If cow cull rates are low, then longevity is high (longevity ≈ 1 / cull rate) which means that the average cow is older and has a lower genetic merit than a younger herd. Thus, low cow cull rates are associated with greater genetic lag. The effect of culling on average genetic merit in the herd is small, however. On the other hand, lower cow cull rates also mean lower herd replacement costs, assuming that the cost to raise a heifer is higher than the cow's cull price. Further, an older herd has more mature cows which affect the herd's performance such as milk production, probability of conception, lameness, etc. Thus, there are opposing forces of replacement costs and genetic progress. The economic optimum balances both forces.

The question is how should genetic progress in replacement heifers affect cow culling and therefore longevity. Is it worth to raise lots of heifers just because we expect them to be genetically better than the cows? Replacing cows with genetically superior heifers is an application of the general problem of asset replacement with technologically improved assets. Standard economic theory says that cows should be replaced sooner when the incoming heifers are

genetically improved (Groenendaal et al., 2004). Specifically, "*the optimum time for replacement of a dairy cow is determined by comparison of the marginal net revenue anticipated from the present cow with the economic opportunity of a replacement. The latter value equals the maximal average discounted net revenue anticipated from replacement cows, also reported as annuities. For a situation with identical replacement or genetically improved replacement, the optimum time of replacement is defined as the first time period in which the annuity value of the cow drops below the maximal annuity value of the replacement animal*." This is easier said than realistically calculated.

Several studies have tried to address the tradeoff question of lower culling vs. genetic progress. A complete analysis considering all effects is complicated because there are interacting effects of (at least): 1) involuntary cow culling, 2) voluntary cow culling, 3) choice of dams to supply the next generation of replacement heifers, 4) number of heifers required to replace culled cows, and 5) genetic progress from sires.

An elegant, rather complete but now old study is from Allaire (1981). He included all 5 factors from the previous paragraph to determine optimal cull rates, as well as increases in milk sold per cow and increases in profitability after 20 years of culling and selection. The model included culling and selection based on milk yield only. In the model, Allaire assumed that youngstock culling was proportional to cow culling, so when cow culling increased, so did youngstock culling. He found that optimal cull rates were 30% to 35% when the objective was maximum milk sold per cow. The gain from keeping heifers from random survivor dams after voluntary culling was slightly smaller than the effect of voluntary culling only for low milk yield around the 35% cull rate. This effect of culling was equivalent to at least 25 years of genetic gain from dam selection. When the calves from the best dams among the survivor dams were used to generate the next generation of heifers, the additional gain was quite small because at 35% cull rate, few surplus dams were available. Thus selection intensity in dams was low at higher cull rates. No genetic progress from sires was considered in these cases. Considering a 0.5% annual increase in milk yield from sires, the gain was equivalent to the gain from breeding the best surviving dams and voluntary culling. These optimums around 35% cull rates to maximize milk yield do not include the cost of raising heifers and the price of cull cows. These herd replacement cost are obviously greater at 35% cull rates then at lower cull rates. So when Allaire (1981) included herd replacement costs that were relevant in Ohio in 1979, the result was that the economically optimal female cull rates were in the range of 20% to 23%, only 0 to 3 percentage points above the 20% involuntary cull rate he assumed. Cow replacement costs were much more important than genetic progress from service sires. Although the Allaire method used is elegant, the results are somewhat outdated because of assumptions in prices, milk yield, and annual genetic progress in sires. It is also not a trivial task to repeat his analysis.

Van Arendonk (1985) studied optimal replacement policies in dairy cattle, including the effects of genetic progress in milk yield from

using superior sires over time. These optimal culling policies were much more detailed than those assumed by Allaire (1981) and were economically optimal, but genetic progress from the dam side, either through voluntary culling or generating offspring from the genetically better dams, was not considered. Annual sire genetic improvement was set be worth $5.45, $10.91, or $16.36 (1985 values, roughly $13, $25, or $38 in 2016 dollars). Optimal annual culling rates changed only from 27% to 30% when genetic progress was included. The proportion of cows for which replacement was voluntary, instead of involuntary, increased from 23% to 32%. He concluded that the effect of changes in genetic improvement in milk revenue minus feed cost on the average herd longevity was small. Reduced involuntary cull rates improved profitability, but also simultaneously increased optimal voluntary culling. Therefore, he further concluded, from an economic point of view, that management and breeding policies should be directed towards reduction of involuntary disposal rather than maximization of the average herd life (= low cull rates) of cows.

A third analysis. A better heifer with an EBV of +$100 Net Merit is expected to generate approximately $33 more per year than an average heifer with an EBV of $0. Considering discounting for future income into today's net present value, and considering that the better heifer's offspring are also expected to be somewhat better than average, we might use a factor of 1.4 to put the EBV of $100 Net Merit into today's net present value of +$140. Subtracting the $140 value from the raising or purchase cost of the average heifer means that the herd entry cost of the +$100 Net Merit heifer are $140 lower than the herd entry cost of the average heifer. If the average heifer costs $2000, then we might consider the better heifer to cost only $1860. So should heifer cost of $1860 vs. $2000 significantly change the cow cull rate?

Using updated inputs for a typical US dairy herd in 2014 and a model (De Vries, 2006) similar to the one used by Van Arendonk (1985) that optimized culling decisions, we varied heifer entry prices and observed cull rates as well as the surplus of dairy heifer calves generated. Surplus dairy heifer calves occur if the number of calves available for replacement is greater than the number needed to replace culled cows. Some key results are in Table 1 for two levels of estrus detection rate leading to pregnancy rates of approximately 25% and 20%. Only conventional semen was used.

Table 1 shows that annual cull rates are somewhat insensitive to heifer prices and therefore insensitive to realistic superiority of genetics in heifers. With lower heifer prices, profitability increased, annual cull rate increased, and the rate of surplus heifer calves decreased. A negative surplus implies that the herd has a shortage of heifer calves and additional heifers need to be purchased. In the case of the lower pregnancy rate (≈20%), surplus = 0 when the heifer price was $1590. Using the culling policy associated with the $1590 heifer price, the profit per cow per year was $518 when the heifer price was $2000, or $66 lower than when the culling policy was optimal for the $2000 heifer price. The results show, in agreement with the older results of Allaire (1981) and Van Arendonk (1985), that genetic progress in sires

is not fast enough to warrant a high cull rate (resulting in a short longevity). The cost of cow depreciation is a bigger factor deciding optimal cull rates. However, genetic progress does reduce the economical optimal longevity somewhat.

Table 1. Optimal annual cull rate and surplus of dairy heifer calves as a function of heifer price and pregnancy rates calculated with a model with economically optimal culling decisions to maximize profitability.

Heifer price ($/head)	Profit ($/cow /year)	Pregnancy rate (%)	Annual cull rate (%)	Surplus heifer calves (%)
1400	818	25%	59%	-22%
1600	720	25%	41%	8%
1800	647	25%	34%	21%
2000	584	24%	30%	28%
2200	526	24%	28%	32%
1400	801	21%	64%	-30%
1600	696	20%	44%	2%
1800	617	20%	36%	15%
2000	550	20%	32%	22%
2200	488	20%	30%	26%

3) Income over variable cost rule versus retention pay off rule

Optimal cull rates including culling decisions for groups of cows could be calculated for individual farms if farm management and prices remained constant for years. But because major factors such as milk, feed, beef and heifer prices may change greatly within a few months, it remains uncertain how many heifers should be raised and how much culling is best. Rapid changes in the milk-feed price ratio should affect stocking density and therefore temporary cull rates. Also, more rapid genetic progress in heifers should increase cull rates by a few percent.

These forces do not dictate which cows should be culled when. First, we'll look at culling late lactation open cows when their milk sales not pay for their variable costs anymore, versus a more inclusive rule that includes also beef and heifer prices (excerpts taken from De Vries, 2009). Finally, we'll look at a method currently used to cull cows on a commercial dairy farm and investigate how differences in milk, beef, and heifer prices, as well as the culling rule, affect the ranking of cows for culling decisions.

One culling rule often employed by dairy farmers is to cull open cows that do not generate enough milk sales to cover their variable costs (including feed, some parlor supplies, perhaps some labor). Let's call this culling rule the Income over (=minus) variable costs (**IOVC**) policy. Open cows may be culled if their daily milk sales are less than the variable costs to keep her as a lactating cow. For example, if variable costs are $6.00 per day and the milk price is $0.19 per lbs,

then the cow needs to produce at least $6.00/$0.19 = 32 lbs per day to pay for her variable costs. This culling rule is greatly affected by the milk price and to a lesser extent by the feed price. For example, if variable costs are $7.00 per day and the milk price is $0.13 per lbs, the cow would have to produce 54 lbs per day to cover her variable costs. When milk prices are low, or variable costs are high, blindly following this culling rule would likely result in many culled cows.

This culling rule does not consider poorly producing pregnant cows. This culling rule also does not consider the cost of replacing culled cows. Rarely does it make sense to leave a slot vacant, so culled cows should be replaced. Cows can be ranked by IOVC, but better culling rules are available.

We used the computer program DairyVIP (De Vries, 2006) to gain some insight in the effect of various culling rules on profitability. DairyVIP allows the user to enter information about prices, milk production, reproduction, feed intake, involuntary culling, and breeding and replacement policy. The program then calculates the technical and economic consequences at the herd level of optimal breeding and culling decisions for individual cows (using dynamic programming), or the effects of non-optimal decisions. The strength is that the dynamic programming algorithm compares many different times for culling the current cow and retains the best. The program either calculates steady state results (the results are the same every year) or results for up to 36 months into the future given some starting situation. For example, the effects of changes in milk prices can be evaluated.

Culling decisions are limited to the decision to keep the cow at least one more month or cull her immediately. Culled cows are immediately replaced with calving heifers if that is optimal. Delayed replacement can be optimal in very seasonal herds. An alternative is to leave the slot open one or more months. Open cows could remain in the herd for up to 24 months after calving, if not culled earlier.

A result of the calculation to determine the optimal time of culling is the retention value, or retention pay-off (**RPO**). The RPO is the net present value of the future cash flow from keeping the cow until the optimum time of culling, considering the risk of premature involuntary culling, minus the future cash flow from culling the cow now and an optimal replacement policy in the future. In other words, the RPO is the extra net return of keeping the cow in the herd compared to immediate culling. The cow should be culled if the RPO < $0 and a replacement heifer is available. If the RPO is, say, $800, the cow should be kept and culling her now results in $800 opportunity costs. The RPO calculation considers all major factors that affect future cash flows, such as milk production, chances of getting pregnant, abortion, calvings, and future replacement animals in the same slot. The RPO is the ideal measure to rank cows for culling decisions, provided cow performance and prices are accurately projected and heifers are available.

Let’s assume that milk prices are either higher ($0.19 per lbs) or lower ($0.13 per lbs) and evaluate the effects of two different culling

strategies. One strategy is to keep open cows as long as they "keep paying for themselves". In other words, cull open cows if their IOVC < $0. If the cow is culled, her milk revenues and the variable costs disappear. The other strategy is to cull open cows if their RPO < $0. Key prices assumed were $1800 per replacement heifer, $0.12 per lbs dry matter intake for lactating cows, $0.07 per lbs dry matter intake for dry cows, and $0.42 per lbs of body weight for culled cows. Maximum conception rate for first parity cows was 37% at day 91 after calving with a gradual decline until 23% at day 456. Older cows had lower conception rates. The risk of involuntary culling was higher after calving and again later in lactation, and increased with parity. Cull cow price was on average $488. Many other inputs, including fixed and variable labor costs and other costs, were also included.

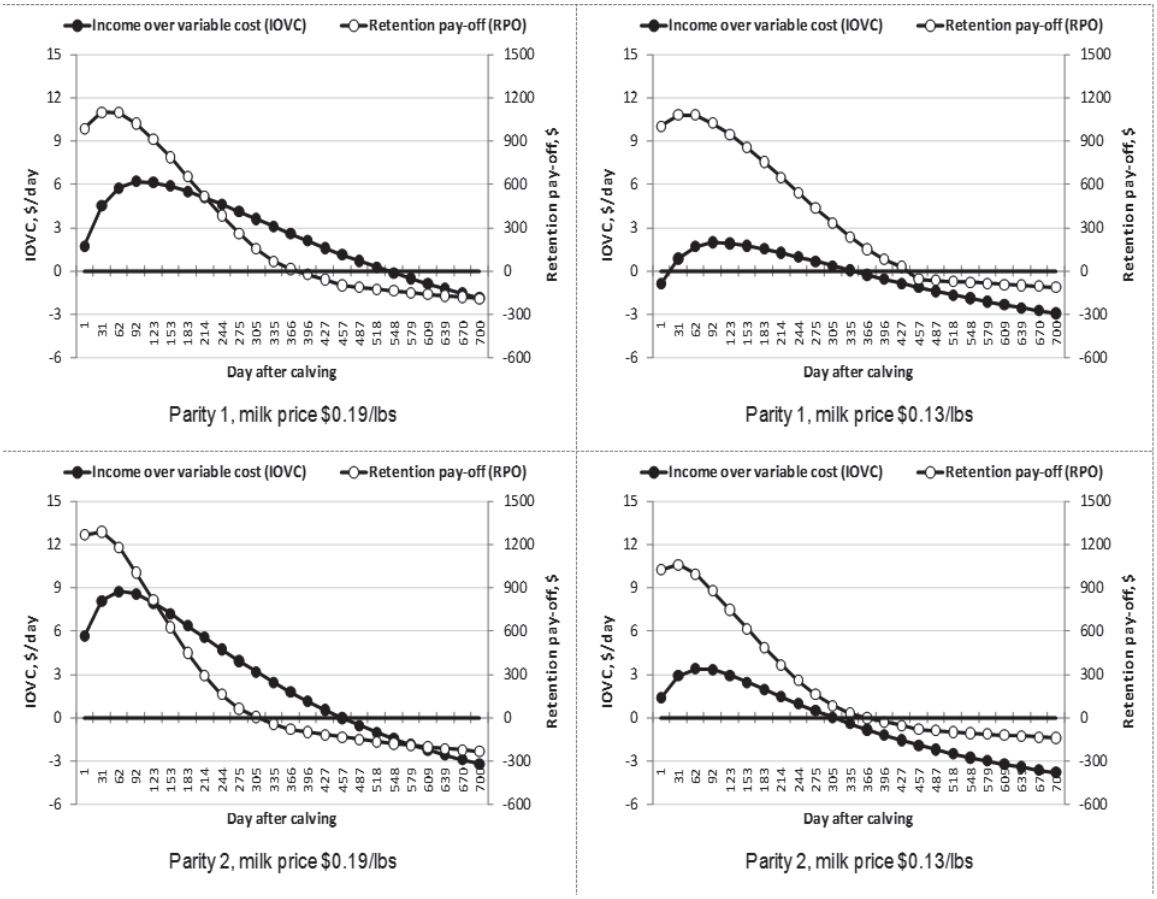

Figure 5. Retention pay-off (RPO) and Income over variable costs (IOVC) by days after calving for average first and second parity cows at higher ($0.19 per lbs) and lower ($0.13 per lbs) milk prices.

Figure 5 shows the RPO and IOVC of average first and second parity cows at higher ($0.19 per lbs) and lower ($0.13 per lbs) milk prices. The IOVC is clearly lower when milk price is $0.13 per lbs than $0.19 per lbs. The RPO reaches approximately $1100 in the 3rd month after calving and then gradually declines until it reaches a little below $0. The RPO

cannot be greatly negative because when it is below $0, it represents the opportunity cost of exactly one month too late culling and replacement with a heifer. This happens because DairyVIP calculates optimal monthly culling decisions.

When the milk price is higher, the RPO reaches $0 faster than when milk price is lower. When milk is $0.19 per lbs, the RPO reached $0 at day 396 when the IOVC was still $2.10 (first parity cows). For second parity cows, the RPO reached $0 at day 335 and the IOVC was $2.46. The IOVC did not reach $0 until day 548 and 457, respectively.

When milk price was $0.13 per lbs, IOVC reached $0 on day 366 while the RPO was still $152 for first parity cows. The RPO dropped below $0 on day 457. The IOVC was then -$1.12. For second parity cows, the IOVC reached $0 on day 335 when the RPO was still $30. The RPO dropped below $0 on day 466. The IOVC was then a loss of $0.81 per day.

It may appear strange that the RPO > $0 rule says to keep a cow at least one month longer when her IOVC may be negative (when milk prices are low). Such cows, at that moment, do not generate enough milk sales to cover their variable costs. But there is still a chance that they get pregnant and make it to the next lactation. Although these pregnant cows would have to go through an extended period of low milk production, at a low milk price, this option is still less costly than culling the cow and replacing her with a heifer. If heifer prices decrease, the cows should be culled faster.

Cows should be culled faster when beef prices are higher and sufficient heifers are available. Higher beef prices do not affect the IOVC rule, but will decrease the RPO. In Figure 5 this means that when milk prices are $0.19 per lbs, the IOVC at culling based on the RPO rule is even greater. At a milk price of $0.13 per lbs, the IOVC at culling may become positive.

4) Ranking cows for culling decisions in practice

The dynamic programming algorithm is able to rank cows for optimal culling decisions, but the current implementations of the method in working software also have some drawbacks. For example, cows with negative RPO have very similar RPO and their relative ranking is not clear. Some cows with negative RPO may be kept a bit longer when replacement heifers are not immediately available.

Secondly, the prediction of future cow performance, and hence her cash flows, is generally simplistic in dynamic programming models and does not include individual cow characteristics beyond parity, level of milk yield, pregnancy status (open or pregnant) and days in milk. For example, differences in milk components, shapes of lactation curves and detailed reproductive data (days bred, timing and chances of conception) are generally ignored.

We developed a Visual Basic Application in Excel (“spreadsheet”) to calculate RPO-like values for individual cows that include more detail about individual cow characteristics and therefore are more accurate cash flow predictions. This spreadsheet does not optimize culling decisions, but does include replacement costs similarly to the dynamic programming model. The spreadsheet is currently used on a 2200-cow

Florida dairy farm with two locations of about 1100 cows each. Culling decision are made monthly a few days after DHIA test day. When new test day results are available, we generate a PCDART report with relevant database items for each cow, such as current milk, protein and fat yields, days in milk, days bred, parity, pregnancy status etc. Some additional inputs are entered, such as prices of milk, feed, cull cows, and heifers. The spreadsheet calculates the RPO (Keep value) for each cow. It also calculates the Keep ranking and Keep percentile, which is the percentile of the ranking of the Keep value in the herd (1% to 99%). Both Keep values and Keep percentiles are imported back into PCDART and included in the PCDART cull report by the farm. This cull report includes other data base items that are not part of the spreadsheet calculations, such as rating and somatic cell count. Experiences are that the Keep percentile is an intuitive value for an initial ranking of the cows. The Keep value, in addition to other items such as somatic cell count, lameness status, and body weight are used to cull within the Keep percentile.

We evaluated the Keep rankings and Keep values of the 1148 cows included in the February 2016 DHIA test of the dairy farm. Figure 6 is a snapshot of the Keep values and Keep percentiles in the PCDART cull report the farm uses.

012 - COMBINATION-- LOW COWS & 30 DAY DRY-OFF barn#2

GRP	Index	Days In Milk	Prev T.D. Milk	Curr T.D. Milk	KEEP VALUE	KEE PCT	Pre SCC Act	Cur SCC Act	LTD Milk	Proj ME Milk	RAT	No Br	CDE	Bred Heat Date	Due Date	Date To Dry
7	1437	434	50.0	48.5	-413	3	1131		24799	22473	D		C			
16	72	64		11.6	-382	4										
7	490	398	53.0	52.4	-361	4	325		22484	21350	C		C			
6	2194	350	58.0	44.6	-310	4	141		21872	26304	B		C			
16	77	63		15.5	-229	5										
7	1330	210	44.0	44.6	-243	5	1600		5621	13553	E		C			
7	409	366	48.0	44.6	-133	6	93		24323	24235	B		C			
16	73	64		31.0	-26	7										
16	61	69		36.9	231	11										
7	1859	289	44.0	34.9	298	14	33		15139	19246	E	1	P	08/07	05/13	03/19
7	2791	242	55.0	36.9	306	14	33		9021	20905	D	2	P	10/08	07/14	05/20
4	2866	202	69.0	68.0	344	15	3430		8401	26884	A		C			
7	1198	310	41.0	34.9	413	17	100		15790	18697	E	1	P	07/17	04/22	02/27
7	2291	279	56.0	34.9	412	17	54		12804	20768	D	1	P	08/14	05/20	03/26
8	1754	281	66.0	46.6	513	22	123		15621	23069	D	1	P	08/14	05/20	03/26
7	2513	394	42.0	29.1	523	23			17904	21607	C	4	P	08/18	05/24	03/30
7	2642	315	45.0	36.9	561	24	141		11638	18001	E	1	P	07/10	04/15	02/20

Figure 6: Keep value and Keep percentile (KEE PCT) added to a PCDART report to rank and cull cows. The report is sorted by KEE PCT. Only part of the report is shown.

Keep values were on average $952 with a maximum of $3314 and a minimum of -$802. Some changes in prices had very small effects on the rankings. For example, the correlation of a ranking based on milk price of $0.20 per lbs ignoring components, and a ranking based on $0.05 per

lbs milk with $1.77 per lbs of fat and $2.94 per lbs of protein, was 0.995. Knowing milk components had little effect on the ranking for culling decisions.

A doubling of the beef price from $0.50 per lbs of body weight to $1 per lbs of body weight reduced the average Keep value to $468. The rank correlation between both rankings for body weights was high at 0.989, however.

A combination of differences in average milk price, milk component pricing, and body weight price resulted in a rank correlation of 0.946. The correlation between the actual Keep values was 0.902 in this case. For cows with a Keep ranking around 600 with one set of inputs (about the middle of the herd), the difference between the highest and lowest rank was about 400 for the other set of inputs (400 to 800). This example shows that changes in prices cannot be ignored when ranking cows for practical culling decisions.

Experiences with the Keep values and Keep rankings are thusfar positive and the spreadsheet is being improved monthly after feedback from the dairy farm.

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Circadian Patterns of Feed Intake and Milk Composition

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SUMMARY

The dairy cow has a well-recognized natural daily pattern of feed intake and milk synthesis, but regulation of these circadian rhythms has not been well described in the literature or well considered in current dairy management. Additionally, even when a total mixed ration (TMR) is fed, ruminal fermentation varies over the day due to daily variation in feed consumption which results in up to 3 to 8 times more fermentable substrate entering the rumen during the high intake periods of the day than the low intake periods of the day. Integrating knowledge of circadian patterns of intake and milk synthesis may improve milk yield and feed efficiency by stabilizing rumen fermentation and temporally matching nutrient absorption and mammary requirements, thereby altering the interactions between central and peripheral circadian clocks. Although this area of research is very new, it has been demonstrated that there is a daily pattern to milk fat and protein synthesis that is dependent on the timing of nutrient intake. It is reasonable to expect that synchrony of the rhythm of nutrient absorption and the metabolic capacity of the mammary gland is essential for maximal efficiency of conversion of nutrients to milk, but dyssynchrony may occur if the rhythm of intake and mammary metabolism entrain to different cues

INTRODUCTION

Circadian rhythms refer to twenty-four hour repeating cycles followed by most physiological functions. These rhythms originate from endogenous timekeepers, which are also called biological clocks. Recent discoveries have clearly described circadian time-keeping mechanisms in metabolically important peripheral tissues (e.g. adipose and liver) that are responsive to environmental factors such as timing of food availability. Interestingly, in many experimental models the timing of food intake can alter the synchrony between the central master timekeeper and peripheral clocks, resulting in development of numerous disorders including obesity, insulin resistance, and metabolic diseases (Reviewed by Takahashi et al., 2008). Milk yield and feed efficiency may be improved by temporally matching nutrient absorption and mammary requirements while maintaining synchrony of endogenous clocks (Figure 1). The daily rhythms of intake, milk synthesis, and the central master and mammary clocks are likely primarily regulated by the light/dark cycle, the timing of feeding, milking, and the daily rhythm of nutrient absorption (Figure 2).

BIOLOGICAL CLOCKS

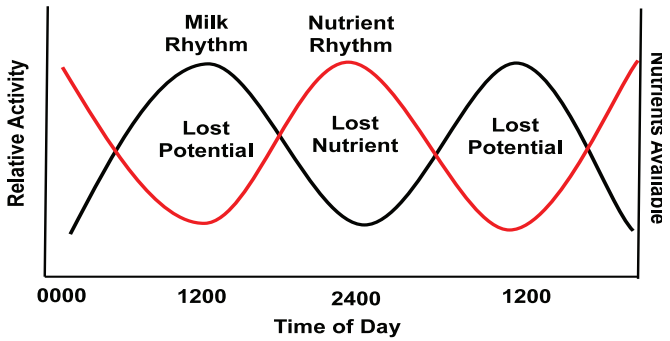

Figure 1: Illustration of the potential impact of unsynchronized rhythms of nutrient absorption and milk synthesis.

The classical characteristic of a circadian rhythm is a repeating 24 h cycle that fits a cosine function. Rhythms can vary in their amplitude, period (length of cycle), or phase (time of peak) and are controlled by endogenous circadian clocks (timekeepers) found in most tissues of virtually all living organisms. Clocks enable the synchronization of behaviors and physiological processes with changes in the external environment. Clocks also permit the coordination of internal activities in one organ with complementary processes that occur in a different organ, all within the same animal. In mammals, the dominant circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus, which organizes the temporal activity of peripheral clocks located throughout different organs of the body by regulating a series of neural and hormonal signals (See review Dibner et al., 2010). Additionally, Plasma glucose, non-esterified fatty acids (NEFA), blood urea nitrogen (BUN), and insulin concentrations are nutritionally important factors that follow daily rhythms in the cow (Lefcourt et al., 1999, Giannetto and Picciano, 2009).

CIRCADIAN PATTERN OF INTAKE AND ITS PHYSIOLOGICAL IMPACT

Discussion of the daily rhythms of metabolism must be integrated with a discussion of the pattern of feed intake and nutrient absorption. Feeding behavior is centrally regulated through integration of many factors including hunger, satiety, physiological state, environment, and endogenous circadian rhythms. Grazing cows have a well described "crepuscular" feeding pattern with a large proportion of intake consumed at dawn and dusk (Reviewed by Albright, 1993). Feeding behavior of lactating dairy cows has more recently been studied using automated observation systems and found to follow a slightly modified crepuscular pattern (e.g. Dado and Allen, 1994, Shabi et al., 2005). We have used an automated feed observation system at Penn State to investigate the impact of feeding

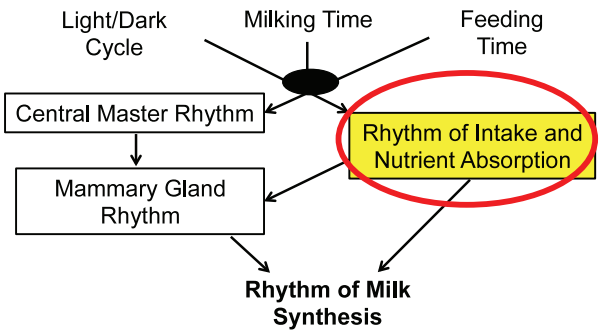

Figure 2: Integrated illustration of environmental cues and interaction of the daily rhythm of intake, mammary metabolism, and milk synthesis. This proposal focuses on the impact of the rhythm of nutrient absorption on milk synthesis.

time and diet composition on the daily rhythm of intake. To provide an example, the daily pattern of intake in high producing cows is shown as the percent of daily intake per hour in cows fed 1x/d at 0830 h or 2030 h [Figure 3; (Niu et al., 2014). Over 20 and 34% of daily intake was consumed in the 2 h after feeding in cows fed at 0830 and 2030 h, respectively. The intake rate at other times of day did not differ greatly, with both groups at a low level of intake overnight (2400 to 0500 h; 2.2%/h ± 0.77 mean ± SD) and a moderate level of intake in the afternoon (1200 to 1700 h; 4.9%/h ± 1.1, Mean ± SD).

The ruminant has a more consistent absorption of nutrients over the day because of more frequent meals, the size of the rumen, and the slow rate of ruminal digestion. However, highly fermentable diets are commonly fed to maximize energy intake and microbial protein production and result in a rapid production of volatile fatty acids (VFA) after consumption (Allen, 1997). Total mixed ration feeding was developed to provide a consistent *concentration* of fermentable substrate in each meal over the day and has become the standard practice for feeding dairy cattle. However, differences in the rate of feed intake over the day results in a large difference in the *amount* of fermentable substrate entering the rumen over the day.

The dynamic nature of rumen fermentation is supported by high resolution observations of rumen pH which clearly show a daily pattern of rumen pH with a nadir approximately 10 h after feeding (e.g. Yang and Beauchemin, 2006, Harvatine, 2012). Ruminal digesta weight and starch concentration were 24% and 87% higher, respectively, 4 h after feeding compared to 1.5 before feeding (Data not shown). Additionally, ruminal starch and NDF concentration over the day fit a cosine function with a 24 h period demonstrating a daily rhythm (Data not shown). A daily rhythm has also been reported for fecal particle size, NDF, iNDF, and starch concentration (Maulfair et al., 2011) and the rhythm of fecal NDF was dependent on the time of feeding (Niu et al., 2014). Changes in the rate or composition of duodenal flow throughout the day has not been

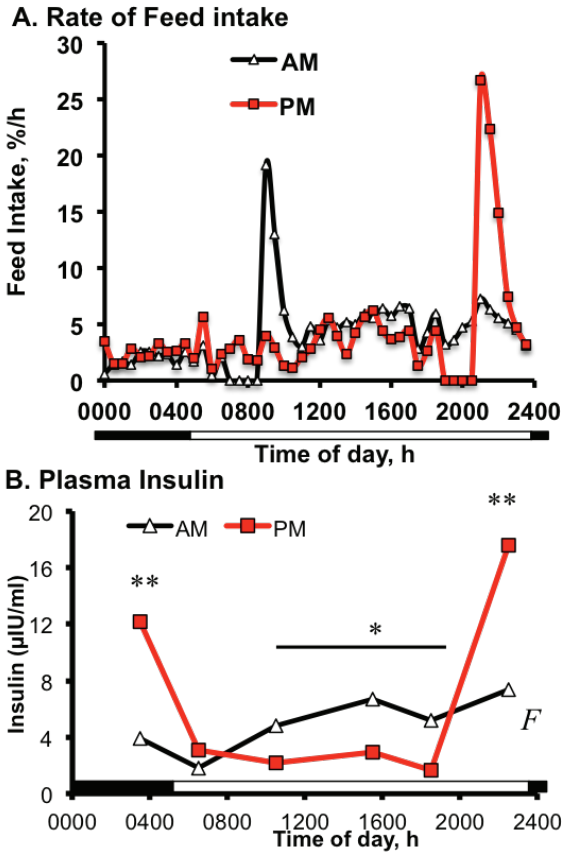

Figure 3: Daily pattern of feed intake (% DMI/h) and plasma insulin in cows fed 1x/d at 0830 (AM) or 2030 (PM) h. Treatment differences at each time point are shown for insulin (* $P < 0.05$, ** $P < 0.01$). Light and dark phases are shown below the x- axis.

reported to my knowledge, but taken together, there is strong support for a circadian rhythm of nutrient absorption in the cow.

Many factors impact feeding behavior of dairy cows (von Keyserlingk and Weary, 2010). The dairy cow may have a preferred feeding rhythm, but must also consume a large amount of feed to meet energy demands and adapt to feeding and milking times selected by farm management. The effect of feeding time on milk yield and intake has been investigated in a limited number of experiments. Piccione et al. (2007) demonstrated that the timing of feed availability entrained the rhythm of urea synthesis in restricted fed cows. We recently observed increased insulin and lower plasma glucose after feeding in cows fed at 2030 h compared to 0830 h, presumably due to higher intake immediately after feeding in the evening (Figure 3). Nikkhah et al. (2008) observes a similar response in cows fed at 0900 and 2100 h.

The variation in intake across the day led us to ask if diet composition should be changed across the day. To test this, we fed a 31.5% NDF TMR 1 x/d at 0800 h or fed the same diet components as a 32.5% NDF diet fed at 0.7 times daily intake and a 29% NDF diet fed at 0.3 times daily intake. When the high NDF diet was fed first (0800 h) followed by the low NDF diet in the evening (2200 h) milk yield and milk composition and empty body weight gain did not change, but dry matter intake decreased ~6% (Data not shown). Previously, Robinson et al. (1997) stabilized ruminal fermentation by feeding a protein supplement (15% DMI) at 0300 h compare to 0830 h in cows fed a TMR twice daily at 0800 and 1800 h. Specifically, they observed increased ruminal organic matter and crude protein digestibility, increased ruminal VFA concentration, a more stable ruminal pH, and increased milk fat with supplementation at 0300 h.

MOLECULAR CLOCKWORK

Mammalian circadian clocks include a network of transcription factors that are referred to as the “core clock” genes (Figure 5). These include Period 1 and 2 (PER1, PER2), Brain-muscle-arnt-like 1 (BMAL1), Cryptochrome 1 and 2 (CRY1, CRY2), and CLOCK (See reviews by Dibner et al., 2010). The transcription and translation of these so called “clock genes” is rhythmic and their collective output regulates the timing of circadian rhythms at the cellular and systems levels.

Recent evidence demonstrates that the time of day in which food is available can entrain circadian rhythms in a variety of animals without affecting the phasing of activity in the SCN. The effect of diet on circadian regulation in peripheral tissues is currently a prolific area of research (See reviews Bass and Takahashi, 2010, Stangherlin and Reddy, 2013, Tahara and Shibata, 2014). When the SCN is removed, mammals will typically exhibit more robust entrainment to feeding cycles, suggesting that 1) food and nutrient availability are potent organizers of the circadian systems and 2) peripheral clocks are impacted more by cyclical availability of food than the central pacemakers (Hara et al., 2001, Escobar et al., 2009). Genetic

disruption of some core clock genes results in obesity and metabolic syndrome in mice, and "shift working" mice and humans who eat outside their natural pattern experience a desynchronization of tissue clocks and increased incidence of obesity and metabolic disorders (See Bass and Takahashi, 2010).

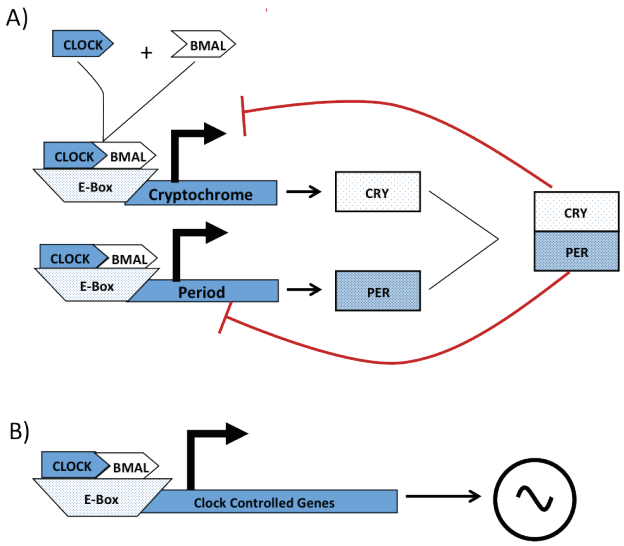

Figure 5: The core circadian clock. A) CLOCK and BMAL heterodimerize and promote transcription of genes containing E-boxes in their promoter regions, including CRY and PER. CRY and PER dimerize to inhibit CLOCK/BMAL E-box activation, effectively shutting down CRY and PER transcription to create a molecular transcription/translation feedback loop. B) CLOCK/BMAL can activate the transcription of "clock controlled genes", which also have E-boxes in their promoter regions. Among these clock controlled genes are those involved in the regulation of lactation.

CIRCADIAN REGULATION OF MILK SYNTHESIS

Dairymen commonly recognize that morning and evening milking differ in milk yield and composition. Gilbert et al. (1972) reported 0.65 kg higher milk yield at the morning milking, but 0.32 and 0.09 percentage unit higher milk fat and protein, respectively, at the evening milking in cows milked at 12 h intervals. More recently, Quist et al. (2008) conducted a survey of the milking-to-milking variation in milk yield and composition on 16 dairy farms. Milk yield and milk fat concentration showed a clear repeated daily pattern over the 5 days of observation in herds that milked 2 and 3 x/d. We have recently observed milk yield and milk component yield at each milking while milking every 6 h and feeding cows 1 x/d at 0800 h or in 4 equal feedings every 6 h [0600, 1200, 1800, and 2400 h; Figure 6;(Rottman et al., 2014)]. This demonstrated the daily pattern of milk synthesis in high producing dairy cows and identified an interaction with the timing of feed intake discussed below (Mean MY = 47.7 kg/d).

Photoperiod also has a well-characterized effect on milk synthesis (Reviewed by Dahl et al., 2000). Milk yield is on average 2.5 kg/d higher with long-days (16 h light), although the mechanism responsible has not been directly demonstrated. In other experimental models the effects of photoperiod are dependent upon biological clocks, thus these data strongly support a role of the circadian system in milk synthesis. The response to long photoperiod is lost when animals are placed under constant light, a condition that is known to abolish circadian rhythmicity further supporting the involvement of a biological clock (Dahl et al., 2000).

Automated milking systems (**AMS**) provide an opportunity to observe a natural preference for milking time. Care is needed in interpretation of cow behavior in AMS because of the confounding factors of demand for the robot and the entrainment of behavior by lighting, feeding, and manager intervention. However, the frequency of cows entering the milking system appears to follow a circadian pattern. For example, Wagner-Storch et al. (2003) reported 2% of cows in the holding area between 0000 and 0500 h compared to 8 to 12% of cow between 0800 and 1900 h. This preference for milking time may be due to a natural circadian synchronization of milking with physiology and other behaviors.

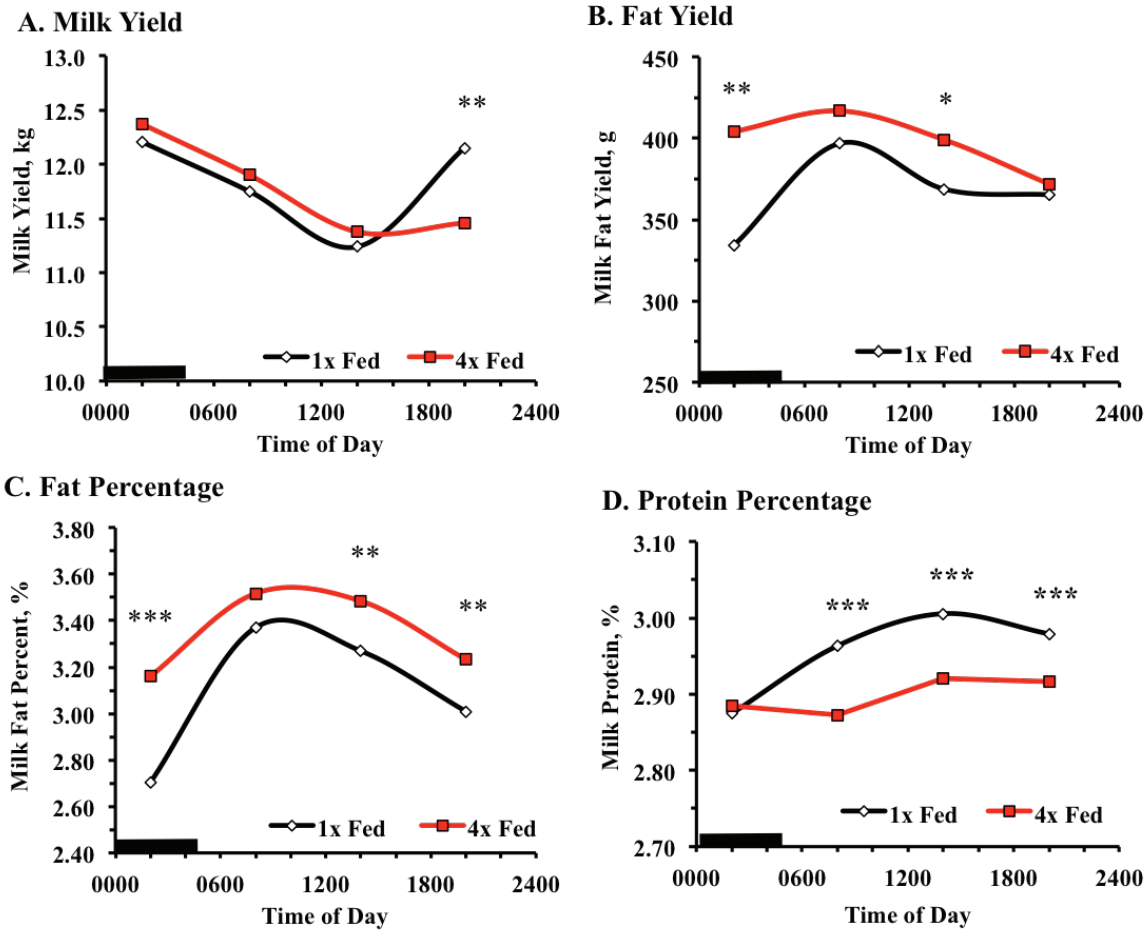

Figure 6: Temporal pattern of milk synthesis in cows fed 1 x/d at 0800 h or in 4 equal meals every 6 h (0600, 1200, 1800, and 2400 h; Rottman-Gredell et al., 2014). Model tested effect of feeding frequency (F), time (T), and their interaction (FxT). Panel A: Milk yield (F $P = 0.75$, T $P < 0.001$, and FxT $P < 0.05$), Panel B: Milk fat yield (F $P < 0.001$, T $P < 0.01$, and FxT $P < 0.05$), Panel C: Milk fat concentration (F $P < 0.001$, T $P < 0.001$, and FxT $P < 0.05$), and Panel D: Milk protein concentration (F $P < 0.001$, T $P < 0.001$, and FxT $P < 0.001$). Treatment differences at each time point are shown (ŧ $P < 0.10$, * $P < 0.05$, ** $P < 0.01$). All fit a cosine function with a 24 h period. Feeding 4x/d modified the phase of all and amplitude of fat and protein percent (Data not shown). Black bar shows the dark phase.

INTERACTION OF THE PATTERN OF INTAKE AND MILK SYNTHESIS

Theoretically, feed efficiency is maximized when nutrient requirements are exactly met by the diet, and nutritionists traditionally use a stoichiometric approach to match *daily* nutrient requirements with predicted nutrient absorption. A day is a meaningful unit, but the rate of milk synthesis varies over the day and is dependent on minute-to-minute availability of substrate (Illustrated in Figure 1). To investigate the effect of the daily pattern of intake on milk synthesis we recently conducted an experiment testing the effect of feeding the same TMR 1 x/d at 0800 h or feeding ad libitum in equal meals every 6 h (Rottman et al., 2014). The daily rhythm of milk synthesis was observed by milking cows every 6 h for the last 7 d of each experimental period. As previously discussed, we observed a daily rhythm of milk, milk fat, and milk protein synthesis, but we also observed treatment by time interactions (Figure 6). Importantly, feeding equal meals every 6 h decreased the amplitude of the daily rhythm for milk fat percent by ~50% and increased daily milk fat yield by 8.3% ($P < 0.001$). Increasing the frequency of offering feed from 2 to 4 or 6 x/d was intensively investigated in the 1980's and normally resulted in little effect on milk yield, but more commonly increased milk fat concentration especially when diets induced moderate or severe milk fat depression (Reviewed by Gibson, 1984, Sutton, 1989). Milk fat synthesis is nutritionally regulated by bioactive *trans* fatty acids formed as intermediates of ruminal biohydrogenation of polyunsaturated fatty acids under conditions of unstable ruminal fermentation (See review Harvatine et al., 2009). Loor et al. (2004) reported a daily pattern of ruminal *trans* fatty acids isomers in rumen fluid of cows fed high oil diets and the daily rhythm of milk fat synthesis may be due to daily dynamics of *trans* fatty acid absorption. Interestingly, in our experiment frequent feeding did not change the ratio of *de novo* to preformed FA in milk fat or the bioactive *trans* FA over the day. Additionally, to test if the mammary gland is more sensitive to bioactive FA at certain times of the day we analyzed the pattern of milk synthesis in two experiments that milked cows 3x/d at equal intervals and induced milk fat depression with *trans*-10, *cis*-12 CLA infusion or a low forage and high oil diet. We observed a rhythm of milk fat synthesis over the day, but milk fat was equally depressed across the day (Ma et al., 2015).

CONCLUSIONS

Most physiological processes exhibit circadian rhythms that are driven by central and peripheral timekeepers that improve fitness by synchronizing physiological processes so that they occur at the optimum time of day and by allowing anticipation of changes over the day. Disruption of central and peripheral rhythms has been shown to have significant implications for metabolism and health in rodents and

humans. It is possible that many feeding and management schedules disrupt circadian rhythms in the cow, resulting in reduced milk yield and feed efficiency. Additionally, TMR feeding was developed just over three decades ago and understanding the regulation of the circadian rhythm of the mammary gland allows development of “second generation” feed management strategies that integrate knowledge of circadian rhythms. The impact the daily pattern of intake and milk synthesis should be considered in dairy management and further experiments will be required to specifically define the role of each factor on the dairy cow.

ACKNOWLEDGEMENTS

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Leaky Gut's Contribution to Heat Stress and Poor Transition into Lactation

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INTRODUCTION

Suboptimal milk yield limits the U.S. dairy industry's productive competitiveness, marginalizes efforts to reduce inputs into food production, and increases animal agriculture's carbon footprint. There are a variety of situations in a cow's production cycle when nutrient utilization is reprioritized from milk synthesis towards agriculturally unproductive purposes. Two well-known examples that markedly reduce milk production are heat stress (HS) and the metabolic maladaptation to lactation (i.e., ketosis) following calving. Heat stress negatively impacts a variety of dairy production parameters including milk yield, milk quality and composition, rumen health, growth and reproduction, and is a significant financial burden (~$1 billion/year for dairy the U.S. alone; St. Pierre et al., 2003). Similarly, ketosis is a costly disorder (estimated at ~$300 per case; McArt et al., 2015) and also represents a major obstacle to farm profitability. While the metabolism of ketosis and HS has been studied for more than 40 years, the actual pathologies of both remain poorly understood. Suboptimal feed intake, experienced during both metabolic disorders, is unable to fully explain the

decrease in productivity. In other words, the initial insult in the cascade of events ultimately reducing milk synthesis in both heat-stressed and ketotic cows has not been identified.

Heat Stress

Many reports indicate the global surface temperature is expected to increase (IPCC, 2007). High ambient temperature, especially when coupled with elevated humidity, imposes severe thermal stress and reduces performance in all agriculturally important species (Baumgard and Rhoads, 2011, 2013; Belhadj Slimen et al., 2015). Heat stress interferes with animal comfort and suppresses productive efficiency (Fuquay, 1981; Strong et al., 2015). Furthermore, it is well-known that selecting animals based on productivity increases their metabolic heat production which makes them less heat resistant. In other words, increased production decreases heat tolerance (Spiers et al., 2004; Brown-Brandl et al., 2004). During periods of HS, animals initiate major thermo-regulatory adaptations in order to maintain euthermia. The result of HS is underachievement of an animal's full genetic potential. It has traditionally been assumed that inadequate feed intake caused by the thermal load was responsible for decreased milk production (Fuquay, 1981; West, 2003; Strong et al., 2015). Presumably, reduced feed intake is a survival strategy as digesting and processing nutrients generates heat, especially in ruminants (i.e., thermic effect of feed; Collin et al., 2001; West, 2003). However, reduced feed intake only explains approximately 35-50% of the decreased milk yield during environmental-induced hyperthermia (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). Therefore, HS affects many production parameters either directly (i.e., decreased milk yield, increased mortality) or indirectly (i.e., via

decreased feed intake; Collier et al., 2006; Adin et al., 2009; Hansen 2009; Baumgard and Rhoads, 2011, 2013; Mahjoubi et al., 2014). The remaining "direct" effects of HS are explained by the fact that heat-stressed animals exploit novel homeorhetic strategies to direct metabolic and fuel selection priorities independent of nutrient intake or energy balance.

Ketosis

The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptations to support milk production. Early lactation dairy cattle enter a normal physiological state during which they are unable to consume enough nutrients to meet maintenance and milk production costs and animals typically enter into negative energy balance (NEB; Drackley, 1999). During NEB, cows mobilize non-esterified fatty acids (NEFA) in order to partition glucose for milk production in a homeorhetic strategy known as the "glucose sparing effect." These NEFA can undergo one of three fates: 1) energy production via complete oxidation through the TCA cycle; 2) partial oxidation to produce ketone bodies (acetone, acetoacetic acid, and β -hydroxybutyric acid [BHBA]; 3) re-esterification to form triglycerides (TAGs), which are either exported as very low density lipoprotein to deliver fatty acids extra-hepatic tissue or "stored" in the liver (Ingvartsen, 2006; Ingvartsen and Moyes, 2013; McArt et al., 2013). Mitochondria available oxaloacetate is needed for fatty acid derived acetate to enter the TCA cycle; however, oxaloacetate exits the TCA cycle because it is a key gluconeogenic precursor during NEB and therefore full NEFA oxidation is limited. The ruminant liver has limited ability to export the large amount of NEFAs mobilized from adipose tissue during NEB, resulting in hepatic TAG accumulation (Grummer, 1993; Drackley, 1999; Gross et al.,

2013). Consequently, ketone body production is a mechanism by which fatty acids can be partially oxidized in the liver and exported into the bloodstream as a water-soluble, transportable form of acetyl units to peripheral tissues. In dairy cattle, ketosis is arbitrarily defined as an excess of circulating ketone bodies and is characterized by decreases in feed intake, milk production, and increased risk of developing other transition period diseases (Chapinal et al., 2012). Epidemiological data indicate about 20% of transitioning dairy cows clinically experience ketosis (BHBA > 3.0 mM; Gillund et al., 2001) while the incidence of subclinical ketosis (>1.2 mM BHBA) is thought to be much higher (> 40%; McArt et al., 2012). Therefore, ketosis is thought to result from an imbalance in energy demand, excessive adipose tissue mobilization, and increased ketone body production in hepatic tissue (Drackley et al., 2001; Garro et al., 2014).

HEAT STRESS ETIOLOGY

Mechanisms responsible for altered nutrient partitioning during HS are not clear; however, they might be mediated by HS effects on gastrointestinal health and function as we and others have demonstrated HS compromised intestinal barrier function (Lambert et al., 2002; Dokladny et al., 2006; Yang et al., 2007; Pearce et al., 2013; Sanz-Fernandez et al., 2014). During HS, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat (Lambert et al., 2002), leading to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al., 2006), resulting in ATP depletion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight junction dysfunction and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013). As a result,

HS increases the passage of luminal content into portal and systemic blood (Hall et al., 2001; Pearce et al., 2013). Endotoxin, otherwise referred to as lipopolysaccharide (LPS), is a glycolipid embedded in the outer membrane of Gram-negative bacteria, which are abundant and prolific in luminal content, and is a well-characterized potent immune stimulator in multiple species (Berczi et al., 1966; Giri et al., 1990; Tough et al., 1997). Activation of the immune system occurs when LPS binding protein (LBP) initially binds LPS and together with CD14 and TLR4 delivers LPS for removal and detoxification, thus LBP is frequently used as a biomarker for LPS infiltration (Ceciliani et al., 2012). For a detailed description of how livestock and other species detoxify LPS see our recent review (Mani et al., 2012). Endotoxin infiltration during HS into the bloodstream which was first observed by Graber et al. (1971), is common among heat stroke patients (Leon, 2007), and is thought to play a central role in heat stroke pathophysiology as survival increases when intestinal bacterial load is reduced or when plasma LPS is neutralized (Bynum et al., 1979; Gathiram et al., 1987). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities to HS; such as an increase in circulating insulin (Lim et al., 2007). Infusing LPS into the mammary gland increased (~2 fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10 fold increase in circulating insulin (Rhoads et al., 2009; Stoakes et al., 2015c,d). Interestingly, increased insulin occurs prior to increased inflammation and the temporal pattern agrees with our previous *in vivo* data and a recent *in vitro* report (Bhat et al., 2014) suggesting LPS stimulates insulin secretion, either directly or via GLP-1 (Kahles et al., 2014). The possibility that

LPS increases insulin secretion likely explains the hyperinsulinemia we have repeatedly reported in a variety of heat-stressed agriculture models (Baumgard and Rhoads, 2013). Again, the increase in insulin in both models is energetically difficult to explain as feed intake was severely depressed in both experiments.

TRANSITION PERIOD INFLAMMATION

Recently, the concept that LPS impacts normal nutrient partitioning and potentially contributes to metabolic maladaptation to lactation has started to receive attention. Although LPS itself has not been the primary causative focus, general inflammation has been the topic of investigations. Increased inflammatory markers following parturition have been reported in cows (Ametaj et al., 2005; Bertoni et al., 2008; Humblet et al., 2006; Mullins et al., 2012). Presumably, the inflammatory state following calving disrupts normal nutrient partitioning and is detrimental to productivity (Loor et al., 2005; Bertoni et al., 2008), and this assumption was recently reinforced when TNFα infusion decreased productivity (albeit without overt changes in metabolism; Yuan et al., 2013; Martel et al., 2014). Additionally, in late-lactation cows, injecting TNFα increased (>100%) liver TAG content without a change in circulating NEFA (Bradford et al., 2009). Our recent data demonstrates increased inflammatory markers in cows diagnosed with ketosis only and no other health disorders. In comparison with healthy controls, ketotic cows had increased circulating LPS prior to calving and post-partum acute phase proteins such as LPS-binding protein, serum amyloid A, and haptoglobin were also increased (Fig. 1; Abuajamieh et al., 2015).

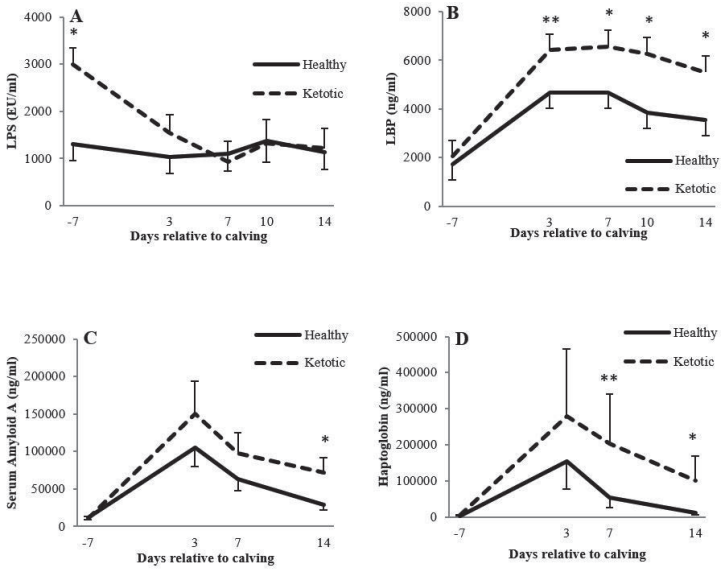

Figure 1. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows.

Endotoxin can originate from a variety of locations, and obvious sources in transitioning dairy cows include the uterus (metritis), mammary gland (mastitis) and the gastrointestinal tract (Mani et al., 2012). However, we believe intestinal permeability may be responsible for inflammation observed in the transition dairy cow. A transitioning dairy cow undergoes a post-calving diet shift from a mainly forage based to a high concentrate ration. This has the potential to induce rumen acidosis which can compromise the gastrointestinal tract barrier (Khafipour et al., 2009). In order to further investigate the effects of intestinal permeability on production and inflammation, we intentionally induced intestinal permeability in mid-lactation dairy cows using a gamma secretase inhibitor (GSI), a compound that specifically inhibits crypt stem cell differentiation into

enterocytes via disrupting Notch signaling (van Es et al., 2005). We anticipated feed intake of GSI administered cows would decrease, so we pair-fed controls in order to eliminate the confounding effect of feed intake. Treatment with GSI decreased feed intake and altered jejunum morphology consistently with characteristics of leaky gut (shortened crypt depth, decreased villus height, decreased villus height to crypt depth ratio). Circulating insulin and LBP were increased in GSI cows relative to controls. Interestingly in our GSI model, acute phase proteins serum amyloid A and haptoglobin increased for both treatments over time, indicating inflammation was occurring in pair-fed controls as well (Stoakes et al., 2014). This is not surprising, as pair-fed controls were receiving ~20% of their ad libitum intake and decreased feed intake has been shown to increase intestinal permeability in feed restricted rodents and humans (Rodriguez et al., 1996; Welsh et al., 1998) and we have also observed this in pigs (Pearce et al., 2013; Sanz-Fernandez et al., 2014). Recently, we confirmed the detrimental effects of feed restriction in mid-lactation cows by demonstrating a linear increase in circulating acute phase proteins and endotoxin with increasing severity of feed restriction. Furthermore, cows fed 40% of ad libitum intake had shortened ileum villous height and crypt depth, indicating reduced intestinal health (Stoakes et al., 2015b). In summary, inflammation is present during the transition period and likely contributes to changes in whole-animal energetics.

METABOLISM OF INFLAMMATION

LPS-induced inflammation has an energetic cost which redirects nutrients away from anabolic process that support milk and muscle synthesis (see review by Johnson, 1997, 1998) and thus compromises productivity and efficiency. Interestingly, immune cells become more insulin sensitive and consume copious amounts of glucose upon activation in order to support rapid proliferation and biosynthetic processes (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). In contrast, inflammation induces an insulin resistant state in skeletal muscle and adipose tissue (Liang et al., 2013; Poggi et al., 2007). Recent data has also demonstrated a decrease in ketone oxidation during LPS infiltration (Suagee et al., 2011; Frisard et al., 2015) which we believe may partly explain increased ketone body concentrations during the transition period.

Endotoxin has previously been recognized to be involved with metabolic dysfunction. For example, metabolic endotoxemia (increased circulating LPS) is frequently reported in obese animals, including humans (Cani et al., 2007, Gregor and Hotamisligil, 2011) and we have confirmed this in the obese Ossabaw pig model. Interestingly, dietary factors can induce metabolic endotoxemia as a high fat diet (saturated fat in particular) increases plasma LPS (Erridge et al., 2007; Mani et al., 2012). Further, LPS is involved with the development of fatty liver (Ilan, 2012), insulin-resistance in rodent models (Cani et al., 2007) and cardiovascular disease (Kelly et al., 2012). Experimentally-induced endotoxemia in dairy cattle has been linked to several metabolic and endocrine disturbances including decreased circulating glucose, termination of pregnancy, leukopenia, disruption of ruminal metabolism, and altered calcium homeostasis (Griel et al., 1975; Giri et al., 1990; Waldron et al., 2003; Jing et al., 2014). The aforementioned pathological

conditions are likely mediated by LPS-induced inflammation and the subsequent changes in nutrient partitioning caused by immune system activation.

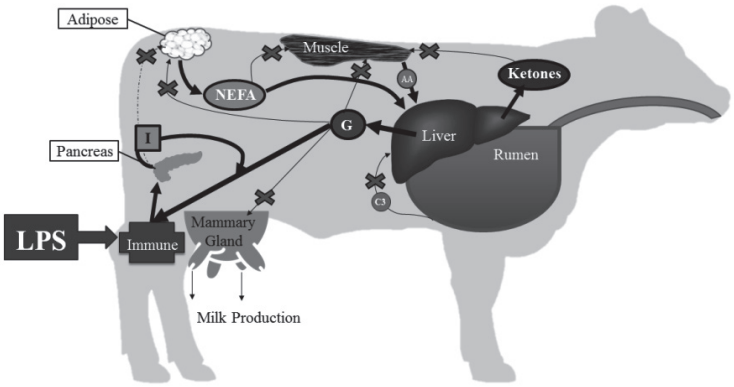

Figure 2. LPS induced alterations in glucose metabolism and insulin sensitivity.

Energetic Cost of Immune Activation

An activated immune system requires a large amount of energy and the literature suggests that glucose homeostasis is markedly disrupted (Leininger et al., 2000) during an endotoxin challenge. Upon immune system activation, immune cells switch their metabolism from oxidative phosphorylation to aerobic glycolysis, causing them to become obligate glucose utilizers in a phenomenon known as the Warburg Effect (Vander Hiden et al., 2009). Our group recently employed a series of LPS-euglycemic clamps to quantify the energetic cost of an activated immune system. Using this model, we estimated approximately 1 kg of glucose is used by the immune system during a 12 hour period in lactating dairy cows. Interestingly, on a metabolic body weight basis the amount of glucose utilized by LPS-

activated immune system in lactating cows, growing steers and growing pigs were 0.64, 1.0, and 1.1 g glucose/kg $BW^{0.75}$/h, respectively; Stoakes et al., 2015a,c,d). Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia obviously decrease the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool).

We and others have now demonstrated that both heat-stressed and ketotic animals have increased circulating markers of endotoxin and inflammation. We believe that the circulating LPS in both maladies originates from the intestine and thus both likely have an activated immune system. This activated systemic immune response reprioritizes the hierarchy of glucose utilization and milk synthesis is consequently deemphasized.

CONCLUSION

Altogether, our studies suggest that ketosis and heat stress may share the same etiology (i.e., decreased gut integrity) as indicated by altered intestinal morphology and increased plasma inflammatory biomarkers. This inflammation can redirect resources normally used for growth, milk production, and reproduction toward agriculturally unproductive purposes. More research is still needed to understand the mechanisms and consequences of intestinal permeability and associated inflammation in order to provide foundational information for developing strategies aimed at maintaining productivity during HS and the transition period.

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The "Goldilocks diet" 10 years on… is it still "just right"?

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INTRODUCTION

Our research group has studied the effects and potential benefits of controlling energy intake during the dry period. Although our initial reports (Grum et al., 1996; Douglas et al., 1998) were met with a great deal of skepticism, these concepts now are being widely applied worldwide with considerable success. The foundation of this approach is that dry cows and close-up cows should be fed to meet their requirements for energy, without either underfeeding or allowing cows to consume an excess of energy. By using bulky, low-energy forages to dilute higher energy of corn silage, cows can still be allowed to consume feed for ad libitum intake and eat to a maximum defined by rumen fill. If the energy density of the total diet is held low, the fill-limited dry matter intake (**DMI**) still provides only the amount of energy intake (Mcal/d) needed by the cow. Our research has shown that excesses of energy are counterproductive to trouble-free transitions. One of my former Ph.D. students working on this area (Nicole Janovick) named our controlled energy program the "Goldilocks diet" because it advocates for "not too much, not too little, but just right".

We and others have continued research to both increase our understanding of the mechanisms behind this approach as well as to determine optimal applications of the concept in the field. The purpose of this article is to provide an update on controlled energy diets in theory and in practice.

THE TRANSITION: TIME OF TROUBLESOME TURMOIL

Cows must make tremendous adaptations in metabolic systems during the transition from pregnancy to lactation. Historically, effectiveness of transition management programs was judged largely on reduction of metabolic disorders. While prevention of fresh cow health problems remains a hugely important part of transition strategies and potential for profit, more recent evidence indicates that even in the absence of clinical disease, suboptimal transitions result in significant losses of milk and decreased reproductive success (Ospina et al., 2010b). Elevated concentrations of nonesterified fatty acids (**NEFA**) in blood before calving and of NEFA and β-hydroxybutyrate (**BHBA**) after calving, indicating negative energy balance (**NEB**), are associated with increased incidence of displaced abomasum and clinical ketosis, decreased reproductive success, and decreased milk production (Ospina et al., 2010a,b). Increased NEFA and BHBA concentrations lead to fatty liver and ketosis interfere with normal function of the liver, which in turn may lead to clinical disease and loss of production independently of the NEB and elevated NEFA and BHBA (Bobe et al., 2004; Loor et al., 2007).

Periparturient diseases and disorders are strongly associated with NEB after calving. Until recently, a great deal of emphasis has been placed on maximizing energy intake during the close-up or pre-fresh period in an attempt to improve energy balance. This approach was designed on the basis of research showing advantages in adaptation of the rumen microbial population and rumen papillae to higher nutrient diets fed after calving, decreased body fat mobilization and fat deposition in liver, and compensation for declining DMI as calving approaches. Although each of these ideas were sound and based on at least some supporting research data, the ability of higher-energy close-up or "steam-up" diets to minimize production diseases in research trials and field experience has been disappointing.

It has now become clear that this approach does not lead to improved postpartum energy balance or transition outcomes, despite increased energy intakes before calving. Moreover, the causative nature of NEB leading to increased NEFA and BHBA has been questioned. Recent research has implicated the role of chronic inflammation as a major factor in periparturient health problems (Roche et al., 2013). Environmental stressors and metabolic imbalances may lead to loss of gastrointestinal tract barrier function, allowing endotoxin entry and resulting in inflammation (Bradford et al., 2015; Mani et al., 2015). Endotoxin administration can lead to many of the changes seen in displaced abomasum and retained placenta (Zebeli et al., 2011).

CONTROLLING ENERGY INTAKE DURING THE DRY AND CLOSE-UP PERIODS

In our view, the simplest and most easily defended principle of nutrition for dairy cows during the dry period and transition is to feed to meet but not greatly exceed requirements (Drackley and Dann, 2008). This concept is really nothing new, as it centers on formulating dry cow rations to dietary energy densities that were established many years ago by the National Research Council (**NRC**). Rethinking what these data and previous knowledge tell us about dry cows led us to a new interpretation relative to the former dogma, and to develop practical systems suitable for modern dairy management practices on both small and large dairies.

Our research group has shown that controlling energy intake during the dry period leads to better transition outcomes (Grum et al., 1996; Drackley, 1999; Drackley et al., 2001, 2005; Dann et al., 2005, 2006; Douglas et al., 2006, 2007; Loor et al., 2005, 2006, 2007; Janovick and Drackley, 2010; Janovick et al., 2011; Vasquez et al., 2011). Our research drew from earlier reports that limiting nutrient intakes to requirements of the cows was a preferable strategy to overfeeding (e.g., Kunz et al., 1985). The data we have collected demonstrate that cows fed even moderate-energy diets (1.50 – 1.60 Mcal NE_L/kg DM) will easily consume 40 – 80% more NE_L than required during both far-off and close-up periods (Dann et al., 2005, 2006; Douglas et al., 2006; Janovick and Drackley, 2010). Cows in these studies generally were about 3.0 body condition score at dry-off (essentially all less than 3.5), were housed in individual stalls, and were fed diets based on corn silage, alfalfa silage, and alfalfa hay with some concentrate supplementation. We have no evidence that the extra energy and nutrient intake was beneficial in any way. More importantly, our data indicate that allowing cows to over-consume energy even to this

degree may predispose them to health problems during the transition period if they face stressors or challenges that limit feed intake.

We have collected a variety of data indicating that prolonged over-consumption of energy during the dry period can result in poorer transitions. These data include whole-animal responses important to dairy producers such as lower post-calving DMI and slower starts in milk production (Douglas et al., 2006; Dann et al., 2006; Janovick and Drackley, 2010), as well as shortened days to pregnancy (Cardoso et al., 2014; Drackley and Cardoso, 2014). We also have demonstrated that overfeeding results in negative responses of metabolic indicators, such as higher NEFA in blood and more TG in the liver after calving (Douglas et al., 2006; Janovick et al., 2011). There are alterations in cellular (Litherland et al., 2011) and gene-level responses (Loor et al., 2005, 2006, 2007) that potentially explain many of the changes at cow level. For example, we recently showed that overfeeding during the close-up period actually *increases* the enzymatic "machinery" in adipose tissue for fat mobilization after calving (Ji et al., 2012). We also showed that controlling energy intake during the dry period improves neutrophil function postpartum (Graugnard et al., 2012, 2013) and so may lead to better immune function.

Our data demonstrate that allowing dry cows to consume more energy than required, even if cows do not become noticeably over-conditioned, results in responses that would be typical of overly fat cows. Because energy that cows consume in excess of their requirements must either be dissipated as heat or stored as fat, we speculate that the excess is accumulated preferentially in internal adipose tissue depots in some cows. We recently demonstrated that moderate overfeeding of non-lactating cows for 57 d leads to greater deposition of fat in visceral adipose tissues (omental, mesenteric, and perirenal) than in cows fed a high-straw diet to control energy intake at requirements (Drackley et al., 2014). The NEFA and signaling molecules released by some of these visceral adipose tissues go directly to the liver (Ji et al., 2014), which may cause fatty liver, subclinical ketosis, and other secondary problems with liver function. Abdominal obesity is a risk factor for disease in humans. Similarly, cows might vary in the degree to which they accumulate fat internally. The mechanisms we have been studying in dry cows are similar to those from human medical research on obesity, type II diabetes, and insulin resistance.

Other research groups in the US (Holcomb et al., 2001; Mann et al., 2015) and in other countries (Agenas et al., 2003; Kunz et al., 1985; Rukkwamsuk et al., 1998, Vickers et al., 2011) have reached similar conclusions about the desirability of controlling energy intake during the dry period, although not all studies have shown clear benefits (Winkleman et al., 2008). Our work has extended the ideas to show that over-consumption of energy is common even when feeding typical dry period diets thought to be "safe", and that this may be a predisposing factor to poor health. We also have extended the ideas of limit-feeding moderate energy diets or ad libitum feeding of high-straw, low-energy rations as simple and practical approaches to achieve the control of energy intake (Janovick and Drackley, 2010) as proposed by others (Beever, 2006).

HIGH BULK, LOW ENERGY DIETS FOR DRY COWS

A workable solution to the potential for cows to over-consume energy is to formulate rations of relatively low energy density (1.30 – 1.38 Mcal NE_L/kg DM) and high fiber that cows can consume free choice without greatly exceeding their daily energy requirements (Janovick and Drackley, 2010). The principle is to feed cows a diet of sufficient fiber (bulk) content that cows will only meet their requirements consuming all the DM they can eat. At the same time, it is critically important that the diet provide required amounts of metabolizable protein (Mann et al., 2015) and all minerals and vitamins.

Controlling energy intake requires that some ingredient or ingredients of lower energy density be incorporated into diets containing higher-energy ingredients such as corn or barley silage, good quality grass or legume silage, or high quality hay. Cereal straws, particularly wheat straw, are well-suited to dilute the energy density of higher-energy feeds, especially when corn (corn) silage is the predominant forage source. Lower quality grass hays also may work if processed appropriately, but still may have considerably greater energy value than straw and so are not as effective in decreasing energy density. Selecting low-potassium grass hay (and even low-potassium straw) is important to help control the DCAD.

We are aware of no controlled data comparing different types of straw, but it is the general consensus among those who have years of experience using straw that wheat is preferred. Barley straw is a second choice, followed by oat straw. While reasons for these preferences are not entirely clear, wheat straw is more plentiful, is generally fairly uniform in quality, and has a coarse, brittle, and hollow stem that processes easily, is palatable, and seems to promote desirable rumen fermentation conditions. Barley and wheat straws lack some of these characteristics and do not process as uniformly. In addition, oat straw generally is somewhat more digestible and thus has greater energy content. Prairie hay also is a good choice.

It is critical that the straw or other roughage actually be consumed in the amounts desired. If cows sort out the straw or other high bulk ingredient, then they will consume too much energy from the other ingredients and the results may be poor. A TMR is by far the best choice for implementing high-fiber diets to control energy intake. Few TMR mixers can incorporate large amounts of straw (30 to 50% of total dietary DM) without pre-chopping and without overly processing other ingredients. An exception that we have worked with is the Keenan paddle mixer with knives. Unless using that type of mixer, however, straw may need to be pre-chopped to 5-cm or less lengths to avoid sorting. Implements such as the “Balebuster” can be used to chop straw to a small and uniform particle length.

ADVANTAGES AND BENEFITS

Based on our research and field observations, adoption of the high-bulk, low-energy TMR concept for dry cows may lead to the following benefits:

- Successful implementation of this program essentially eliminates occurrence of displaced abomasum. This may result from the greater rumen fill (Drackley et al., 2014), which is maintained

for some period of time even if cows go off feed for some reason, or from the stabilizing effect on feed intake (Janovick and Drackley, 2010).

- A consistent finding in our studies cited earlier and in studies from others (Vickers et al., 2011; Mann et al., 2015) is a marked reduction in BHBA concentrations during the early postpartum period.
- Field survey data collected by the Keenan Co. in Europe (courtesy of D. E. Beever, Richard Keenan and Co., Borris, Ireland) indicate strongly positive effects on health. In 277 herds (over 27,000 cows) in the United Kingdom, Ireland, France, and Sweden, changing to the high-straw low-energy TMR system decreased assisted calvings by 53%. In addition, the change decreased milk fevers by 76%, retained placentas by 57%, displaced abomasum 85%, and ketosis by 75%. (Colman et al., 2011). Using standard values for cost of these problems, the average increase in margin per cow in these herds was $114 just from improved health alone. While these are certainly not controlled research data, they are consistent with our research results and field observations in the USA.
- The same sources of observational data indicate that body condition may cycle less with fewer extremes, and that reproductive success is improved (perhaps because of less change in body condition).
- Although data are limited, milk production appears to be similar to results obtained with higher-energy close-up programs (Vasquez et al., 2011; Mann et al., 2015). There is speculation in the field that persistency may be improved, with cows reaching slightly lower and later peak milk. Producers should be careful to not evaluate the system based on early peaks and should look at total lactation milk yield, daily milk, and, over time, indices of reproduction and other non-milk indicators of economic value.
- Straw and corn silage generally are lower in potassium and calcium, and thus help control the dietary cation-anion difference (DCAD) without excessive addition of anionic salt mixtures.
- The program may simplify dry cow management and ration composition in many cases.
- Depending on straw cost, rations based on corn or barley silage and straw likely will be no more expensive than the average cost of traditional far-off and close-up diets, and could be cheaper where straw is plentiful. Remember that even when straw appears expensive it replaces something else in the diet so marginal cost is the key criterion. Furthermore, total DMI per cow may be lowered by addition of straw, so that feed cost per cow per day can actually be decreased substantially.
- A recent “meta-analysis” of all of our research with transition nutrition and controlled energy diets during the dry period, including individual data from 404 cows, showed that overfeeding during the last 4 wk of the dry period resulted in an odds ratio for pregnancy of 0.69 compared with controlling energy. Thus, we now have data to show that overfeeding during late pregnancy not only does not improve milk production and increases risks of metabolic disorders, but also is associated negatively with reproductive success (Cardoso et al., 2013).

SINGLE DIET DRY COW PROGRAMS

An opportunity resulting from this work is use of single-group or single-diet dry period management strategy. Although not the primary focus of this paper, aspects of management such as overcrowding, feeding space, cow comfort, and movement among groups have emerged as hugely important determinants of transition success (Cook and Nordlund, 2004; Cook, 2007). Avoiding a group change during the late dry period would be a positive from the standpoint of cow behavior and social stressors as well as simplifying management on the farm.

Some of our research (Richards et al., 2009; Janovick and Drackley, 2010; Janovick et al., 2011; Vasquez et al., 2011) and research at Cornell University (Mann et al., 2015) as well as considerable field experience indicates that a single-diet dry cow program can be successful using these principles. Dry matter intakes remain more constant as cows approach calving when fed the high-straw low energy diets (Dann et al., 2006; Richards et al., 2009; Janovick and Drackley, 2010) than in cows fed high-energy close-up diets (Grummer et al., 2004). Single-group management may work particularly well for producers managing for shorter dry periods (45 – 50 days) and for smaller herds where trying to maintain separate far-off and close-up pens is difficult. A variation that some farmers may prefer is to maintain far-off and close-up groups, but use essentially the same diet for both except that a different concentrate mix or premix is used for the close-ups, which may incorporate anionic salts, extra vitamins and minerals, additional protein, or selected feed additives. The optimal high-forage low-energy dry cow ration will contain the primary forages fed in the lactation diet, but diluted with straw or low-quality forage to lower energy density. This way, the rumen remains adapted to the types of ingredients to be fed after calving without excessive energy.

We explored whether moving dry cows to a higher-energy close-up diet at 3 wk before calving benefits cows during the transition compared with a single high-bulk diet fed all the way through to calving (Richards et al., 2009). We included an overfed group, which received the higher-energy close-up diet during the entire dry period. The overfed group had greater DMI during the dry period but not during lactation; cows gained body condition during the dry period but lost more body condition after calving. Overfed cows had increased fat in the liver, greater and more prolonged increases in NEFA and BHBA after calving, and had greater milk fat production than the other two groups. The single-diet group had the least change in DMI around calving, and the lowest concentration of fat in the liver after calving. Surprisingly, the group provided the close-up diet had fat content in the liver that was intermediate to the single-diet group and the overfed group, but did not have any advantages to the single-diet group. A second experiment confirmed these findings (Vasquez et al., 2011). We have little evidence, therefore, that the two-group strategy offered any advantage compared with the single-diet (controlled-energy high-fiber) strategy. Conclusions from recent Cornell research (Mann et al., 2015) from an experiment very similar to ours (Richards et al., 2009) are the same.

If producers prefer to manage dry cows in the conventional two-group or “steam-up” philosophy for dry cow feeding, our research indicates that the most critical factor is to ensure that the energy density of the far-off dry period diet is decreased to near NRC (2001)

recommendations (NE_L of 1.25 – 1.30 Mcal/kg DM) so that cows do not over-consume energy (Dann et al., 2006). Although data are limited, we also would recommend that nutrient densities of the close-up diet be no more than half-way between the far-off and lactation diet, to minimize the extent of overfeeding.

SPECIFICATIONS FOR SINGLE-DIET DRY PERIOD RATIONS

Most of the research available on the controlled energy system was obtained with diets relying on corn silage as the primary forage. Typical rations generally contain roughly one-third of the DM as corn silage, one-third as chopped straw, and the remaining third split between some other hay or silage and a small amount of concentrate to meet protein, mineral, and vitamin needs. The combination of straw and corn silage is complementary for many reasons, including energy content, low potassium contents, starch content, and feeding characteristics.

The NE_L requirement for 700-kg Holstein dry cows is between 14.5 and 15 Mcal per day (NRC, 2001). Some suggested guidelines for formulation of controlled energy diets to meet that requirement are as follows, on a total ration DM basis.

- Dry matter intake: 12 to 12.5 kg per day. For far-off cows, intakes by individual cows often exceed 13.5 kg DM per day.
- Energy density: 1.30 – 1.38 Mcal NE_L/kg DM.
- Protein content: 12 to 15% of DM as CP; >1,000 g/day of metabolizable protein as predicted by the NRC (2001) model or CNCPS Dairy model. This usually requires addition of high-RUP sources such as blood meal or heat-treated soybean meal.
- Amino acid nutrition: Our recent research with methionine supplementation suggests that the Lys:Met ratio should be <3:1. Add a source of rumen-protected methionine.
- Starch content: 12 to 16% of DM. If starch is poorly fermentable diets should be at the upper end of this range.
- Forage NDF: 40 to 50% of total DM, or 4.5 to 5.5 kg daily (0.7 to 0.8% of body weight). Target the high end of the range if more higher-energy fiber sources (like grass hay or low-quality alfalfa) are used, and the low end of the range if straw is used.
- Total ration DM content: 45 – 48% (add water if necessary). Additional water will help hold the ration together and improve palatability. When ration DM exceeds 55%, DMI will decrease and sorting may increase.
- Follow standard guidelines for mineral and vitamin supplementation. For close-ups, target values are 0.40% magnesium (minimum), 0.35 – 0.40% sulfur, potassium as low as possible, a DCAD of +25 to +50 meq/kg, 0.27 – 0.35% phosphorus, and at least 1,500 IU of vitamin E. Calcium is typically set at about 0.9% of DM. Note that we do not aggressively attempt to lower the DCAD to negative values.
- Rumensin: should be included in the ration. Our recent research shows that milk production was increased by more than 2 kg/d when Rumensin was included in the dry period diet (Vasquez et al., 2011). Aim for intakes of at least 300 mg/d.

An area where our thinking and recommendations may be changing deals with calcium management at calving and use of anionic salts. The

controlled energy diet approach as worked well without aggressively trying to lower the DCAD to recommended negative values. This likely results from selection of low-potassium and low-calcium ingredients such as corn silage and wheat straw. Typically, we have recommended 0.35 – 0.40% sulfur, potassium as low as possible, and moving the DCAD toward zero (+25 to +50 meq/kg), with calcium set at about 0.6 to 0.9% of DM. A recently completed but unpublished experiment at Cornell University (T. R. Overton, personal communication) obtained evidence that an aggressive negative DCAD program resulted in less subclinical hypocalcemia and more milk than the typical partial acidification. We are undertaking experiments to further clarify these issues. We have seen good results with negative DCAD programs in the field.

As long as the lactation diet is formulated appropriately, there seems to be little difficulty in transitioning to the lactation diet immediately after calving. Many producers have found that inclusion of 0.25 to 1 kg of chopped straw in the lactation diet improves rumen function and animal performance, particularly when physical fiber is borderline adequate. Addition of the straw postpartum also may help to ease the transition from the lower-energy dry cow diet.

COMMON PROBLEMS IN FIELD IMPLEMENTATION

Sorting of the straw or lower-energy roughage is the key problem that may hamper success. The straw must be chopped into a particle size that cows will not sort, which in general means less than 5 – 7 cm particles. If the straw is pre-chopped, an appropriate chop is indicated by having about 1/3 of the particles in each of the three fractions of the Penn State shaker box. Because of the bulky nature of straw and the resulting TMR, producers may think that cows are sorting excessively when they are not. To verify that cows are not sorting, the feed refusals should be monitored carefully and compared to the original TMR. One simple way to evaluate sorting is to shake out the TMR with the Penn State box and then repeat the analysis on the feed refusals the next day. Results should not differ by more than 10% from TMR to refusal. Another way to monitor sorting is to collect samples of the feed refusal from several areas of the feedline and have it analyzed for the same chemical components as the TMR fed. Again, composition of NDF, CP, and minerals should not vary by more than 10% between ration and refusal if cows are not sorting. If cows sort the straw, some cows will consume a higher energy diet than formulated, and some (the more timid cows) will be left with a much lower quality ration than desired. Herds where sorting is a problem will be characterized by pens of dry cows that range widely in body condition: some will be over-conditioned and some under-conditioned, while of course some may be “just right”.

Another common pitfall is barn design or poor feedbunk management that limits the ability of cows to consume feed ad libitum. Because of the bulky nature of the diet, cows spend more time eating to consume enough feed to meet energy and nutrient requirements. As a result, having adequate bunk space in 6-row barns is problematic. Bunk space must be adequate and feed pushed up frequently. If feed is not pushed up, cows likely will not be able to consume what they need to meet requirements. Other common problems arise when the DM content of straw, hay, and silages changes markedly from assumed values. This may happen, for example, if the straw is rained on or the DM content of silage changes without the feeders knowing it. Changes in DM of the

ingredients mean changes in the DM proportions of the total diet unless the mix is corrected. Thus, energy intake may increase or decrease relative to the target, and a rash of calving-related health problems may occur until the situation is corrected.

SEPARATE CLOSE-UP DIETS

For producers using two-group dry cow management, our recent findings offer some guidance for proper formulation. If the far-off diet is appropriately low in energy (ca. 1.30 Mcal NEL/kg DM), then the energy density of the close-up diet can be set intermediate to the far-off and lactation rations. Make sure that metabolizable protein needs are being met; Holstein cows should receive a minimum of 1100 g/d of MP, with heifers consuming at least 950 g/d. Where producers are struggling with metabolic problems, efforts to boost the MP intake in mature cows to >1200 g/d sometimes have been successful in decreasing health problems.

Recently our group has shown that supplementation with protected methionine (Smartamine; Adisseo) or HMBi (Metasmart; Adisseo) during the close-up and post-fresh period improved lactation performance (Osorio et al., 2013). Cows fed either methionine source had greater DMI postpartum and greater milk yield. These results highlight the potential for strategic supplementation with limiting amino acids to improve production and metabolic outcomes.

OTHER CONSIDERATIONS

As mentioned earlier, the combination of straw and corn silage, along with other lactation ration ingredients, works well because of the complementary features of the components in the total diet. Straw has many desirable characteristics that seem to improve health and digestive dynamics in the rumen. The slow digestion and passage rate of straw certainly seems to be important in prevention of DA. Control of energy intake is a critically important factor in maintaining a more constant energy intake during the dry period and in preventing other disorders around calving such as ketosis and fatty liver.

Whether other low-energy ingredients will produce the same desirable results remains uncertain. We are not aware of research that has compared other low-energy ingredients such as poor-quality hay, oat hulls, cottonseed hulls, corn stalks, soybean residue, or flax shives to straw or to conventional rations, although we have anecdotal reports from producers and nutritionists with varying reports of success. With roughage-type materials, the key consideration is uniform processing and palatability so that cows do not sort and the formulated profile of nutrients is actually consumed. Care must be taken to not use moldy or weather-damaged materials or those that have excessive amounts of soil contamination. For concentrate-type or finely ground ingredients, energy content is low but particle size is so small that rate of passage can be too fast, allowing particles to escape more quickly even though they are not digested. In this case, DMI by the cows may increase so that total energy intake still exceeds requirements considerably.

Just because straw or other low-energy ingredients are “low quality” by conventional standards based on protein or energy content does not mean that other measures of “quality” can be ignored. Straw

or other feeds that are moldy, severely weather-damaged, or have fermented poorly should not be fed to dry cows, especially the close-ups. Producers are advised to lock in supplies of high-quality and consistent straw to minimize these problems.

SUMMARY AND CONCLUSIONS

While the single-diet dry period strategy does represent more of a "traditional approach" in terms of only having one group of dry cows, the nutritional considerations to make this successful should not be thought of as traditional in many senses. Controlling energy intake is exciting for its potential to markedly improve health during the transition period. The key concept is to strive to meet the requirements of cows for energy and all other nutrients, but to not allow cows to exceed their requirements for energy by large amounts for the duration of the dry period. A major aim is to provide consistent intake of all required nutrients. Provided that high-bulk low-energy rations are formulated, mixed, and delivered properly, results have been positive and consistent. Research and field observations indicate that the rations result in better energy balance after calving, with subsequent reductions in lipid-related health disorders. Milk production is maintained, and field observations suggest that reproductive success may be improved also, although data are lacking to date. Importantly, there is little evidence to indicate that the two-phase system (far-off and close-up) provides any advantage in terms of health or milk production.

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UPDATE ON STRATEGIES FOR PREVENTION OF MILK FEVER AND SUBCLINICAL HYPOCALCEMIA

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INTRODUCTION

Calcium is necessary for proper contraction of muscle. Severe hypocalcemia prevents skeletal muscle contraction to the point that the clinical syndrome known as milk fever occurs. While milk fever is a severe form of hypocalcemia, Reinhardt et al., (2011) found more than half of older cows developed subclinical hypocalcemia (blood Ca < 8.0 mg/dl) shortly after calving. Cows with sub-clinical hypocalcemia had elevated concentrations of non-esterified fatty acids compared with normocalcemic cows. Martinez et al (2012) demonstrated that sub-clinical hypocalcemia (blood calcium below 8.6 mg/dl) was a major risk factor for metritis. Cows with sub-clinical hypocalcemia were at a greater risk of developing fever, metritis, and puerperal metritis compared with normocalcemic cows. Prevention of hypocalcemia should be a major goal of all transition cow management and feeding programs.

PREVENTING HYPOCALCEMIA

Ca homeostasis is primarily controlled by the parathyroid glands, which respond to hypocalcemia by secreting parathyroid hormone (PTH). The primary target cells are bone osteoblasts and osteocytes, and kidney tubular epithelium cells. PTH action on bone cells is to bring calcium out of bone to support normal blood calcium. PTH action on the kidney is to reduce urine calcium losses and to stimulate the kidney to produce a hormone derived from vitamin D — 1,25-dihydroxyvitamin D. This hormone activates mechanisms in intestinal cells to enhance absorption of diet calcium. When the system is working normally, there is a small decline in blood calcium at the onset of lactation and then Ca homeostasis kicks into gear and blood calcium returns to normal in a matter of hours.

WHY DOES CALCIUM HOMEOSTASIS FAIL IN SOME COWS?

Mounting evidence suggests the key to milk fever prevention lies in reducing the degree of metabolic alkalosis experienced by the cow just before calving. Cows fed high potassium diets (High DCAD) are in a state of compensated metabolic alkalosis. We fed late gestation cows a High DCAD, alkalinizing diet or a Low DCAD, acidifying diet and treated the cows with synthetic exogenous PTH. The cows fed the alkalinizing diet had a greatly diminished response to the PTH compared to cows fed the acidifying diet. Their kidneys did not produce as much $1,25(OH)_2D$ and serum Ca did not rise as quickly. It appears the tertiary structure of the PTH receptor is altered during metabolic alkalosis, reducing its affinity for PTH and resulting in a state of pseudohypoparathyroidism (Goff et al., 2014). In highly alkaline cows,

despite the fact that bone and kidney cells are exposed to very high concentrations of PTH at the onset of lactation, they respond only poorly to the PTH. Addition of anions to a diet to counteract cations in the diet of a cow reduces the alkalinity of the blood and restores tissue responsiveness to PTH at the onset of lactation. Hypomagnesemia can also interfere with PTH function. It also affects the ability of tissues to respond to PTH and it can also inhibit PTH secretion (Goff, 2014).

DESIRED MINERAL PROFILE OF PRE-PARTUM DIET

The difference between the number of cation and anion particles absorbed from the diet determines the pH of the blood. The cation-anion difference of a diet is commonly described in terms of mEq/kg of just Na, K, Cl, and SO_4 (S) as follows:

> Dietary Cation-Anion Difference (DCAD) = (mEq Na^+ + mEq K^+) - (mEq Cl^- + mEq S^{--}).

This equation is useful, although it must be kept in mind that Ca, Mg, and P absorbed from the diet will also influence blood pH. Evaluation of the relative acidifying activity of dietary Cl vs. SO_4 demonstrates SO_4 is only about 60% as acidifying as Cl (Goff et al., 2004). The DCAD of a diet and its acidifying activity is more accurately described by the following equation: (Na^+ + K^+) - (Cl^- + 0.6 S^{--}). While DCAD equations provide a theoretical basis for dietary manipulation of acid-base status they are not necessary for formulation of mineral content of prepartum dairy cow rations because, with the exception of K and Cl, the rate of inclusion of the other macrominerals can be set at fixed rates.

The NRC (2001) requirement for Na in the diet of a late gestation cow is about 0.12%. A small amount of salt is added to the diet to prevent pica, which often is manifest as a desire to drink urine from the floor. Exceeding the requirement for Na using NaCl is to be avoided in late gestation because it will increase the risk of udder edema, not because it greatly affects acid-base status.

At least two studies have clearly demonstrated that inclusion of Ca in the diet at NRC required levels or several fold above NRC required levels does not influence the degree of hypocalcemia experienced by the cow at calving (Goff and Horst, 1997; Beede et al., 2001) . Beede et al. (2001) fed 0.47, 0.98, 1.52, and 1.95 % Ca diets to cows in late gestation being fed a high Cl diet to prevent milk fever. Cows fed 1.5% Ca diets had slightly reduced feed intake when compared to control cows while those fed the 1.95% Ca diet had significantly lower feed intake. Dietary Ca did not influence the degree of hypocalcemia experienced at calving or milk production in the subsequent lactation. It appears from this study that a close-up diet Ca concentration of 1% is optimal. This is similar to the level the cow will receive in the lactating diet and though higher diet Ca may contribute some extra Ca to the blood via the paracellular route of intestinal Ca absorption, higher dietary Ca could negatively impact feed intake.

Magnesium can only be absorbed from the rumen in cows. Potassium can inhibit the magnesium transporter in the rumen wall. To ensure adequate concentrations of Mg in the blood of the periparturient cow the dietary Mg concentration should be 0.35-0.4%. This level of Mg

ensures the Mg transporter in the rumen will work even in the face of the inhibitory effects of dietary potassium. This is particularly important in the pre-partum diet and the early lactation diet. Further, hypomagnesemia is the primary cause of mid-lactation milk fever in cows. The details behind this rationale will be discussed in detail in the section on Mg.

Dietary P concentration should meet but not exceed the NRC requirement for P in the late gestation cow. This is generally about 0.35% P for most cows. A diet supplying more than 80 g P/day greatly increases the risk of milk fever. Keeping dietary phosphorus below 50 g / day seems to be safe, though lower levels (35 g /day) improve Ca homeostasis (Peterson, et al., 2005). Keeping diet P low in the prepartum diet does not contribute to the hypophosphatemic downer cow condition, which is sometimes observed as a sequelae of milk fever.

Dietary S must be kept above 0.22% (to ensure adequate substrate for rumen microbial amino acid synthesis) but below 0.4% (to avoid possible neurological problems associated with S toxicity). Ca sulfate and Mg sulfate are good sources of sulfur that may also supply any needed Mg and Ca.

Now, with the exception of K and Cl, the “variables” in the various proposed DCAD equations have become “fixed”. The key to milk fever prevention (Holstein cows) is to keep K as close to the NRC requirement of the dry cow as possible (about 1.0% diet K). The key to reduction of subclinical hypocalcemia, not just milk fever prevention, is to add Cl^- anions to the ration to counteract the effects of even low diet K on blood alkalinity. For formulation purposes the concentration of Cl required in the diet to acidify the cow should first be set at 0.5% less than the concentration of K in the diet. In other words, if diet K can be reduced to 1.3%, the Cl concentration of the diet should be increased to 0.8%. This will adequately acidify about 20% of herds in this author’s experience. Ultimately in many herds the amount of chloride added will have to be brought to within 0.3% of the diet potassium for proper acidification. A conservative approach should be taken when formulating the diet of close-up cows — going immediately to the higher chloride diet will cause over acidification of 20% of herds, which can reduce feed intake creating many other metabolic disease challenges. Move to the higher dose of chloride only if urine acidification (described as a monitoring tool below) is not achieved at the lower chloride level. There is also a limit on how much anion can be added to a diet without affecting feed intake. In this author’s experience, when diet potassium exceeds 1.4% it is difficult to add enough chloride to the diet using the traditional chloride salts (Ca, ammonium, and magnesium chloride) to acidify the cow and maintain adequate dry matter intake. With some of the more palatable commercial anion supplements it is possible to acidify the diets and maintain feed intake when diet potassium is as high as 1.8%. If dietary K can only be reduced to 2.0% the diet Cl would need to be roughly 1.5% to acidify the cow. Raising Cl to this level in the diet is likely to cause a decrease in dry matter intake. Chloride and sulfate sources differ in their palatability and since achieving low dietary K can be difficult it is prudent to use a palatable source of Cl or sulfate when formulating the diet. Ammonium chloride (or ammonium sulfate) can be particularly unpalatable when included in rations with a high pH. At the higher pH of high forage (low corn silage) rations where pH of the diet exceeds 5.5, the ammonium cation is converted to ammonia, which is

highly irritating when smelled by the cow. Prilling the Cl (and SO_4) salts can reduce the unpleasant taste of the salts and allows improved anion supplementation success. In this author's experience hydrochloric acid has proved the most palatable source of anions as well as the strongest acidifying agent. Hydrochloric acid can be extremely dangerous to handle when it is procured as a liquid concentrate. Several companies now manufacture anion supplements comprised of hydrochloric acid adsorbed onto feed particles, which are safe to handle and palatable.

MONITORING URINE PH

These are simply guidelines for anion supplementation used by this author and are based on inclusion of Ca, Na, S, Mg, and P at the levels outlined above. Urine pH of the cows provides a cheap and fairly accurate assessment of blood pH and can be a good gauge of the appropriate level of anion supplementation. Urine pH on high cation diets is generally above 8.2. Limiting dietary cations will reduce urine pH only a small amount (down to 7.5-7.8). For optimal control of subclinical hypocalcemia the average pH of the urine of Holstein cows should be between 6.2 and 6.8 during the last week of gestation, which essentially requires addition of anions to the ration. In Jersey cows the average urine pH of the close-up cows has to be reduced to between 5.8 and 6.3 for effective control of hypocalcemia. If the average urine pH is between 5.0 and 5.5, excessive anions have been added and there is the danger they have induced an uncompensated metabolic acidosis and the cows will suffer a decline in dry matter intake, even if a palatable anion supplement is used.

HYPOMAGNESEMIA

Insufficient dietary Mg supply leads to hypomagnesemia. Hypomagnesemia is a major risk factor for milk fever. Hypomagnesemia affects Ca metabolism by reducing tissue sensitivity to PTH and by reducing PTH secretion in response to hypocalcemia. Adding Mg to diets before and after calving should eliminate hypomagnesemia as a contributor to hypocalcemia. Unfortunately issues with bioavailability of magnesium sources have arisen.

Magnesium is included in dairy rations for two reasons: to maintain adequate levels of Mg in the blood and as a rumen fluid alkalinizer. Mg sulfate.7 H2O and Mg chloride.2 H2O are very soluble, very available sources of Mg,. They are acidifying salts of magnesium and are often included in close-up diets as part of a low DCAD diet. Many anion supplements on the market include Mg in the sulfate or chloride form and easily meet the dry cow requiremtns for magnesium. Once the cow begins lactation the magnesium supplement often switches over to magnesium oxide. MgO takes up little room in the ration, costs less, and is more palatable than some other sources of Mg. It can help alkalinize rumen fluid so it is more appropriately used in lactation diets in conjunction with sodium bicarbonate and other rumen buffers. The feed industry utilizes MgO which is about 54-56% Mg (>58% Mg MgO often indicates the ore was overly heated in the calcining process and the Mg will be poorly available). Unfortunately, there is tremendous variability in MgO quality. For ruminants, MgO should be ground to a fine dust. A quick test can estimate the relative availability of MgO sources. Place 3 g of a MgO source in a container and slowly add 40 ml 5% acetic acid (white vinegar). Cap container and shake well for 15 seconds and let sit. Check the pH after 30 minutes. Vinegar alone is

pH 2.6-2.8. The best MgO sources will bring the pH up to 8.2; the worst to just 3.8. pH is a log scale so this represents >10,000 fold difference in the number of hydrogen ions buffered. Remember in lactating rations, MgO is relied upon to combat rumen acidosis- and we are not getting that action from these insoluble MgO sources. In an experiment with four cows with rumen fistulas, the solubility of MgO in vitro (tested in various ways) was found to parallel their solubility in the rumen and their urinary excretion (schonewille, 1998).

ORAL CALCIUM TREATMENTS AT CALVING

Ca administered to the fresh cow may arguably be called a treatment rather than a preventative measure for hypocalcemia. Contrasts between the effects observed with intravenous, subcutaneous, and oral Ca treatments have been described elsewhere (Goff, 1999). Briefly, the concept behind oral supplementation is that the cow's ability to utilize active transport of Ca across intestinal cells is inadequate to help her maintain normal blood Ca concentrations. By dosing the animal with large amounts of very soluble Ca it is possible to force Ca across the intestinal tract by means of passive diffusion between intestinal epithelial cells. Best results are obtained with doses of Ca between 50 and 100 g Ca / dose. Ca chloride has been used but can be very caustic. Ca propionate is less injurious to tissues and has the added benefit of supplying propionate, a gluconeogenic precursor. Ca carbonate is not soluble enough to induce a rapid rise in blood Ca. For best control of hypocalcemia a dose is given at calving and again 12- 24 hrs later. Toxic doses of Ca can be delivered orally — a single dose of 250 g Ca in a soluble form will kill some cows. The benefit of adding oral Ca drenches/gels in addition to a properly formulated low DCAD program is becoming easier to justify as recent studies link even moderate hypocalcemia with decreased health and performance of the cow (Martinez et al., 2012; Chamberlin et al., 2013). It has been estimated that about 4 g Ca entered the blood of cows given 50 g Ca as CaCl2 in a drench in the first hrs following treatment [73].

Author Disclosures

Prior to joining the faculty at Iowa State University, Goff was Director of Research at West Central Farmer's Co-operative. In this capacity he developed anion supplements for prevention of hypocalcemia in cows and Goff continues to consult for this company. Goff also holds a patent using calcium propionate paste to prevent hypocalcemia.

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Overview of the Methane Prediction Module in the AusBeef Rumen Model

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Improved livestock nutrition modelling can enable better description and prediction of the physiological and environmental effects of specific production processes, thereby improving productivity and efficiency. Although there are several models of livestock methane emissions (e.g., Ellis et al. 2007) few mechanistic whole-animal models exist. The AusBeef rumen model, initially proposed and developed by Nagorcka and Zurcher (2002), is one of the few whole-animal dynamic and mechanistic models that can be used to predict both productivity and enteric fermentation from ruminants. The AusBeef model is an integration of several sub-models including those for body growth, voluntary feed intake, rumen, and lower gut. Each of these models contains additional sub-models, allowing for more accurate prediction of a variety of biological processes. The methane (CH_4) module within the rumen submodel of AusBeef is discussed below.

There are seven equations that explicitly deal with CH_4 production. The main equation relates rumen hydrogen (H_2) balance to CH_4 production. Hydrogen is produced from rumen fermentation of hexose and amino acids and from microbial growth. Hexose is fermented to volatile fatty acids (VFAs) by cellulolytic and amylolytic bacteria, as well as by protozoa, with some VFAs produced by the fermentation of protein. Hydrogen is produced by fermentation of hexose and amino acids as well as by microbial growth; H_2 production from microbial growth is related to soluble protein uptake in the rumen. Hydrogen is also consumed by VFA production, biohydrogenation of dietary lipids, and microbial growth, with uptake into microbes related to ammonia utilized for growth by amylolytic and cellulolytic bacteria. The model requires improvement in dealing with the effect of nitrates and dietary lipids in reducing enteric methane production. A major redevelopment of the AusBeef rumen sub-model is underway, so that the sub-model will run within AusFarm, a software tool to analyze whole-farm simulation studies.

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Why are conception rates of California dairy cows so low?

Characteristics of cows that were pregnant or not pregnant 39 days post AI

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California is the highest producing dairy state with ~1.8 million lactating dairy cows, each producing about 22,000 lbs of milk per lactation (National Agricultural Statistics Service, 2013). Although these high production levels are the result of decades of intensive research yielding advanced genetic and nutritional understanding of lactating cows, much of their biology remains obscure. For instance, although milk production and overall energetic efficiency have increased over the last few decades, a decline in pregnancy rates of dairy cattle has also been noted.

This study used five replicated early lactation pens on a commercial dairy near Hanford (CA). Multiparous dairy cows, 32, were enrolled at -10 days relative to anticipated artificial insemination (AI) and underwent a common 3 shot synchronization protocol of Factrel®, Lutalyse® and Factrel® at -10, -3 and -1 day from AI. Of these 32 cows, 14 were determined pregnant by rectal palpation on day 39 post AI. Coccygeal blood draws of the 32 cows were collected on days -10 and -3 from AI to determine cyclicity of the cows, as well as at AI, 6, 13 and 20 days post AI. Milk progesterone was measured at 20 days post AI, and body condition was scored at days -10, 0, 13 and 27 days relative to AI.

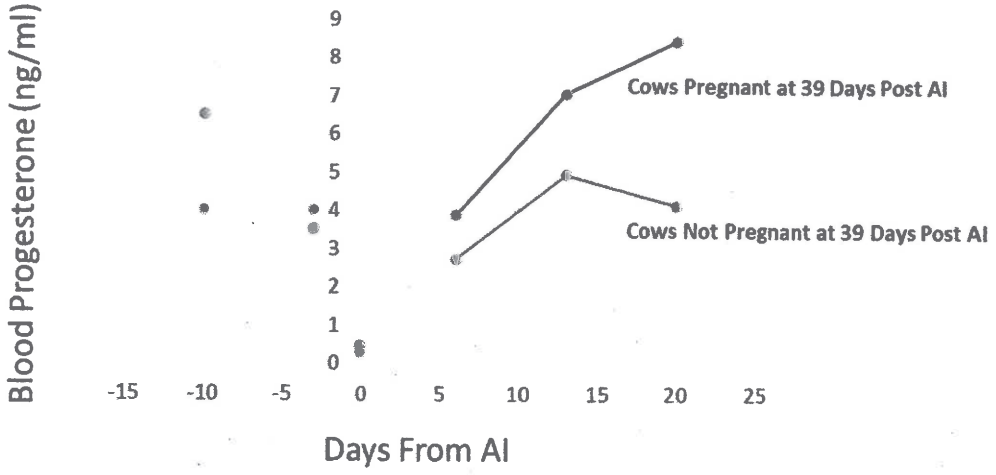

Figure 1. Blood serum progesterone (ng/ml) vs. days from AI for cows determined pregnant or not pregnant 39 days post insemination. Values at -10, -3 and 0 days did not differ (P>0.05), but there was a time by days interaction (P=0.06) for days 6, 13 and 20, and values differed (P<0.05) by pregnancy status for these 3 days.

Serum progesterone levels of cows pregnant at day 39 post AI did not differ on or prior to AI ($P>0.05$) but were higher ($P<0.05$) on days 6, 13 and 20 (Figure 1). Milk progesterone at 20 days post AI was also higher (3.82 vs. 6.89 ng/ml; $P<0.01$) for cows pregnant at 39 days post AI. The body condition score (BCS) of cows pregnant at day 39 post AI was higher (2.42 vs. 2.71; $P=0.02$) on the day of AI vs. cows not pregnant at 39 days post AI. It was clear that cows with BCS of 2.5 or lower on the day of AI had a much lower pregnancy outcome than cows with a BCS > 2.5 (Figure 2), but the change in BCS from day -10 to AI was not related ($P=0.18$) to pregnancy outcome.

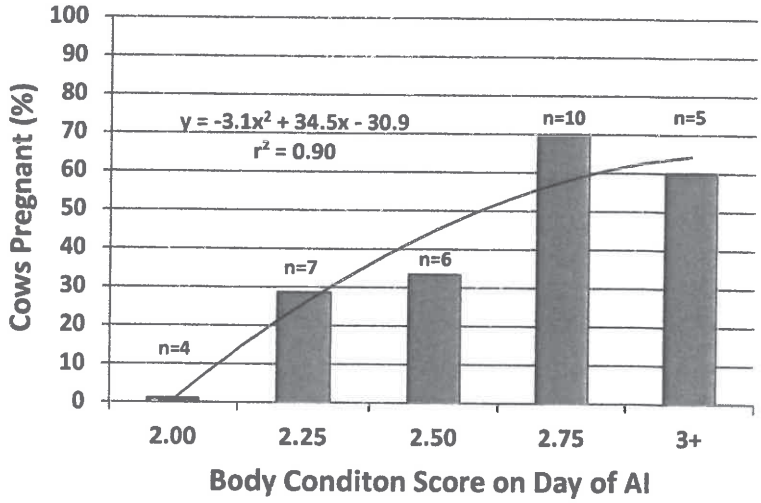

Figure 2. Percentage of cows pregnant 39 days post AI vs. BCS on day of AI.

Most reproductive failures in dairy cows occur during very early pregnancy. Progesterone is critical during this time in order to ensure homeostatic uterine conditions. Therefore, it is not surprising that cows retaining pregnancies through 39 days post AI had higher levels of blood serum progesterone on days 6, 13 and 20 post AI. Nonetheless, abortions within 20 days of AI in cows not pregnant at 39 days post AI, thereby leading to early resumption of a new estrous cycle, likely influenced the differences in progesterone levels between groups at day 20 post AI. In addition to endocrine factors influencing the probability of pregnancy at day 39 post AI, the importance of a cow's BCS at AI was more important than the energy balance during the 10 days prior to AI. This demonstrates the importance of a nutritional management system that provides cows with the higher BCS needed at AI in order for cows to get pregnant, and retain pregnancy during early embryonic fetal development.

Adequate progesterone levels and higher BCS are important factors influencing pregnancy. There is value in use of blood serum and milk progesterone as a fertility marker in genome/breeding databases or integrated into commercial milking systems. Similarly, the practical implementation of BCS as a criterion for breeding cows is not complex on commercial dairy farms.

Effects of Precision Feeding Dairy Cattle on Enteric Emissions and Milk Production

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ABSTRACT

The objective of the present experiment was to evaluate the effect of supplementing diets with rumen-protected Lys and Met on dairy cow performance and enteric emissions. The trial was conducted for 6 weeks with 20 Holstein cows beginning with a 2-week acclimation period. Cows were blocked by days in milk (DIM) and parity. Cows were randomly assigned to 1 of 4 dietary treatments (n=5). The dietary treatments were corn silage based and supplemented with differing amounts of rumen-protected methionine (Smartamine, Adisseo USA Inc., Alpharetta, GA; RPM) and lysine (AjiPro-L 2, Ajinomoto, Fort Lee, NJ; RPL). The diets included: 15% crude protein (CP), 15% CP with added RPM and RPL, 18% CP, and 18% CP with added RPM and RPL. Diets were formulated for similar metabolizable protein content with a 3:1 ratio of lysine to methionine for direct absorption by the duodenum. Measurements taken included body weight (BW), dry matter intake (DMI), milk yield and milk components, feed efficiency, milk urea nitrogen (MUN), plasma urea nitrogen (PUN), urinary urea nitrogen (UUN), and enteric gases from head chambers. The gases of interest included nitrous oxide (N_2O), methane (CH_4), carbon dioxide (CO_2), methanol (MeOH), ethanol (EtOH), hydrogen sulfide (H_2S), and sulfur dioxide (SO_2). For a 12-hour period, each cow was secured in a ventilated head chamber with *ad libitum* feed and water. Cows underwent two separate measurement periods on d 14 and d 28 throughout the treatment period. Enteric gases were captured and measured by gas analyzers that were connected to the head chambers. The experimental design used was a split-split plot completely randomized design with a 2x2 factorial arrangement of treatments. Data were analyzed using PROC GLM in SAS (version 9.4, SAS Inst. Inc., Cary, NC). Over the course of the trial, cows in all treatments groups had similar milk yield, DMI, milk fat, and milk protein. The MUN, PUN, and UUN measurements in cows fed 18 vs.15% CP diets were higher, but no effects were found for RPAA supplemented rations. In terms of enteric emissions, there were no differences found in cows fed the different diets, except for H_2S, which was affected by CP only. Cows fed the 18% CP diets emitted higher H_2S emissions. The lack of effect on production traits could be due to the method of supplement administration or term of the trial.

Key Words: dairy cow, enteric emission, environmental impact, rumen-protected amino acid, nitrogen utilization

Assessing the environmental effect of vermifiltration on dairy lagoon water emissions

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In Central California, dairy lagoon water is traditionally stored in anaerobic lagoons, which emit a variety of greenhouse gases (GHGs) and other volatile organic compounds (VOCs). To mitigate the environmental impact of its lagoon water, a commercial dairy in Central California has installed a vermifilter, a wastewater management system that uses earthworms embedded in woodchips to enhance removal of solids and contaminants. The objective of this study was to quantify the environmental impact of the vermifilter by comparing the GHG and VOC profiles from the lagoon water (L), influent (I), effluent (E), over the surface of the filter (S), and the bottom of the filter (B). Thus, ammonia (NH_3), nitrous oxide (N_2O), carbon dioxide (CO_2), methane (CH_4), and hydrogen sulfide (H_2S) gases were captured using flux chambers for L, I, and E, a triangle sampling tunnel for S, and an inlet threaded to the bottom of the filter for B. All gases were measured using gas analyzers housed in the UC Davis Mobile Agricultural Air Quality (MAAQ) Lab. The resulting emission profiles suggest that the vermifilter reduces NH_3 emissions from lagoon water by 90.2% without increasing the emission of N_2O, a potent GHG that is the byproduct of incomplete denitrification. However, the vermifilter does not affect CO_2, CH_4, or H_2S emissions from lagoon water. The vermifilter's ability to reduce NH_3 emissions might render vermifiltration a potential environmental solution for other dairy lagoons.

Abstract
Stocking density effects on production qualities of antibiotic free broilers
Key words: stocking density, litter moisture, antibiotic free.

In antibiotic free (ABF) broilers, enteric diseases, mainly coccidiosis and necrotic enteritis (NE), pose the biggest threat to the intestinal health. Through knowledge of litter condition and management, these diseases can be minimized. Stocking density for ABF houses are typically lower than those raised in conventional housing. With coccidiosis and NE being the main source of mortality and morbidity in ABF flocks, it is important to understand the relationship stocking density has on the prevalence of those enteric diseases. Enteric diseases will directly affect the feed conversion efficiency, creating a less efficient bird. If there is no relationship between stocking density, coccidiosis and NE, broiler houses could maintain similar stocking densities as conventional housing.

Two stocking densities were selected for the study, a high stocking density (0.76) and a low density (0.90). The study had a total of 19,740 straight run Cobb broilers split into four pens. The experiment consisted of two pens with 4,203 broiler chickens and the other two with 5,355 broilers. All chickens were ad libitum fed the same diet from start to processing. Thirty chickens (15 males and 15 females) were randomly selected per pen for feed conversion. Disease involvement, body weights, cellulitis, and fecal contamination were analyzed as a one-way ANOVA.

The greatest amount of significance exists between pen locations rather than between stocking densities. There was a difference in litter moisture ($P<0.05$) and body weights after day 42 ($P<0.05$). Overall, feed conversion was not affected by the difference in stocking density.

Environmental factors such as location in the house were more influential in feed efficiency, mortality, and carcass yield. While the stocking density differences were influential in body weights and litter moisture.

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Glucose precursor supplementation in Holstein and Jersey cows as a preventative treatment for ketosis in the transition period

Kelly Mitchell

Subclinical ketosis, a common metabolic issue in the transition period, is estimated to cost $78 per case due to decreased milk production, reduced fertility, displaced abomasum, and other health issues (Geishauser, T., Leslie, K., Kelton, D., & Duffield, T., 2001). This cost increases when subclinical ketosis continues or escalates to clinical ketosis. Glucogenic substances can treat ketosis by lowering β-hydroxybutrate (BHBA) levels (subclinical ketosis ≥1.0 mmol/L, clinical ketosis >1.2 mmol/L) and raising glucose (Glu) levels (subclinical/clinical ketosis, <60 mg/dl). However, preventative measures to lower incidence would be more profitable. The objectives of this study are to determine if supplementation with a glucose precursor powdered product (Glucose Booster, Stuhr Enterprises, LLC.; GP) during the transition period has an effect on Glu and BHBA levels, and to observe effects on animal health and milk production. Holstein and Jersey cows at a commercial dairy were systematically enrolled into either a control (C) or GP treated prepartum pen (PreP) and then moved the corresponding C or GP postpartum pen (PostP) after calving. Cows in GP pens were supplemented daily with 300g/head of GP mixed into the TMR. Daily feed samples were taken of dropped and residual feed, pooled weekly, and sent to a commercial lab (Analab: Agriking, Fulton, IL) for nutrient analyses. Weekly BCS were recorded and weekly blood samples were collected and tested for Glu (mg/dl) and BHBA (mmol/L) using Nova Max® (Nova Diabetes Care, Inc., Billerica, MA) test strips. Weekly milk samples were taken during the first 3 weeks of lactation followed by monthly tests thereafter. Cows that remained in the correct PreP and PostP for at least 2 wks were included in the data analysis (n=211). The Holsteins (n_{GP}=52, n_C=54) and Jerseys (n_{GP} =53, n_C=52) responded differently to treatment, thus results were analyzed separately by breed. Data was analyzed using the MIXED procedure of SAS (v. 9.4, SAS Institute 2015) with cow as the experimental unit and treatment, previous lactation milk fat or

protein yield, period of lactation or month of lactation and DIM as fixed effects. Parity was a random effect and blood glucose, BHBA, and milk, fat and protein yields were repeated measures by cow. Holstein cows treated with GP did not show a significant difference in blood values when compared to C Holsteins. Jerseys treated with GP had lower Glu in the PreP (Glu_{GP}=58, Glu_{C}=62, P_{Glu}=0.0008), but PostP GP cows showed no difference in Glu levels. For milk production Holstein cows had a noticeable increase since they produced +4.05 milk, +0.22 fat, and +0.12 protein (kg/d) during PostP (P_{Milk}=0.0020, P_{Fat}=0.0002, $P_{Protein}$=0.0049). Milk production in GP Holsteins continued to be higher than C Holsteins until 120 DIM (P_{Milk}=0.067, P_{Fat}=0.1331, $P_{Protein}$=0.049). Milk production and components were not different between treatments for PPost Jerseys. Total health events in the first 60 DIM for GP Holstein cows decreased (N_{GP}=32, N_{C}=44) and the incidence of subclinical and clinical ketosis decreased by 15%. Holsteins and Jerseys responded differently to treatment; therefore, breeds face different issues during early lactation. Holsteins tend to have a difficult transition period and are more likely to benefit from GP during the first few weeks of lactation when ketosis and other health issues are more common. Despite the variations is response, supplementation with GP in the TMR prevented ketosis and health events for Holsteins.

Physically effective fiber threshold for the hindgut-fermenting leopard tortoise (*Stigmochelys pardalis*)

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The objective of this study was to investigate the effects of ingesting one of two cellulose fiber lengths (2.0 mm and 0.2 mm) on fecal particle size distribution of a hindgut-fermenting tortoise species as a means of measuring physically effective fiber with possible implications for captive diet formulation. Particle size distribution of offered diet, excreted feces, and the change from diet to fecal particle size of 16 female leopard tortoises, born of the same clutch and housed individually in identical pens, was analyzed using a vibratory wet sieve shaker (Retch® AS 300). Each tortoise was randomly assigned to one of three diets. All diets consisted of a nutritionally complete, high fiber (30% NDF, DMB) herbivorous tortoise diet (Mazuri® 5M21) ground through a 2.0 mm screen, and differed as follows: 5M21 only (control, CNTRL), added 2.0 mm length cellulose (2.0 mm), and added 0.2 mm length cellulose (0.2 mm). Researchers were blind to the cellulose-added treatments; only the control diet was known. With only one batch (observation) for each diet type, statistical differences could not be tested; however, one cellulose-added diet retained a greater amount of particles on the 2.0 mm sieve, and the other cellulose-added diet retained a greater amount of particles on the 0.125 mm sieve, corresponding with the added cellulose fiber lengths and reinforcing the validity of the wet sieving method to accurately recover particles of known length from a source of unknown particle size distribution. No significant difference among the fecal particle size distributions was found ($P = 0.1227$); however, a significant difference among the change from diet to fecal particle size was found ($P < 0.0001$). The fecal particle size distribution similarities suggest a physically effective fiber threshold greater than 2.0 mm. Additionally, the significant difference among the change particle size distributions suggest the plant fiber digesting capability of the leopard tortoise. This information may be important for the feed industry, where finely ground ingredients are often used in pelleted and extruded feed formulas targeted for hindgut-fermenting herbivores.

Effects of Nutrition, Husbandry and Environment on Reproductive Outcomes in a Specific Pathogen Free (SPF) Feline Facility

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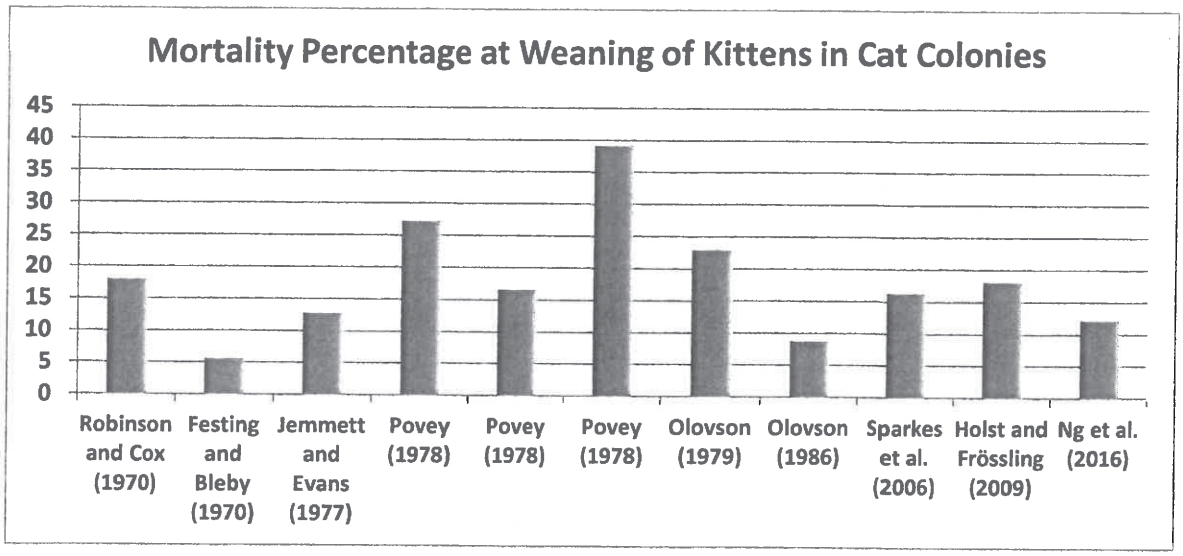
Understanding characteristics of feline reproduction is important in accurate evaluation of colony performance, and to minimize animal losses. Environment, animal husbandry and diet are known to greatly affect the breeding success of a colony. However, colony location can impact day length and temperature, which in turn can influence the breeding cycle of the queens (i.e., mothers) and mortality in the litters. Reproductive data from laboratory animal breeders in different countries demonstrated an inverse relationship between annual reproduction phases, defined as the time between the first and last litters in the year, and proximity to the equator. Remarkably, the reproductive period of cats decreases from 12 to ~6 months as colonies get further away from the equator where daylight is the most variable within year. Research to better understand the metabolic idiosyncrasies and nutrient requirements of cats has improved feline diets and reduced nutritionally associated diseases. Researchers did not understand other unique protein and amino acid requirements of cats until 1976 and the amino acid requirements of growing kittens were not defined until 1979. Mortality in kittens in a research cat colony decreased from 23% in 1979 to 8.8% in 1982 after adjusting the feeding routine and composition of the diet fed to a group of queens and kittens (Olovson, 1986). The advent of specific pathogen free (SPF) cat breeding colonies, first reported by Bleby and Lacey (1969), led to reduced mortality rates from infectious disease. However since animal lab management standards were not established until 1977 by Clarke et al., animal husbandry and breeding practices in earlier studies varied widely. Therefore, common infectious agents in cats, such as feline viral rhinotracheitis, feline calicivirus, feline immunodeficiency virus, and feline leukemia virus, may have impacted prior studies.

Given the widespread changes in facility husbandry, infectious disease control and nutrition, we hypothesized that reproductive performance may be impacted. In our study, reproductive outcomes of cats fed diets appropriate for gestation/lactation and growth, and housed in a temperature and light controlled SPF colony were evaluated over a 5 year period.



In general, our observations in this study competed at UC Davis are consistent with previous findings on cat reproduction in colony environments. Seasonality had some effects on reproductive cycles, and selective breeding appeared to minimize kitten mortality while maximizing birth weight and growth rate. The male to female ratio was 1.03 (n = 515 males, 502 females) and the median litter size was 5 kittens/litter (range: 1 to 11). The survival rate to weaning age was 87.8%. Median birth weight of kittens was 117 g (n = 920; range: 46 to 225 g) and median growth rate was 13.1 g/d (n = 612; range: 6.0 to 19.3 g/d). Fewer litters were born in February and more litters were born in October (n = 238; $P < 0.01$), fewer kittens were born in February, April, June, and September and more kittens were born in October and December (n = 1,073; $P < 0.01$). Birth weights of kittens were lower in January, February, April, and August (n = 920; $P < 0.01$; median = 117 g). Smaller litters (i.e., < 3 kittens) produced more male kittens (n = 237; $P < 0.01$), had lower mortality rates (n = 238; $P = 0.01$), larger kittens at birth (n = 172; $P < 0.01$), and faster growth rates (n = 86; $P < 0.01$). Larger kittens at birth had faster growth rates than smaller kittens (n = 612; $P < 0.01$), while male kittens were heavier at birth (n = 909; $P < 0.01$) and had faster growth rates than females (n = 612; $P < 0.01$). Our results can be used as a model for breeding cats in controlled environments.

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