

Impact of the Tri-State Dairy Nutrition Conference (2014)

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The success of the Tri-State Dairy Nutrition Conference is demonstrated by attendance (Figure 1) and citation or reprinting of proceedings manuscripts in the scientific, international, and popular press literature. The Conference has resulted in major impacts to the feed industry and dairy producers, and influenced students seeking careers in animal nutrition and the direction of some research programs. The results from the 2014 survey distributed to attendees revealed the following (number in parentheses indicates number of responses; std = standard deviation):

1. **Attended the Conference on average for 11.6 years** (std = 6.8; $n=42$)
2. **What percentage of the farms with which you work have regular or intermittent problems with ruminal acidosis?** ($n = 38$)
0 - 2.6%, 10 - 29.0%, 20 - 18.4%, 30 - 36.9%, 40 - 2.6%, 50 - 7.9%, 60 - 0.0%, 70 - 0.0%, 80 - 0.0%, 90 - 0.0%,
100 - 2.6%
3. **What are the primary signs you look for in accessing the presence of ruminal acidosis?** ($n = 40$)
Manure (quality, loose, bubbles, gray, inconsistent, diarrhea, mucous, corn washouts, score) (31); butterfat depression, fat: protein inversions, F:P ratio (24); cud chewing/rumen contractions decreased (20); feed intake depression or fluctuation (11); poor feet/laminitis, redness above the hoof, sore feet (3); sorting (2); displaced abomasum (2); low rumen pH (2); low amount of feed on top screen of Penn State shaker box (2); fecal starches (2); fluctuating production (2); MUN levels (2); sick cows (2); forage changes; reduced fiber digestion; decreased ear temperature and higher rectal temperature
4. **At what age or stage of development do your clients introduce forage into the diets for heifers?** ($n=39$)
Post weaning (9); 3 to 6 months (7); 2 to 3 months (6); 300 to 600 pounds (5); 6 to 8 weeks (4); at weaning (2); 4 to 6 weeks (2); pre-weaning (2); 1 to 3 weeks (2); 5 to 12 weeks
5. **What are the primary methods used by the farms you work with to mitigate heat stress?** ($n=40$)
Fans (37); sprinklers/soakers/misters (21); dietary changes: DCAD balancing rations, buffers, potassium carbonate, rehydration products, yeasts, sugars (10); extra waterers (7); shade (2); sand bedding; ventilation; higher barn heights; increased corn silage; no overcrowding
6. **What is the primary basis for your decision to include rumen protected amino acids in a diet?** ($n=37$)
Increase milk protein, butterfat, and/or milk (12); IOFC (8); increase milk protein, decrease CP in diet (5); management level (4); Lys:Met ratio predicted by ration balancing (3); producer requests/personality (2); milk protein price (2); health and production; source of glucose and energy; availability of AA; MUN
7. **On average, how many pen moves occur for cows after calving among the farms that you work with?** ($n = 40$)
1 - 17.5%, 2 - 40.0%, 3 - 30.0%, 4 - 12.5%
8. **Relative to the feeding of distillers grains:**
 - a. **What percentage of your clients feed distillers grains?** ($n = 37$)
0 - 0.0%, 10 - 8.1%, 20 - 5.4%, 30 - 8.1%, 40 - 10.8%, 50 - 8.1%, 60 - 2.7%, 70 - 13.5%, 80 - 24.3%, 90 - 8.1%,
100 - 10.8%
 - b. **What is the typical level of inclusion of distillers grains in diets?** ($n=37$)
5 - 46.0%, 10 - 32.4%, 15 - 10.8%, 20 - 5.4%, 25 - 5.4%
9. **What is the typical herd feed efficiency (milk/DMI) for the herds for which you work?** ($n=37$)
1.2 - 2.7%, 1.3 - 8.1%, 1.4 - 29.7%, 1.5 - 32.5%, 1.6 - 18.9%, 1.7 - 2.7%, 1.8 - 5.4%

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10. Changing what single factor on dairy farms would have the greatest impact on improving feed efficiency within a dairy herd? ($n = 38$)

Forages: quality, increase amount fed (15); improving management (4); feed consistency and delivery; and monitoring changes (4); environmental stress reduction (3); cow comfort (2); DIM (2); pushing up feed more frequently (2); stop selecting for large cows; TMR, OM digestibility; particle size; education of all feed personnel, delivery personnel, pen cleaners; grouping/feeding based on stage of lactation; appearance of TMR; feeding sugars to enhance diet energy; additives selected.

11. On what basis do you decide how much milk yield to use in formulating diets for a herd or groups?

Goals, management, and requests of dairy or owner (12); animal factors: DIM (5), DMI (3), age (2), size (1), reproduction status (1); lead factor over actual production: 7% over, 15% above group average, 20% over mean, 110% of group average if more than 2 groups, 120% group average if one group, 5 lb over actual production, tank average plus 10% or group average, 15% above ytd RHA, 1.25 to 1.3 group average, plus 20% and BCS, 10 lb above desired group average, milk shipped/cow*1.25; forage digestibility, forage quality (2); actual tank or pen weights (2); evaluate herd and forage available; quality of cows, forages, facilities, and management; target milk production; current dairy markets; 80 lb; one group: 90 pounds, multiple groups: 12 to 15% over pen average, then adjust to MUN tank level ($n=34$)

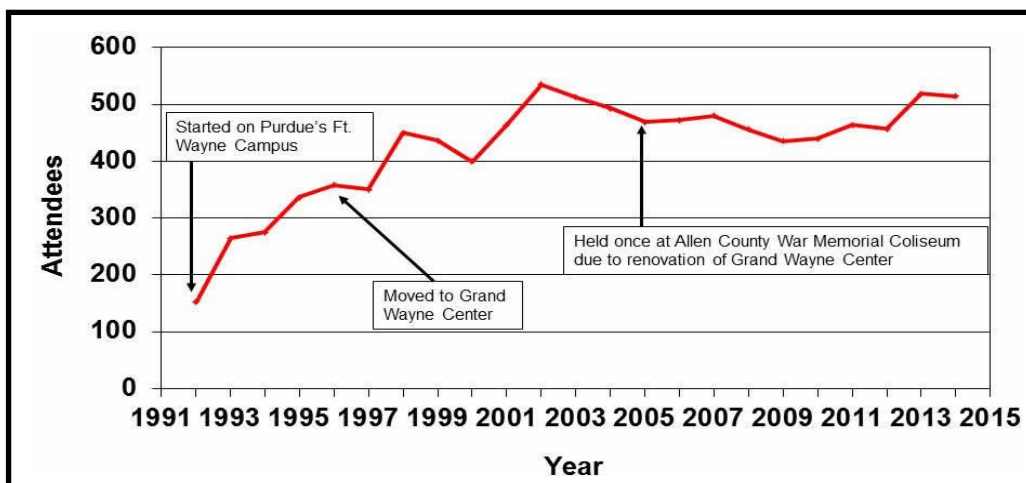


Figure 1. Attendance at the Tri-State Dairy Nutrition Conference

Abbreviations that may be found in this publication include:

AA = amino acids	FCM = fat-corrected milk	r = correlation coefficient
ADF = acid detergent fiber	ME = metabolizable energy	R ² = coefficient of determination
BCS = body condition score	MCP = microbial crude protein	RDP = rumen degradable protein
BW = body weight	MP = metabolizable protein	RFV = relative feed value
CP = crude protein	NEFA = non-esterified fatty acids	RMSE = root mean square error
CV = coefficient of variation	NE _g = net energy for gain	RUP = rumen undegradable protein
DE = digestible energy	NE _m = net energy for maintenance	SCC = somatic cell count
DIM = days in milk	NE _l = net energy for lactation	SD = standard deviation
DHI = dairy herd improvement	NDF = neutral detergent fiber	SE = standard error
DM = dry matter	NFC = nonfiber carbohydrates	SEM = standard error of the mean
DMI = dry matter intake	NRC = National Research Council	TDN = total digestible nutrients
ECM = energy corrected milk	NSC = nonstructural carbohydrates	TMR = total mixed ration
FA = fatty acids	OM = organic matter	VFA = volatile fatty acids

Note: Most of the units of measure in this publication are expressed in U.S. equivalents; however, in some cases, metric units are used.

Use the following to make conversions:

$$1.0 \text{ lb} = 0.454 \text{ kg} = 454 \text{ g}$$

$$1.0 \text{ ft} = 0.3 \text{ m} = 30 \text{ cm}$$

$$^{\circ}\text{F} = (^{\circ}\text{C} \times 1.8) + 32$$

$$1 \text{ U.S. ton} = 2000 \text{ lb} = 909 \text{ kg}$$

$$1 \text{ metric ton} = 1000 \text{ kg} = 1.1 \text{ U.S. ton (2200 lb)}$$

$$1 \text{ acre} = 0.4 \text{ hectare}$$

Abbreviations for metric units are:

ppm = parts per million

mg = milligrams

g = grams

kg = kilograms

cm = centimeters

mm = millimeters

m = meters



Labor Management on Dairy Farms: The Interface Between the Employer and the Employee

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Introduction

Dairy farming is a multi-faceted enterprise wherein producers confront a myriad of challenges as they seek to maintain sustainability and grow in operations and profitability. Among the top challenges facing dairy producers is the difficulty of hiring and retaining qualified employees (Timms et al., 2012). An additional challenge is the increasing cost of labor – a dairy farm's second greatest expense (behind feed expense) – which continues to rise (Laughton, 2014). Overall, dairy farm profitability is impacted by the close relationship between labor productivity and cow productivity; in other words, those operations that increase labor productivity see increased profits (Laughton, 2014).

The single factor with the greatest impact on dairy labor productivity is employee turnover. The costs of turnover can be staggering, with research showing that losses can be measured in multiple categories: productivity; recruitment, selection, and hiring; safety issues; and investment in new employee orientation and training (Billikopf and Gonzalez, 2012). Some labor experts estimate the cost of turnover at 150 to 250% of an employee's annual wages.

Employee exit interviews and follow-up surveys categorize reasons provided by

former employees for leaving employment with compensation and benefits topping the list.

Employees may also cite employment conditions, including working schedules and lack of time off (National Center for Farmworker Health (NCFH), 2014; Harrison, et al., 2009). There is no doubt that the dairy farm employer should give proper weight to such factors cited by departing employees as their reasons for leaving. Working conditions related to wages, benefits, schedules, housing, transportation, job duties, and general job satisfaction should be regularly reviewed and adjusted as appropriate (Moore, 2012).

Research indicates that employees tend to make early decisions regarding whether to make a long-term commitment to an employer (Aberdeen Group, 2006). In fact, this research shows that as many as 5% of workers make the decision to stay with an employer on the first day, and another 20% make this decision within the first week of employment. Overall, 90% of employees make their decision to stay at an employer within the first 6 months (Aberdeen Group, 2006). In light of this research, the real significance of reasons later given by departing employees as the impetus for leaving is somewhat decreased. Rather, it appears that the employer needs to take early action that persuades a new employee to make a long-term commitment at the earliest stages of employment.

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Employee orientation is generally thought of as those activities which take place in the initial moments and days of employment, which activities tend to socialize the new employee and prepare the individual for ongoing training activities. Another term for the process is “onboarding” – defined as: “[A] support process for new employees designed to manage a variety of tasks and requirements initiated when a new applicant is hired and has accepted the position” (Aberdeen Group, 2006). Further:

“New employees often feel that the attention they receive during the pre-hire stages is abandoned once they are onboard. As a result, these individuals are left with a negative impression of their new work environment. In order to improve retention rates and time to productivity, [employers] need to focus on developing a comprehensive onboarding process” (Aberdeen Group, 2006).

While it is impossible to control for all factors during the early days of employment, research indicates that significant reduction in employee turnover can be achieved through effective employee orientation programs (Nobel, 2013). This research involved dividing entry-level customer service employees into 2 groups (herein Alpha and Beta) and providing those two groups with similar but distinctive orientation and socialization experiences during the first minutes or hours of employment. The key elements of these early orientation exercises are described herein:

Alpha group – early orientation exercises

- The senior leader spent 15 minutes with the Alpha Group, discussing ways in which “working here will enable you to express your individuality.”

- Alpha Group employees were asked to complete an exercise ranking the individual strengths they would exhibit if stranded on a life raft at sea. Group members spent time discussing and considering how their responses might differ from those of their colleagues.
- Alpha Group members answered questions about their individual strengths. A representative question would be, "What is unique about you that leads to your happiest times and best performance at work?" Alpha Group members then spent time discussing and sharing responses to these individual strength questions.
- At the end of this session, Alpha Group members were presented with new fleece sweatshirts. These shirts were embroidered with the new employee’s individual name. Each Alpha Group member was also provided with an organization name badge. The new employees were asked to wear the shirt and name badge throughout the initial orientation and training period.

Beta group – early orientation exercises

- In the Beta Group, a Senior Leader plus a lead worker spent 15 minutes talking to the new employees about “why our company is a great place to work.”
- Beta Group members were presented with a set of written questions and asked to spend 15 minutes writing answers. A representative question would be, "What did you hear about our Company today that you would be proud to tell your family about?"
- Beta Group members then spent time discussing their answers to the written questions.

- Beta Group employees were given fleece sweatshirts embroidered with the company name. They were also presented with a company name badge. Beta Group members were asked to wear the shirt and name badge throughout the initial orientation and training period.

Results for Alpha Group and Beta Group, Application

While similar, the initial orientation and socialization experiences of the Alpha and Beta employee groups varied in the focus. Alpha Group activities focused more on the employee, while Beta Group experiences focused on the company and why the employee should be happy to work there.

Alpha and Beta Groups were tracked over the next 7 months. Turnover rate in Beta Group was 47.2% higher than that of the Alpha Group. Also of note is that the Alpha Group earned significantly higher customer satisfaction scores during the 7 months than those in the Beta Group (Cable, 2013; Nobel, 2013).

These results can be applied to the dairy farm setting. A dairy farm that is able to significantly reduce worker turnover by a basic change in focus will likewise reduce the high labor costs associated with turnover. Similarly, increased customer satisfaction rates, a result of higher worker satisfaction and better training can translate into better cow care conditions and improved key performance indicators (**KPI**).

Establishment of Orientation and Training Protocols

Farm employers spend significant efforts and resources to carefully recruit candidates, interview, check references, evaluate, and select a new employee. Best practices in regard to the

hiring process are beyond the scope of this paper, but such protocols should be established and followed to increase the likelihood that the best candidate is found for the dairy farm position. However, assuming that goal is achieved, these efforts can quickly dissipate without making the effort to get that new employee off to a good start on the very first day (or before) through a carefully planned orientation and training plan.

When the employment offer has been accepted, a start date should be agreed upon as soon as possible. Inform the employee of what will happen on the first day of work. Clearly communicate when they are expected to arrive.

While it may seem fundamental to the dairy farm employer, new workers are assisted by providing the answers to basic questions common among new employees. The farm employer should send new employees a letter by US mail or an e-mail with the answers to what might seem like very elementary questions such as the following:

(1) **What should I wear?** Provide guidelines on footwear, gloves, or other appropriate attire. More and more, new farm employees do not have farm backgrounds and need guidance so that they arrive for the first day of work appropriately attired. Particularly in dairy operations, there are biosecurity guidelines, and some attire may be provided. Inform the new employee that they will be trained on these biosecurity procedures. Dairy farm employers should not assume that new employees know what they should wear to work.

(2) **Should I bring my lunch or snacks and beverages?** Some farm work sites provide a noon meal, or snacks and beverages. Others do not. Some groups of farm workers stop in town for lunch each day. Let that new employee know what the practice is at the farm and what they should bring to work.

(3) Vehicles and parking questions: If the new employee is expected to have a vehicle to use in the position, this should have been communicated during the pre-employment process. Other employees may have concerns as basic as where they are expected to park at the farm site. Provide this information.

(4) What documents should I bring on my first day of work? The new employee will complete a Form I-9, as well as other basic forms on the first day of work. Inform the new employee of what documents should be brought to work on the first day to assist in completing these forms necessary for compliance with state and Federal law. Consult the US Citizenship and Immigration Services (USCIS) website: http://www.us-immigration.com/?utm_source=bing-yahoo&utm_medium=cpc&utm_campaign=uscis-srch&utm_term=usci for the most current I-9 forms and instructions. Note however, filling out forms and paperwork should not be the first on-the-job activity. Instead, follow the protocols of the Alpha and Beta Group research – focus on the new worker, what skills they bring to the workplace, and the personal satisfaction they will achieve on the farm. Paperwork and forms can be completed later.

(5) What should I bring (or not bring) to work? If the employee is expected to have a cellphone, that should be communicated. Some employees may need to be instructed that electronic music devices cannot be used on the job. Likewise, if the farm is tobacco or smoke-free, the new employee should be so instructed.

(6) What will I do on my first day of work? Tell the new employee what they will do on the first days (or weeks) of work. Confirm that work hours (including break policies) have been clearly communicated. Provide a general outline of initial orientation and training activities. This

will decrease the new employee's apprehension or confusion and help to get the new employee off to a good start with a planned orientation program, as well as initial and ongoing training opportunities.

First Day on the Dairy Farm

The new employee should be promptly greeted on the first day of work. Employers should not make the mistake of saying to the new employee – in essence – “we forgot you were coming, we’re not really prepared for you, just follow this guy around today, and we’ll check back with you later.” Introduce the employee to other workers and family members. Nametags can be very helpful to the new person, as it can be very confusing when meeting multiple people in the early days of employment. Immediately, show the new worker the location of restrooms and break areas. Until the employer is certain that the new employee has been thoroughly trained in farm safety practices and procedures, the new employee should be accompanied by a properly trained person.

Name tags/badges:

Even smaller dairy farm employers should consider having laminated clip-on photo identification name badges for all owners and employees. Recall the Alpha Group orientation protocols. There are a variety of systems that can generate badges. Such identification increases worker socialization, and farm security and biosecurity protocols are enhanced when each individual present on the farm is clearly identified.

The First Day of Work – Expectations of Millennials

There is an increasing volume of research regarding the expectations of millennials

(defined as persons born during the period 1981 through 1996) in the workplace (Friedell et al., 2013). One commentator summarized the expectations of millennials after the first day on the job in the form of 4 key questions to which these new workers should have good answers. (Chester, 2013).

1. Why did they hire me for this job?
2. Will I enjoy working here?
3. Are any of my coworkers “friend” material – or in other words, have I made a personal connection with someone else who works here?
4. Who can I talk to about my general questions and concerns?

The dairy farm employer should plan the first hours of the employment experience so that the new worker has positive answers to these questions at the end of the day.

In summary, at the end of the first day, the new employee should be asked if there are any questions or concerns. Offer the new employee assurances about how the first day went, and again offer information about what will happen in those early days of orientation and training.

Further Planning: Orientation and Training Programs

Orientation programs

All employees need orientation and training as they begin new employment. While training is an ongoing process that continues throughout employment, the orientation phase begins with the first day on the job and is generally completed within the first week or so of employment. That first day on the job will fly by quickly. The smart farm employer will have a plan in place for employee orientation and training.

Purposes of farm employee orientation

Employee orientation helps employees become socialized to the farm business which helps to reduce a new employee’s natural anxiety that comes with starting any new job. A new employee who becomes comfortable in the workplace is more likely to develop and maintain a positive attitude toward the job and the employer. This positive attitude translates into earlier and higher productivity. When the new worker is assisted in becoming quickly familiar with the work environment, the stress level decreases and the individual is better able to learn new job duties, skills, and expectations. This socialization aspect of employee orientation prepares a new worker for job training. If a new employee is relieved of general stress and worry, that individual is able to concentrate and absorb substantive information about new job assignments and tasks.

Planning and content of the orientation program

If the dairy producer has not previously conducted an employee orientation program, planning may seem like an overwhelming task. One way producers can think about orientation is to consult with current employees for input. Current employees should be surveyed regarding what they wish they had been told when they first started working on the farm. The producer should ascertain what current employees view as important information for newcomers. Every farm business is different, but some possible content areas to consider including the following:

Farm Background and Overview:

Provide new employees with the dairy farm’s story – the history and development of the farm business. This should include information about key people in the farm’s history, as well as present-day leadership. Share the dairy

farm's mission statement, goals, and objectives. While a dairy farm tour may have been part of the pre-employment process, this should be repeated, perhaps over a series of days as the new employee is introduced to the layout of facilities, fields, and operations. Throughout the process, emphasize the role and importance of the employee in the farm's success.

Employee Policies: Even the smallest dairy farm should consider development of an employee handbook or policy document. As part of the employee orientation process, all key policies, compensation, and benefits information should be reviewed. Producers should not just present a new employee with stacks of documents and instructions to read. Orientation is the farm employer's opportunity to review the policies, explain rationale, and provide opportunities for questions or clarification.

Introductions: As mentioned earlier, employee identification badges (or even embroidered shirts/apparel) can be very helpful in the farm workplace. Provide new employees with an organizational chart. Provide names of people who visit the farm on a regular basis, such as drivers, veterinarians, suppliers, service personnel, neighbors, or relatives.

Job Duty Information: While a position description was most likely discussed during the employment process, this is a key part of the new employee orientation phase. Producers should provide the written position description, and use it as a guide to discuss specific tasks, including training that will be provided to the new employee. Emphasize basic safety and indicate the importance of ongoing safety training and awareness. New employees should be assisted to understand the relationship and importance of the position to other jobs and functions on the farm.

Who Should Conduct New Employee Orientation?

To assure a consistent message to new employees, it is useful to have the same person conduct orientation. However, identifying other supervisors or more experienced co-workers to participate in the process will also assist in the socialization aspect of orientation. All members of the farm orientation team should be those who will share a positive attitude with the new employee. Especially during the early days of employment, the new worker needs to hear constructive, upbeat messages geared toward making those good, early impressions.

Outcomes – Conclusion

A well-planned orientation program takes an investment of time and effort on the part of the dairy farm employer. Providing a positive orientation experience during the early hours and days of employment sets the stage for a satisfying, long-term employment relationship on the farm. Surveys show that employees find job satisfaction when they feel that they are being treated with respect. The dairy producer who treats the new employee with respect from the very beginning will reduce turnover and labor costs while increasing productivity and profits, resulting in long-term employment relationships of benefit to all, especially the cows.

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An Updated Meta-Analysis of Bovine Somatotropin: Effects on Health and Welfare of Dairy Cows¹

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Abstract

Recombinant bovine somatotropin is a technology that allows a liter of milk to be produced using fewer nutrients and a lower carbon footprint. Twenty years of commercial use of POSILAC[®] (**rbST**) in the US provides the backdrop for an updated reevaluation of the effects on cow health and welfare. Our evaluation involved a meta-analysis of data from peer-reviewed publications or regulatory reports with the criteria being that rbST use was according to label specifications (St-Pierre et al., 2014). Twenty six studies were identified which had usable data (13,784 cows). Results indicated milk yield was increased by 8.8 lb/day, whereas milk fat, protein, and lactose contents were unaltered. Likewise, the use of rbST had little or no effect on variables associated with cow health and welfare. Overall, these results and 20 years of commercial experience demonstrate that management practices used by US dairy producers are adequate for the effective use of POSILAC to increase milk production with no adverse effects on cow health or well-being.

Introduction

Recombinant bovine somatotropin is a production-enhancing technology that allows the dairy industry to produce milk more efficiently. The commercial formulation is recombinant sometribove-zinc (**rbST**) which

is marketed under the trade name POSILAC[®]. Cows treated with rbST produce a liter of milk with less feed resources and a reduced carbon footprint. As the first recombinant protein approved for use in production animals, rbST received unprecedented scrutiny. In the US, this included the traditional evaluation by FDA, as well as public hearings, science evaluations and legislative reviews (Bauman, 1992). After a thorough review of well-controlled studies, FDA concluded that rbST could be used safely and effectively by the US dairy industry. Use commenced in February 1994 and to date an estimated 35 million US dairy cows have received the commercial formulation of recombinant bovine somatotropin (St-Pierre et al., 2014).

Not all agreed with the above conclusions on the use of rbST. Health Canada requested that the Canadian Veterinary Medical Association (**CVMA**) evaluate if “rbST used in accordance with label directions will increase milk production without resulting in serious health problems which cannot be adequately controlled by current management practices”. CVMA formed a task force and addressed their mandate by using a meta-analysis of studies that used recombinant bovine somatotropin. The CVMA Report (Dohoo et al., 1998), subsequently published in the Canadian Journal of Veterinary Research (Dohoo et al., 2003a; 2003b), concluded that use of bST would

¹Text and data derived from St-Pierre et al. (2014).

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increase yields of milk and milk components but would also adversely impact cow health and welfare, especially udder health, lameness, body condition, reproduction, and lifespan (Dohoo et al., 2003a; 2003b).

Since the CVMA report, there have been several large scale rbST investigations relating to various aspects of cow health and welfare (e.g., Ruegg et al., 1998; Bauman et al., 1999; Judge et al., 1999; Collier et al., 2001; Santos et al., 2004). Results from these investigations and commercial experience on US dairy farms seem at odds with the conclusions reached by the CVMA (Dohoo et al., 2003a; 2003b). Thus, we undertook an updated evaluation of the impact of rbST on the efficacy, health and welfare of dairy cows.

Approach

To provide an updated evaluation of the efficiency and safety of rbST, we formed an expert panel made up of a data manager and project coordinator, a professional statistician, and 6 domain experts (St-Pierre et al., 2014). The evaluation involved a set of meta-analyses. Criteria to be included was that data were from peer-reviewed scientific publications or regulatory agency reports where rbST was used according to label. Data from studies involving off-label use of rbST or studies that used unapproved formulations or doses of rbST were excluded.

Potential data for the analysis were identified by an extensive literature search using PubMed (US National Library of Medicine, US National Institute of Health, Bethesda, MD), Agricola (National Agriculture Library, US Department of Agriculture, Beltsville, MD), Web of Science (Thomson Reuters Science, New York, NY), and CAB Direct (CAB International, Wallingford, UK). Potential studies were

identified and their abstracts obtained (Figure 1). All studies that were not conducted using the commercial formulation of rbST or that clearly did not report results pertinent to the analyses (e.g., dairy market analyses) were immediately discarded. The remaining studies were numbered and corresponding full publications were obtained. Twenty-six studies met the criteria and data from these formed our meta-database (Figure 1). Specific details of the methodology for the meta-analysis can be found in St-Pierre et al. (2014), and results of this analysis are presented in the following sections.

Results and Discussion

Milk Yield and Composition

Seven variables were analyzed to characterize milk and milk composition responses to rbST: milk yield, percent milk fat, percent milk true protein, percent lactose, 3.5% fat-corrected milk yield, fat yield, and protein yield. Except for the percentage of lactose in milk, responses across studies were heterogeneous ($P < 0.10$), indicating that unidentified factors associated with individual studies affected the magnitude of the response.

Results demonstrated that yield of milk and milk components were all increased by rbST treatment. Milk yield (8.8 lb/day) and 3.5% fat corrected milk (8.9 lb/day) were increased by about 15% over control cows (Table 1). However, milk composition for fat ($P = 0.09$), protein ($P = 0.07$), and lactose ($P = 0.26$) was not affected (Table 1). Thus, yield of these milk components increased in parallel to milk production with daily yields of fat ($P < 0.001$) and protein ($P < 0.001$) being increased by an average of 0.317 and 0.301 lb/day, respectively. In agreement with the present meta-analysis, other summaries demonstrate that values for milk responses to rbST tend cluster

about a range of 9 to 11 lb/day (Bauman, 1999). Likewise, other investigations have consistently observed that milk composition is not altered by rbST-treatment and factors which affect milk composition do so in an identical manner in rbST-treated cows (Bauman, 1992; National Research Council, 1994).

Udder Health

Milk SCC is an indicator of inflammation in the mammary gland, and the SCC of milk will increase in response to both subclinical and clinical mammary infections (Hogan and Smith, 2012). In our meta-analysis, tests for heterogeneity indicated significance for both milk log SCC ($P < 0.001$) and mastitis incidence rate ($P < 0.04$); thus, unidentified factors associated with individual studies affect the observed values. In the case of SCC, the control group averaged nearly 100,000 SCC/mL, and there was no effect of rbST treatment ($P = 0.54$; Table 1). Likewise, the mastitis incidence rate was not different between the control and rbST-supplemented groups ($P < 0.12$; Table 2). These results are consistent with the systematic review of the effects of rbST on mastitis incidence and SCC conducted by JEFCA (2013). Their review of clinical and epidemiological studies found no effect of rbST on mastitis incidence. In the case of subclinical mastitis, they reported that the “vast majority of studies reported no effect of rbST treatment on SCC values, although a few studies reported small transient increases” (JEFCA, 2013).

Environmental and management factors are the major causes of mastitis, and they impact both SCC and mastitis incidence. In addition, genetic studies have demonstrated a small positive relationship between mastitis risk and milk production. However, high producing herds are better managed so that effects of increased milk production on mammary health are minimized or negated (Hogan and Smith, 2012).

Body Condition

Dairy cows need to maintain an adequate body condition over the lactation cycle. Thus, it was of interest whether rbST-treated cows would become thin and emaciated due to the use of body reserves to support the increased milk production. Data for body condition score (BCS) were available for 15 studies, and the test for heterogeneity of responses among studies approached significance ($P = 0.10$). The BCS data used in the meta-analysis consisted of the BCS obtained during and after rbST treatment. Mean BCS was lower in cows treated with rbST as compared to control cows ($P = 0.04$), with the difference being -0.064 ± 0.031 points (mean \pm SE; Table 1). Published studies indicate that 1 point of BCS represents about 110 lb BW (see St-Pierre et al., 2014), so the difference in BCS for the rbST-treated cows represents about 7 lb BW. While significant, this difference would not be visually detected and is about equivalent to the change in BW associated with a typical feeding or drinking episode for a dairy cow. Consistent with the meta-results, research has demonstrated that rbST-treated cows increase voluntary intake in an amount energetically comparable to the rbST-induced increases in milk yield (Chilliard, 1989).

Lameness

Lameness reflects altered locomotion or mobility caused by a range of foot and leg disorders that result from disease, management, or environment factors (Shearer et al., 2012). For our meta-analysis, data regarding the number of cows that were clinically lame are presented in Table 1. Where possible, data were separated into 2 categories - lameness lesions and traumatic lesions. Lameness lesions are lesions that directly cause clinical lameness (e.g., laminitis, sole ulcers, or digital dermatitis), whereas traumatic lesions are lesions that rarely,

cause or result in lameness (e.g., mechanically induced skin lesions) (Shearer et al., 2012). The test for heterogeneity was not significant for any of the 3 outcome variables ($P = 0.999$). Likewise, incidence rates for cows that were clinically lame, had lameness lesions, or had traumatic lesions did not vary significantly between cows that were and were not treated with rbST ($P = 0.99$; Table 1).

Reproduction

A significant 5.4% improvement in pregnancy proportion was observed in the rbST supplemented cows for the first 2 breeding cycles after the voluntary wait period ($P < 0.01$; Table 2). When compared over the full length of the trial, the pregnancy proportion was reduced 5.5% for the group receiving rbST ($P < 0.05$; Table 2), a reduction that was likely due to reduced estrous behavior. The fact that rbST-treated cows were more likely to become pregnant during the first 2 breeding cycles, the period when cows are generally enrolled in a timed-AI protocol, suggests that rbST did not impair, and might even have a positive effect on the reproductive performance of dairy cows during this period.

There was no effect of rbST on fetal loss, days open, services per conception, or twinning (Tables 1 and 2). Similarly, the incidence rate of cystic ovaries did not differ between controls and rbST-treated cows ($P = 0.43$; Table 2). The lack of effect on ovulation failure and cystic ovaries in dairy cows is consistent with the results in which rbST-treated cows ovaries with healthy estrogen-active follicles (De La Sota et al., 1993).

Culling

Results of our meta-analysis indicated that culling density did not differ between

controls and cows treated with rbST ($P = 0.34$; Table 1). These findings corroborate those of a large longitudinal field study conducted over 4 years on 340 commercial dairy herds in the Northeastern US; results demonstrated that rbST use had no effect on stayability or herd-life (Bauman et al., 1999). Culling rate is often incorrectly assumed to reflect the quality of the production and management system. The optimal culling rate increases when there is a relative abundance of replacements and the cost of a replacement cow is similar to the slaughter value of the cow being replaced (St-Pierre et al., 2014).

Summary and Conclusions

Results of the meta-analysis carried out by St-Pierre et al. (2014) indicated that administration of the commercially available rbST formulation to lactating dairy cows according to FDA-approved label directions resulted in an increase in milk, fat, and protein yields with no unmanageable adverse effects on milk composition (percentages of fat, protein, and lactose), udder health, body condition, lameness, reproduction, or culling. These findings are contrary to the meta-analysis conducted by the CVMA (Dohoo et al., 2003a; 2003b). There are several reasons for conclusion differences as discussed by St-Pierre et al. (2014). Briefly, our meta-analysis was able to include studies conducted subsequent to the CVMA report (Dohoo et al., 1998), and several of these were large scale studies conducted on commercial dairy farms. Consistent with our objective, we included all studies which followed “label directions for use”, whereas the CVMA Report combined rbST studies that varied in formulation, dose, administration route, and period of use. In addition, we identified several errors in the CVMA database that would effect results (see discussion in St-Pierre et al., 2014).

Overall, our meta-analysis provided no evidence that use of rbST causes any unmanageable adverse effects on milk composition, udder health, reproduction, body condition, lameness, or longevity (St.-Pierre et al., 2014). These results are consistent with the various FDA evaluations (US FDA, 2014a; US FDA 2014b), numerous scientific reviews (e.g., Crooker and Otterby, 1991; Bauman, 1992; National Research Council, 1994), and large-scale studies conducted on commercial dairy operations (e.g., Ruegg et al., 1998; Bauman et al., 1999; Collier et al., 2001; Santos et al., 2004). Collectively, these results and 20 years of commercial experience involving rbST-treatment of over 35 million US dairy cows provide definitive evidence that management practices used by US dairy producers are adequate for the safe and effective use of rbST.

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Table 1. Estimates of responses to rbST and associated statistics from the meta-analyses of continuous traits.¹

Variables	Number of Studies	Mean of Control Cows	Response Estimate	Standard Error of Estimate	P Value	95% Lower CL ⁵	95% Upper CL
Milk production and composition							
Milk yield (lb/day)	15	59.8	8.8	0.9	<0.001	3.21	4.79
Fat (%)	13	3.64	-0.073	0.043	0.09	-0.156	0.011
Protein (%)	13	3.15	0.025	0.013	0.07	-0.001	0.051
Lactose (%)	11	4.82	0.023	0.021	0.26	-0.017	0.063
3.5% FCM (lb/day)	13	64.2	8.9	0.9	<0.001	3.24	4.84
Fat yield (lb/day)	13	2.38	0.317	0.046	<0.001	0.104	0.185
Protein yield (lb/day)	13	1.89	0.301	0.046	<0.001	0.101	0.173
Reproduction (all parities)							
Days open	5	104.2	-0.21	4.18	0.96	-8.39	7.98
Services per conception	4	1.66	-0.25	0.162	0.12	-0.57	0.07
Udder health							
Log ₁₀ somatic cell count	9	4.99 ⁶	-0.034	0.055	0.54	-0.141	0.074
Lameness and lesions²							
Clinical lameness	7	0.38	0.13	1.14	0.99	-2.18	2.21
Lameness lesions	3	1.12	0.32	29.2	0.99	-55.4	56.0
Traumatic lesions	5	0.11	0.093	7.59	0.99	-15.5	15.7
Body condition							
Body condition score ³	15	3.31	-0.064	0.031	0.04	-0.124	-0.004
Culling							
Culling density ⁴	6	4.64	0.603	0.633	0.34	-0.637	1.018

¹From St. Pierre et al. (2014).

²Expressed as incidence rate per 1,000 cow-days at risk.

³Body condition score is expressed on a 1 to 5 scale, with 5 being severely over-conditioned.

⁴Culling density is expressed as incidence rate per 10,000 cow-days at risk.

⁵CL = confidence limit.

⁶Log₁₀ somatic cell count of 4.99 = 97,734 somatic cells/mL milk.

Table 2. Estimates of responses to rbST expressed as odds ratios and associated statistics from the meta-analyses of non-continuous traits.¹

Variables	Rate of Control Cows	Estimates of Odds Ratio	<i>P</i> Value	95% Lower CL ⁴	95% Upper CL
Reproduction, all parities					
Pregnancy rate in LRP ²	0.291	1.281	0.01	1.072	1.530
Pregnancy rate in ERP ³	0.761	0.753	0.05	0.568	0.997
Fetal losses rate	0.115	1.065	0.65	0.812	1.397
Twinning rate	0.065	1.107	0.68	0.685	1.787
Cystic ovaries rate	0.065	1.171	0.43	0.795	1.725
Udder health					
Mastitis incidence rate	0.174	1.249	0.12	0.942	1.655

¹From St. Pierre et al. (2014).

²Limited response period (first and second AI inseminations).

³Extended response period (full duration of the trial).

⁴CL = confidence limit.

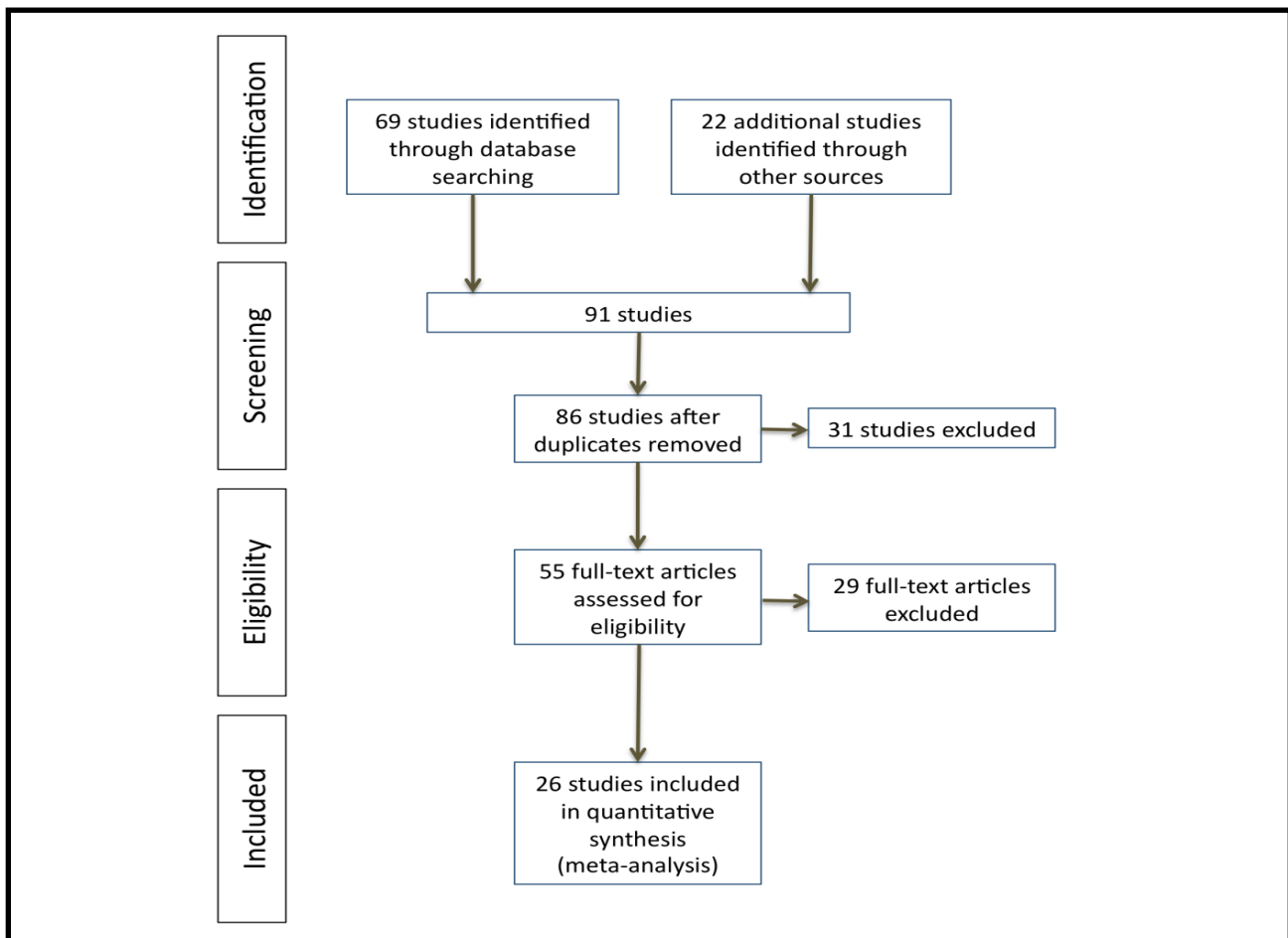


Figure 1. Flow diagram for studies considered in a meta-analysis of the effects of rbST administration on the production and health of lactating dairy cows (St-Pierre et al., 2014).

Using Decision Tree Analysis to Make Herd Management Decisions

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Introduction

Dairy producers and dairy consultants are continually faced with decisions. Making a decision between 2 alternatives, when both the costs and projected returns of each alternative are predictable with moderate to high accuracy, is fairly straightforward and can be performed with the aid of simple tools (e.g., partial budgets). More commonly, however, life is not so straightforward. Decision trees are formal quantitative tools that may be used to select the best course of action in situations where the decision is complex and outcomes are uncertain (Overton, 2004). Indeed, decision trees are particularly useful when there is uncertainty, since the probability of each potential outcome is factored into the analysis.

How to Construct a Decision Tree and Make a Decision Therefrom

Decision trees are constructed from left to right and begin with a square box called a root node or decision node. Lines are drawn from the box projecting toward the right, ending at a circle representing each of the decision alternatives that are available. Only one of these alternatives may be selected. Each circle may represent an outcome in itself or several possible outcomes that might result from that decision alternative may then be drawn, projecting still further to the right from each

circle. Values (in dollar amounts) are assigned to each outcome. Values may be positive or negative. A probability is then assigned to each potential outcome (when there is more than one) within a given decision alternative. Probabilities are sometimes available from the research literature. More often, probabilities must be estimated. This would seem to be a problem, but it is a problem that can be overcome (more on that later). Note that within a given decision alternative, the probabilities of the outcomes must sum to 1. Any costs associated with a given decision alternative are inserted and considered along that decision pathway. Finally, an “expected value” (again, in dollars- often this is instead referred to as “expected monetary value”) is calculated for each decision alternative by folding back the decision tree (doing calculations from right to left). Folding back to an expected value for each decision alternative involves subtracting any costs associated with that decision and then multiplying each outcome by its probability. The decision with the highest expected value is the recommended action to take. It is important to realize that the “expected value” is not the expected return (\$) if that alternative is chosen. The expected value is the average expected return (\$) of many iterations of the same set of circumstances.

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Examples

Figure 1 is an example of a simple decision tree constructed to evaluate the question of what to do with a cow with a left-displaced abomasum (LDA). The roll and toggle procedure is the preferred decision based upon the tree since the expected value of the roll and toggle (\$2195) is greater than that of surgery (\$2081) or shipping to beef (\$1503). Drilling down into the decision reveals that the cost of the surgery versus the cost of the roll and toggle is what makes the tree lean toward roll and toggle. Figure 2 is a re-evaluation of the same decision in an attempt to determine how much the success rates of the 2 procedures would need to change to shift the decision toward surgery. Readers of this monograph are encouraged to disagree with the assumptions contained in the examples and to draw their own trees to reach their own conclusions.

An example of a problem with the LDA example in Figure 1 is that it only values the profit from the current lactation and does not reward any profits from future lactations to the cow that survives and is kept. The LDA decision in Figure 1 was deliberately kept simple for purposes of illustration - a more complex analysis is certainly possible. Indeed, a more complex analysis reveals that surgical LDA correction is generally a better investment into a younger cow, due to the longer potential time available to recoup the cost of the intervention (Overton, 2004). However, surgery is still not necessarily better than roll and toggle.

Advantages of Decision Tree Analysis:

- Simple, easy and fast- can be done with paper and pencil.
- Decision trees offer an easy to understand visual representation of the decision.

- Can be applied to complex decisions involving many alternatives.
- Provide a more robust analysis of the decision, given that the likelihood of each outcome is taken into account.
- During construction of a decision tree, issues surrounding the decision that may have historically gone unrecognized may become apparent. Furthermore, issues that were previously thought to be rare and therefore not even considered in the decision process, may become recognized to be important enough to change the decision.

Disadvantages (Some With Rebuttal) of Decision Tree Analysis:

- The probabilities associated with outcomes are often unknown. However, this is only a potential disadvantage since it is easy to adjust the probabilities up or down and then see how likely or unlikely a given outcome would need to be to alter the decision. If a probability would need to get into the unrealistic range to change the decision, then the original decision should be reasonable.
- Failure to consider a potential outcome can invalidate the tree, and therefore, lead to a spurious decision.
- It is difficult to use decision trees when an outcome is a continuous variable – e.g., the expected effect on milk production of an input under consideration. This can be partially overcome by assigning several possible outcomes over a range, each with an associated probability. This can be even better overcome by utilizing either advanced mathematics or appropriate computer software.

- Constructing a decision tree for the first time often requires several attempts. This is not a bad thing, since as noted above, the process of construction is often instructive in itself. However, some clients may not have the curiosity or patience to endure through the process and thus become frustrated and ultimately lose confidence in the tool. Note that this can be overcome by preparing a tentative tree ahead of time.
- Decision trees are not appropriate when catastrophic failure (e.g., bankruptcy) is a potential outcome. For example, in the case of a single game of Russian roulette where you had to pay \$10 to play and would receive \$1,000,000 if you won, a decision tree would lead to the decision to play since there is only a 1 in 6 chance of failure. However, most of us would choose to not risk death, even if the odds were only 1 in 6.

Summary

Decision tree analysis is a simple yet powerful tool. Decision trees offer a robust method of analyzing decisions, given that the likelihood of each of potentially many outcomes is taken into account. Furthermore, the actual construction process of building a decision tree can help to elucidate issues surrounding the decision that might have gone overlooked or inadequately considered.

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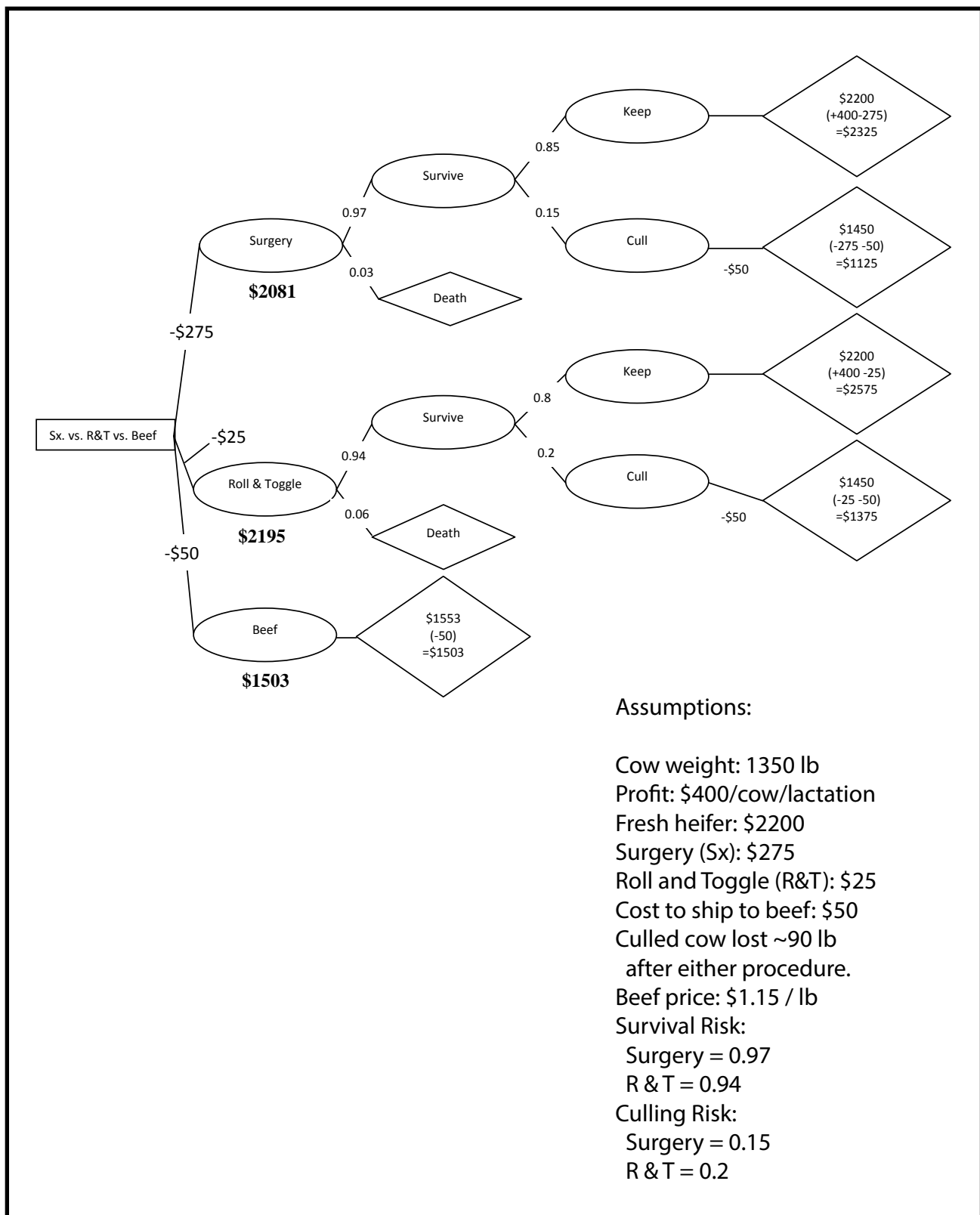


Figure 1. Decision due constructed to evaluate what to do with a cow with a left-displaced abomasum.

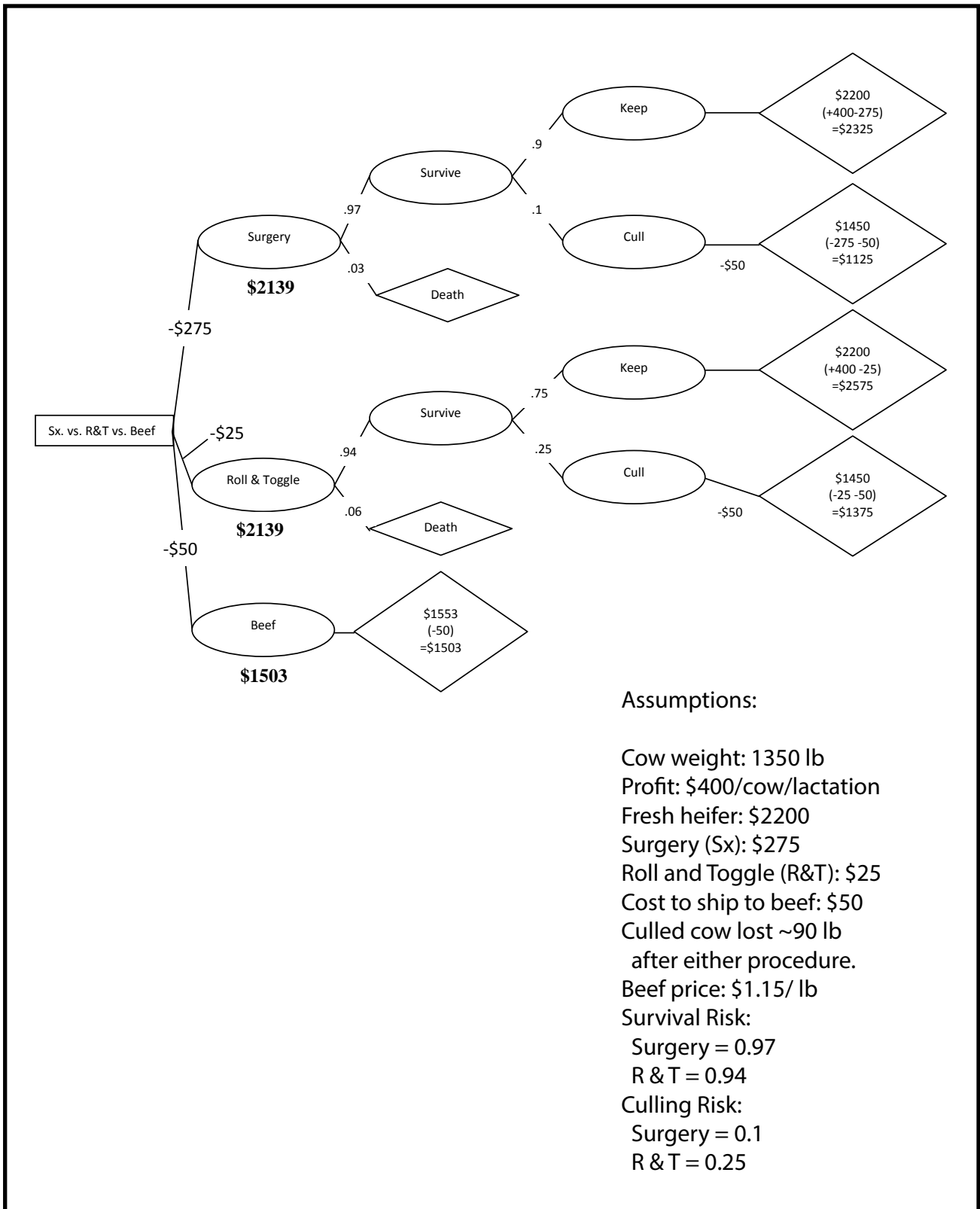


Figure 2. Decision tree contrasted to evaluate what to do with a cow with a left-displaced abomasum, using different assumptions than for Figure 1.



New Insights on Feeding Post-Weaned Dairy Heifers

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Abstract

Nutrition of post-weaned heifers is important to continue to promote the growth and development of heifers. Even though there is a lot of focus placed on feeding milk-fed calves, little research information is available regarding the best strategies for feeding post-weaned dairy heifers. As feed costs are the greatest expense for raising dairy heifers, nutritional strategies to encourage growth and development, while improving feed efficiency, will be beneficial for both the animals and heifer raisers. Numerous recently conducted research studies continue to show the importance of feeding post-weaned heifers quality, grain-based diets as a way to increase growth and improve feed efficiency. Continuing to component feed heifers as they entered the growing phase was found to be advantageous as compared to switching young heifers (~300 lb) onto a TMR feeding system. In addition, continuing to feed diets containing a higher level of grain and concentrates (60:40 grain to forage ratio) was found to improve average daily gain (ADG) and growth, while decreasing the costs per pound of gain. Further research has shown that feeding heifers diets containing greater levels of non-fiber carbohydrates (NFC) resulted in greater ADG in heifers from 12 to 28 weeks of age. Diets of post-weaned heifers are important to continue to promote the proper growth and development of these heifers to ensure that they will be ready for breeding.

Introduction

Even though much emphasis continues to be placed on the nutrition of milk-fed calves, these animals continue to grow and develop. Paying close attention to the diets of post-weaned heifers helps to make sure they are growing at a rate to make sure that they will be ready for breeding and that they are efficiently utilizing the diets they are fed.

Heifer diets are often forage-based diets that are formulated with a goal of being inexpensive. As heifers are fed for approximately 2 years without any economic return, they do comprise a significant cost for dairy operations, and heifers are usually either the second or third greatest expense for dairy herds (Heinrichs et al., 2013). As compared to lactating cattle, dairy heifers have relatively low nutrient requirements and are often fed diets with higher forage levels. However, young heifers require greater dietary nutrient concentration than older heifers and, therefore, need to be fed differently.

Nutrition of dairy heifers is often talked about as a whole without referring to the age and growth stage of the heifer. Similar to lactating cows in various stages of lactation, the nutrient requirements of dairy heifers vary substantially during their 2 years of development. Although milk-fed calves have obviously different feed requirements, the nutrient requirements of

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heifers continue to change, especially over the 6 months after weaning. It is important to keep in mind that calves recently weaned have very different nutrient requirements from year-old heifers, and thus, need to be fed differently. Starter intake does help to promote the growth and development of the rumen in calves, but making the assumption that weaned calves are fully functional ruminants is not correct. Therefore, continuing to pay close attention to how post-weaned heifers are fed will allow for the rumen to continue to develop and will maximize the growth and development of these heifers.

Feeding Post-Weaned Heifers

Grain and forage ratios

In most dairy systems today, calves are fed ad libitum amounts of palatable grain-based starters within a few days of birth. As calves grow, they continue to increase their starter intake until they are to the point where they are able to consume enough nutrients from the starter to support their growth without consuming milk. Once calves are weaned, their starter intake continues to increase substantially to make up for the nutrients that are no longer being consumed through milk and to cover the increased nutrient needs of the calf as they continue to grow. At this time, calves are often fed a diet that consists of only starter or starter and some forage. The timing as to when calves should begin to receive forage, the type of forage they should receive, and how much of that forage they should be given is still of some debate. Some recommendations are that calves do not need to receive any forage until a couple of weeks after weaning, though there is some evidence that having some forage available at weaning may be beneficial (Bach, 2011). In addition, information as to how to continue transitioning these heifers to higher forage diets has been even less available.

Research was conducted at Purdue University to look at different grain-to-forage ratios to help determine the best strategy for feeding post-weaned dairy heifers. Heifers began the study when they were approximately 330 lb and 4.5 months of age and were assigned to diets containing either 80, 60, or 40% concentrate (DM basis) for 56 days before abruptly being switched to a common diet that was 40% concentrate.

In this study, increasing grain inclusion from 40 to 80% of the dietary DM resulted in a linear increase in BW (Table 1). Total BW gain during the treatment period averaged 76.8, 104.9, and 136.0 lb for heifers fed 40:60, 60:40, or 80:20, respectively; whereas, total gain on the common diet averaged 108.2, 106.9, and 96.4 lb for heifers previously fed 40:60, 60:40, or 80:20, respectively. Average daily gain was improved overall for heifers fed 80:20 during the treatment period compared with heifers fed 40:60 or 60:40, though following a diet change, ADG was improved for heifers previously fed 40:60 or 60:40 compared to heifers fed 80:20. Frame growth exhibited similar responses to those observed for BW and ADG. Hip heights, heart girth circumference, and body condition score linearly increased with increasing grain inclusion ($P < 0.01$) during the treatment period, resulting in higher growth overall during the study for heifers fed 80% grain during the treatment period. Peri et al. (1993) reported increased BW for dairy heifers fed ad libitum compared to restricted energy diets. However, Buskirk et al. (1996) fed early-weaned beef heifers either a moderate- or high-energy diet and reported similar ADG and skeletal growth, most likely due to increased intake for heifers fed the moderate-energy diet, resulting in similar energy intake between treatments.

Feed costs averaged \$0.11, 0.12, and 0.13/lb of DMI for heifers fed 40:60, 60:40, and

80:20, respectively, during the treatment period (Table 2). Daily feed costs per head were 44.7 and 21.9% greater for 80:20 than 40:60 and 60:40, respectively, on day 14 of the trial and subsequently increased with increased DMI. On day 56 prior to switching to a common diet, feed costs per head were 68.1 and 32.5% greater for 80:20 than 40:60 and 60:40. Feed costs per pound of ADG were lowest for 60:40 heifers over the duration of the study compared to heifers fed 40:60, though they were statistically similar to the feed costs for the 80:20 heifers. When heifers were fed 60:40 or 80:20 during the treatment period, savings were \$0.24 and 0.22/lb of ADG compared to heifers fed 40:60.

This study demonstrated that feeding higher grain levels to post-weaned dairy heifers can improve growth and can actually decrease the cost of gain over higher forage diets. In addition, it reinforced that heifers fed high grain levels can be negatively impacted by abrupt changes to higher forages diets, with the heifers on the 80:20 treatment showing a definite decline in intake when they were switched to a 40:60 diet from which it took some time to recover (Figure 1).

Non-fiber carbohydrates in heifer diets

Even though previous research found that feeding higher concentrate diets improved gain and feed efficiency, the concentrate portion of the diet may be made up of a wide variety of different ingredients and nutrient compositions. Understanding the best strategies for designing the concentrate portion of the diet could further help to improve the gains and feed efficiency of dairy heifers.

Previous research has found that butyrate and propionate are the most important volatile fatty acids for developing the rumen in young heifers (Tamate et al., 1962; Lesmeister

and Heinrichs, 2004). Therefore, diets that provide greater amounts of readily fermentable substrates could potentially increase the production of butyrate and propionate in the rumen and may help to further promote rumen development and increase the growth and development of heifers.

In order to evaluate the effects of the composition of the concentrate portion of the diet on heifer growth, intake, and feed efficiency, studies were conducted to look at the effects of feeding concentrates that were formulated to provide either high or low levels of non-fiber carbohydrates (NFC). In the first study, heifers were fed a low NDF diet (LNFC), a high NFC diet (HNFC), and a low NFC diet with added fat (LNFC+) formulated to provide the same amount of Mcal of energy as the HNFC diet.

Heifers fed LNFC+ were heavier on day 56 and 112 of the study compared to heifers fed LNFC (Table 3). Heifers on the HNFC diet were intermediate and tended to be lighter on day 56 and 112 compared to heifers fed LNFC+. Overall, heifers fed LNFC+ gained 19.4 lb more BW than heifers fed LNFC during the study ($P = 0.05$). Average daily gain in the first 56 days was 14.9 and 8.9% greater for heifers fed LNFC+ compared to heifers fed LNFC ($P < 0.01$) or HNFC ($P = 0.05$), respectively. Several studies have illustrated increased growth rates with increasing energy concentration for growing dairy heifers (Radcliff et al., 1997; Davis Rincker et al., 2008), though increased body condition likely accounted for some of the differences in this study as energy intake increased.

During the first 56 days, treatment tended to affect feed efficiency (FE), as heifers fed LNFC+ were 12.7% more efficient than heifers fed LNFC and 9.3% more efficient than heifers fed HNFC, with a trend ($P = 0.07$)

towards improved FE for LNFC+ from day 0 to 112 as compared to HNFC. Net efficiency of fiber utilization, whether from forage or non-forage sources, is generally lower than that of starch and fat (VandeHaar and St-Pierre, 2006), though there were not any differences between the FE of high and low NFC diets in this study. However, there was an advantage in FE when fat was added to the higher fiber diet during first half of the study when heifers were younger.

During the NFC study, heifers fed LNFC maintained the lowest cost per heifer per day throughout the study as was expected due to the high inclusion rates of by-product feeds. However, feed costs per pound of ADG were lowest for heifers fed LNFC+ compared to HNFC, resulting in a cost savings of \$0.12/lb of gain (Table 4). However, feed costs per pound of ADG were similar overall among treatments. In our study, a larger proportion of the HNFC diet included corn and distillers dried grains, resulting in greater costs per ton for the grain mix, especially due to higher corn prices from the 2012 crop year. Paired with increased DMI for heifers fed HNFC, our data suggest that alternative energy sources, such as supplemental fat, may be more cost-effective for feeding growing heifers.

A second study was conducted to evaluate the effect of NFC level in the diets of post-weaned heifers after being started on either a conventional (22:20) or higher plane of nutrition (28:20) milk replacer. One of the goals of this study was to determine if how a calf was raised pre-weaning affects subsequent heifer growth and performance. In this study, animals receiving the HNFC diet had greater weight gain during the growing period from 12 to 28 weeks. Interestingly, when the animals were started on a higher plane of nutrition during the milk feeding period and subsequently fed LNFC diets, their BW gain was significantly

decreased as compared to animals that were started with a conventional milk replacer program (Table 5). This study indicates that when calves are started on diets with a higher level of nutrition, maintaining a greater level of nutrition into the growing period may be even more important than when calves are started on a conventional milk feeding program.

Intake of post-weaned heifers

When formulating diets for heifers, having a knowledge of intake is important to help determine dietary concentrations needed to ensure that the animals are consuming the recommended amounts of nutrients. The current dairy NRC (2001) model utilizes only $BW^{0.75}$ and NE_m content of the diet when predicting intake of non-pregnant growing heifers and does not consider other dietary or non-dietary factors. Estimates of DMI for large breed heifers according to the dairy NRC (2001) are 2.8% of BW or less. In our research, intakes of post-weaned heifers averaged 3.0% or more of their BW when they were fed diets containing at least 60% concentrate (Figure 1).

In a study designed to look at feed delivery methods, diets formulated according to the NRC (2001) requirements for 2.0 lb/day of ADG for Holstein heifers estimated DMI of 13.6 lb/day for heifers at the conclusion of the study. Actual DMI observed at the end of the study averaged 20.6 lb/day among treatments, a 51% increase over the NRC predicted intake. While ADG was similar to NRC predictions in the current study, particularly for heifers fed using a TMR, the gross under-estimation of DMI by the model suggests factors other than dietary energy content are required for more accurate estimations of intake in heifers.

Other estimations for intake of heifers have been made. Hoffman et al. (2008)

proposed that replacement heifers will restrict their overall intake to 1.0% of BW as NDF intake; however, in the feed delivery study, NDF intake ranged from 1.3 to 1.4% of BW during the transition period and reached over 2.0% of BW during the grower period. Similarly, NDF intakes ranged from around 1.0 to over 1.6% of BW for heifers receiving different grain to forage ratios (Figure 2), suggesting that factors other than total dietary NDF have the potential to influence intake in replacement heifers. However, when just forage NDF intake was determined as a percentage of BW, heifers did not consume above 1% of BW (Figure 3), indicating that forage NDF and not total NDF may be a better estimator of intake in younger heifers.

Feed delivery methods for post-weaned heifers

Dietary composition is an important aspect of feeding heifers, but the delivery method can also have an impact when feeding heifers. A study was conducted to evaluate the effects of feeding heifers a TMR, feeding them concentrate and hay side-by-side in a feed bunk (SBS), or feeding grain in a bunk and hay in a feeder (HF) on growth and intake of post-weaned heifers (Table 6). In this study, heifers fed using HF were significantly heavier ($P \leq 0.05$) than heifers fed using SBS from day 49 throughout the end of the study. Delivering feed using HF resulted in heifers that were, on average, 12.1 and 7.3 lb heavier than heifers fed using SBS and TMR, respectively, over the course of the study. Heifer weights at the conclusion of the grower period were 605, 576, and 575 lb for HF, SBS, and TMR, respectively.

Average daily gains did vary depending on the time period of the study, as heifers fed using a TMR had lower ADG from day 7 to 14 ($P = 0.05$) and day 14 to 21 ($P = 0.07$) compared with HF and SBS, but higher ADG compared to

SBS from day 21 to 28 ($P = 0.03$). These results suggest that post-weaned heifers require more time to adjust to new diets when feeding a TMR compared with component-feeding.

During the grower period, heifers fed using HF averaged 1.1 lb/day more DMI compared with SBS and TMR ($P < 0.01$). However, heifers fed using a TMR consumed more DMI daily from day 63 to the conclusion of the study. The results of this study suggest that, along with responses in ADG, component-fed heifers maintained intake and weight gains when transitioning to a new diet, while TMR-fed heifers caught up in terms of ADG and efficiency towards the end of the transition period and throughout the grower period. This study indicates that there may be a certain point during the growth of a heifer when it is ideal to be able to switch over to feeding a TMR.

Conclusions

Using the best feeding strategies for post-weaned dairy heifers allows them to continue to meet their growth potential while reducing costs per pound of gain and reducing the overall costs of raising dairy heifers. Continuing to feed heifers high levels of grain post-weaning provides them with a digestible source of nutrients that facilitates growth and improves feed efficiency. At young ages, heifers appear to continue to need readily available energy sources as their rumen continues to develop. Realizing that post-weaned heifers are still developing and are not yet ready to be fed like cows facilitates an understanding that specific feeding strategies need to be developed to allow for optimal growth and development of these heifers.

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Table 1. Weight, skeletal measurements, and intake responses of prepubertal dairy heifers fed increasing levels of grain during the treatment period and then switched to a common diet.¹

Item	40:60 ²	60:40	80:20	SEM	P-value
Body weight, lb					
day 57	369 ^c	399 ^b	429 ^a	6	< 0.01
day 112	476 ^c	505 ^b	525 ^a	6	< 0.01
ADG ³ , lb/day					
day 0 to 56	1.37 ^c	1.87 ^b	2.29 ^c	0.09	< 0.01
day 57 to 112	1.94 ^a	1.92 ^a	1.72 ^b	0.06	0.07
day 0 to 112	1.65 ^c	1.90 ^b	2.07 ^a	0.04	< 0.01
DM intake, lb/day					
day 0 to 56	9.3 ^c	10.7 ^b	12.7 ^a	0.2	< 0.01
day 57 to 112	14.3	14.1	13.7	0.3	0.31
day 0 to 112	11.8 ^c	12.4 ^b	13.2 ^a	0.2	< 0.01
DM intake, % of BW					
day 0 to 56	2.73 ^c	2.96 ^b	3.35 ^a	0.044	< 0.01
day 57 to 112	3.26 ^a	3.00 ^b	2.80 ^c	0.062	< 0.01
day 0 to 112	2.99 ^{xy}	2.98 ^y	3.07 ^x	0.035	0.18
Feed efficiency ⁴					
day 0 to 56	0.147 ^c	0.178 ^b	0.196 ^a	0.008	< 0.01
day 57 to 112	0.136	0.139	0.128	0.005	0.31
day 0 to 112	0.142 ^b	0.158 ^a	0.161 ^a	0.004	0.02
Hip height, in					
day 56	43.7 ^c	44.4 ^b	45.1 ^a	0.1	< 0.01
day 112	45.8 ^c	46.8 ^b	47.2 ^a	0.1	< 0.01
Heart girth, in					
day 56	51.3 ^b	52.6 ^a	52.9 ^a	0.3	< 0.01
day 112	55.6 ^b	57.1 ^a	57.4 ^a	0.3	< 0.01

¹Day refers to day of experiment.

²Grain:forage ratio.

³Average daily gain.

⁴Feed efficiency expressed as lb of ADG per lb of daily DM intake.

^{abc}Means with differing superscripts are significantly different at $P \leq 0.05$.

^{xy}Means tend to differ at $0.10 \geq P > 0.05$.

Table 2. Daily feed costs for heifers fed increasing levels of concentrate during the treatment period (day 0 to 56 of experiment) followed by a common diet (day 57 to 112).

Item	40:60 ¹	60:40	80:20	SEM	<i>P</i> -value
Daily feed cost per head ²					
day 0 to 56	1.03 ^c	1.29 ^b	1.67 ^a	0.024	< 0.01
day 57 to 112	1.48	1.45	1.41	0.030	0.31
day 0 to 112	1.26 ^c	1.37 ^b	1.54 ^a	0.018	< 0.01
Cost of gain ³					
day 0 to 56	0.96 ^a	0.73 ^b	0.73 ^b	0.061	0.03
day 57 to 112	0.82	0.79	0.87	0.052	0.62
day 0 to 112	0.89 ^a	0.76 ^b	0.80 ^{ab}	0.040	0.10

¹Grain:forage ratio.

²All values given in US dollars (\$).

³\$/lb of average daily gain.

^{abc}Means with differing superscripts are significantly different at $P \leq 0.01$.

Table 3. Weight, skeletal measurements, and intake responses of prepubertal dairy heifers fed diets containing high non-fiber carbohydrate (HNFC), low NFC (LNFC), or LNFC with added fat (LNFC+) grain fractions.

Item	HNFC	LNFC	LNFC+	SEM	P-value ¹	
					T	T×S
BW ² , lb						
day 56	438 ^{ab,y}	431 ^b	448 ^{a,x}	4	0.02	--
day 112	552 ^{ab,y}	544 ^b	563 ^{a,x}	4	<0.01	--
ADG ³ , lb/day						
day 0 to 56	2.14 ^b	2.03 ^b	2.34 ^a	0.06	0.02	0.01
day 56 to 112	2.05	2.01	2.05	0.07	0.86	< 0.01
day 0 to 112	2.09 ^{ab}	2.01 ^b	2.21 ^a	0.06	0.13	< 0.01
DM intake, lb/day						
day 0 to 56	12.7	12.6	12.9	0.1	0.45	0.01
day 56 to 112	16.5 ^a	15.3 ^b	15.4 ^b	0.4	0.06	< 0.01
day 0 to 112	14.6 ^x	14.0 ^y	14.2 ^{xy}	0.2	0.15	< 0.01
DM intake, % of BW						
day 0 to 56	3.26	3.24	3.22	0.04	0.73	0.03
day 56 to 112	3.25 ^a	3.03 ^b	2.96 ^b	0.05	< 0.01	< 0.01
day 0 to 112	3.25 ^a	3.14 ^b	3.09 ^b	0.03	< 0.01	< 0.01
NDF intake, % of BW						
day 0 to 56	1.15 ^b	1.42 ^a	1.42 ^a	0.02	< 0.01	< 0.01
day 56 to 112	1.34 ^b	1.41 ^a	1.39 ^a	0.02	0.09	< 0.01
day 0 to 112	1.25 ^b	1.42 ^a	1.41 ^a	0.01	< 0.01	< 0.01
Feed efficiency ⁴						
day 0 to 56	0.166 ^{ab,y}	0.161 ^b	0.181 ^{a,x}	0.006	0.06	0.20
day 56 to 112	0.123	0.132	0.133	0.007	0.52	0.10
day 0 to 112	0.144 ^y	0.146 ^{xy}	0.157 ^x	0.004	0.12	0.07
Hip height, in						
day 56	44.8 ^{ab}	44.7 ^b	45.1 ^a	0.1	0.06	--
day 112	47.6 ^a	47.2 ^b	48.0 ^a	0.1	< 0.01	--

¹T = treatment effect; T×S = treatment × time interaction.

²Body weight; day refers to day of experiment.

³Average daily gain.

⁴Feed efficiency expressed as lb of ADG per lb of daily DM intake.

^{abc}Means differ at $P \leq 0.05$.

^{xy}Means tend to differ at $0.10 \geq P > 0.05$.

Table 4. Daily feed costs for heifers fed diets containing high non-fiber carbohydrate (HNFC), low NFC (LNFC), or LNFC with added fat (LNFC+) grain fractions.^{1,2}

Item	HNFC	LNFC	LNFC+	SEM	P-value
Daily feed cost per head					
day 0 to 56	1.63 ^a	1.49 ^c	1.58 ^b	0.02	< 0.01
day 57 to 112	1.83 ^a	1.59 ^b	1.65 ^b	0.04	< 0.01
day 0 to 112	1.73 ^a	1.54 ^c	1.61 ^b	0.02	< 0.01
Cost of gain ³					
day 0 to 56	0.84 ^a	0.81 ^{ab}	0.72 ^b	0.04	0.09
day 57 to 112	1.08	0.95	0.98	0.11	0.70
day 0 to 112	0.96	0.88	0.85	0.06	0.39

¹All values given in US dollars (\$).

²Day refers to day of experiment.

³\$/lb of average daily gain.

^{abc}Means with differing superscripts are significantly different at $P \leq 0.05$.

Table 5. Weight and skeletal growth responses of dairy heifers and steers at 28 wks of age fed a milk treatment (MILK) of either conventional milk replacer (CONV) or high nutrition plane milk replacer (HIGH) and fed a grower diet (GRWR) of high non-fiber carbohydrate (HNFC) or low NFC (LNFC) post-weaning grower diets from 12 to 28 wk of age.

Item	CONV		HIGH		SEM	P-value ¹		
	HNFC	LNFC	HNFC	LNFC		MILK	GRWR	MILK × GRWR
BW ² , lb								
28 wk ³	516 ^a	503 ^{ab}	522 ^a	495 ^b	8	0.88	< 0.01	0.04
ADG ⁴ , lb/day								
0 to 28 wk	2.12	2.03	2.14	1.98	0.05	0.95	0.01	0.49
Hip height, in								
28 wk	47.6	47.2	47.4	47.3	0.2	0.91	0.24	0.60
Hip width, in								
28 wk	13.9 ^{ab}	13.9 ^{ab,x}	14.1	13.7 ^{b,y}	0.1	0.85	0.15	0.08
Heart girth, in								
28 wk	56.1	56.5	56.7	56.5	0.4	0.34	0.90	0.59

¹MILK = Effect of pre-weaning milk treatment; GRWR = effect of post-weaning diet; and MILK × GRWR = interaction of milk treatment vs. post-weaning diet effects.

²Body weight.

³Weeks of age.

⁴Average daily gain.

^{ab}Means with differing superscripts significantly differ at $P \leq 0.05$.

^{xy}Means with differing superscripts tend to differ at $0.10 \geq P > 0.05$.

Table 6. Body weight, intake, and skeletal measurements of prepubertal dairy heifers fed common diets using different feed delivery methods.¹

Item	HF	SBS	TMR	SEM	<i>P</i> -value
Body weight, lb					
day 28 ²	397	392	388	4	0.37
day 133	605 ^a	576 ^b	575 ^b	4	<0.01
ADG ³ , lb/day					
day 0 to 28	2.29	2.09	1.96	0.12	0.21
day 29 to 133	2.05 ^a	1.83 ^b	1.85 ^b	0.06	0.06
day 0 to 133	2.09 ^a	1.90 ^b	1.87 ^b	0.06	0.02
Hip height, in					
day 133	47.6	47.8	47.9	0.3	0.81
Heart girth, in					
day 133	58.8 ^{a,x}	57.8 ^b	58.1 ^{b,y}	0.3	0.03
DMI ⁴ , lb/day					
day 0 to 28	9.57	9.08	9.72	0.22	0.15
day 29 to 133	18.04 ^a	17.00 ^b	16.96 ^b	0.21	<0.01
day 0 to 133	16.16 ^a	15.26 ^b	15.34 ^b	0.18	<0.01
Feed efficiency ⁵					
day 0 to 28	0.224 ^a	0.228 ^a	0.188 ^b	0.010	0.03
day 29 to 133	0.114	0.111	0.109	0.003	0.58
day 0 to 133	0.124 ^{ab}	0.127 ^a	0.115 ^b	0.004	0.10

¹HF = hay feeder; SBS = side-by-side; TMR = total mixed ration; and SEM = standard error of the mean.

²Day of study.

³Average daily gain.

⁴Dry matter intake.

⁵Feed efficiency expressed as lb of ADG per lb of daily DMI.

^{ab}Means differ at $P < 0.05$.

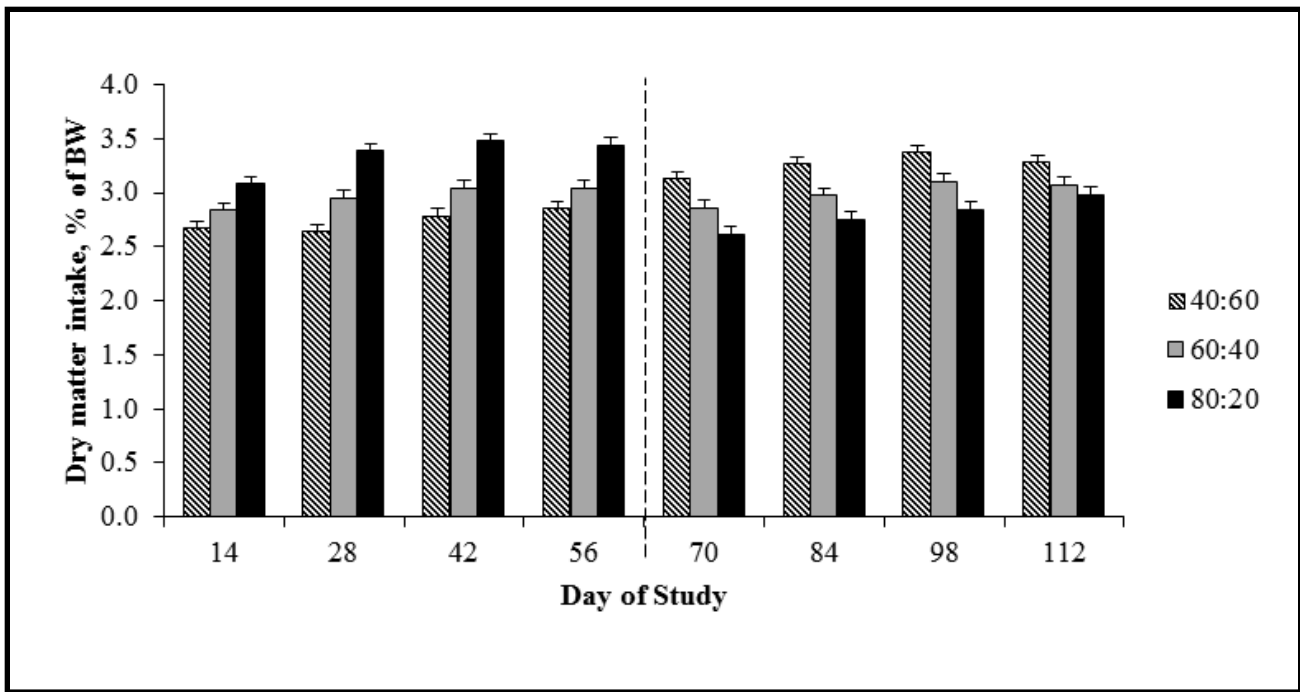


Figure 1. Effects of increasing grain inclusion during the treatment period, followed by a rapid switch to a common diet on DM intake as a percentage of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. Treatment differences were not apparent overall ($P = 0.18$); however, a treatment \times time interaction was observed ($P < 0.01$), as heifers fed 40:60 consumed the least amount of DM during the treatment period as a % of BW compared to heifers fed 80:20, but consumed the most DM during the grower period compared to 60:40 and 80:20.

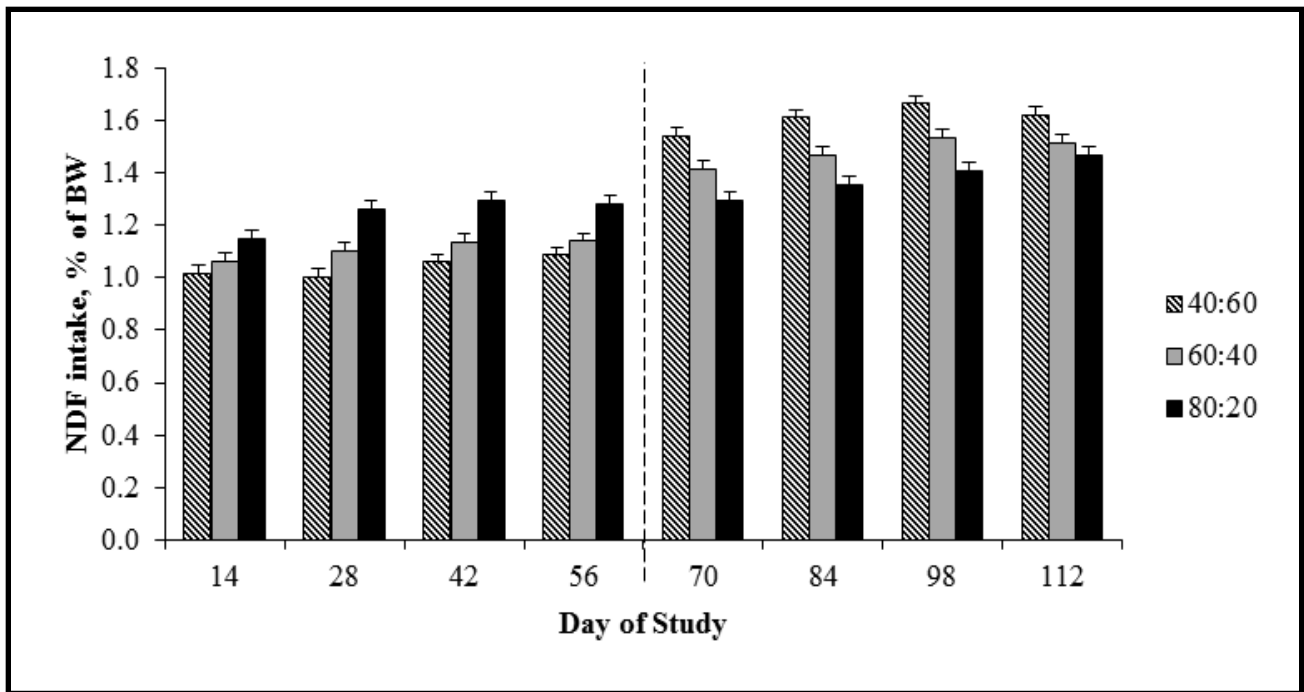


Figure 2. Effects of increasing grain inclusion during the treatment period followed by a rapid switch to a common diet on NDF intake (DM basis) as a percentage of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. There were no overall treatment differences ($P = 0.46$); however a treatment \times time interaction was observed ($P < 0.01$), as heifers fed 40:60 consumed the least amount of total NDF during the treatment period as a % of BW compared to heifers fed 80:20, but they consumed the most total NDF during the grower period compared to 60:40

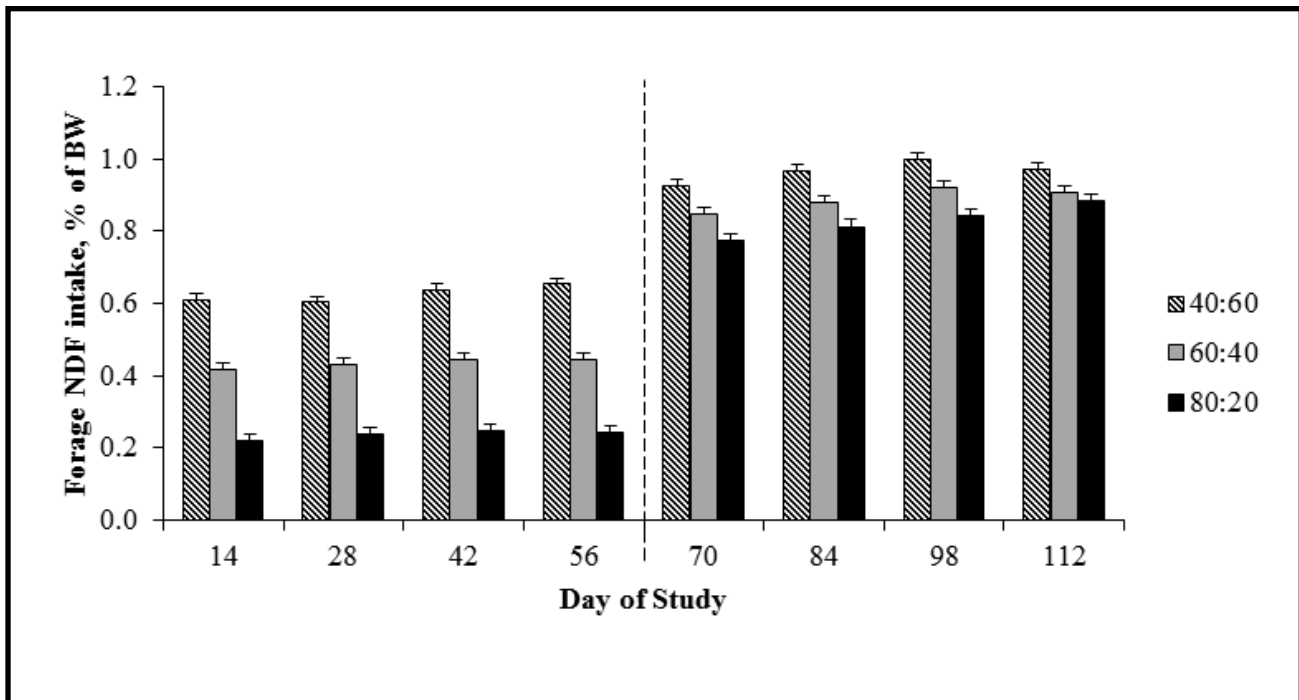


Figure 3. Effects of increasing level of concentrate inclusion during the treatment period followed by a rapid switch to a common diet on forage NDF intake (DM basis) as a percentage of BW over time. Vertical dashed line indicates time of diet switch relative to day of study. Forage NDF intake increased linearly overall as grain inclusion was reduced in the treatment period ($P < 0.01$), and a treatment \times time interaction was also observed overall ($P < 0.01$). As expected, forage NDF intake linearly increased as grain inclusion decreased; however, forage NDF intake was greatest throughout the grower period for heifers previously fed 40:60.

Practical Recommendations for Trace Minerals for Lactating Dairy Cows

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Summary

Providing adequate trace minerals to dairy cows is essential for high production and good health. Providing excess trace minerals inflates feed costs and could be detrimental to production and cow health. This paper provides suggested strategies for formulating diets to meet the trace mineral requirements of cows. Basal ingredients, such as corn silage and hay, provide absorbable trace minerals to cows. Concentrations of trace minerals in basal ingredients should not be set to 0. However, single samples of feeds probably will not provide an accurate estimate of the true concentrations of trace minerals in feeds. The NRC (2001) recommendations for most trace minerals (Mn is an exception) appear adequate and should be the starting point for ration formulation. Because of uncertainty regarding absorption and requirements, a modest safety factor of 1.2 to 1.5 X NRC requirements is appropriate for most trace minerals under normal conditions. The NRC does not consider antagonism, and for Cu, antagonism can be quite common (high intake of S from diet or feed, grazing, and dietary Mo). In those cases, absorption coefficients should be reduced (perhaps more than 50%) so that cows are fed diets with adequate absorbable Cu. However, feeding excess Cu over the long term (months or years) can result in high concentrations of Cu in the liver, which may be detrimental to cows.

The NRC (2001) recommendation for Mn is too low. Some data suggest that Mn requirements for lactating cows should be increased by a factor of 1.8. The NRC recommendation for Co may be too low. Total diet Co may have to be 1 to 1.3 ppm (current NRC requirement is about 0.1 ppm), but in many cases, the basal diet may be adequate. The NRC did not establish a requirement for Cr, but the majority of production studies with transition cows have shown increased milk yield. The decision to supplement Cr is largely an economic decision based on cost of feed, cost of supplemental Cr (Cr propionate is the only approved source of Cr in the U.S.), and price of milk.

Introduction

When this paper was written (March, 2015), the National Research Council was in the process of updating the Nutrient requirements of Dairy Cows publication. The previous version was published in 2001 (NRC, 2001) and provided an up-to-date review of the scientific literature on mineral nutrition of dairy cattle and the requirements were based on the knowledge available at that time. Several nutrition models are used in the U.S. (e.g., NRC, CNCPS, Amino Cow, etc.) to formulate diets for dairy cows and they often differ substantially in their recommendations regarding energy and protein. However, mineral requirements from essentially every nutrition model currently used in the US

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are derived directly or almost directly from the NRC (2001) recommendations. Because of space limitations, this paper will concentrate on six trace minerals (Cr, Co, Cu, Mn, Se, and Zn). The upcoming NRC may or may not reflect the opinions in this paper.

Currently Used Requirement System (e.g., NRC, 2001)

The requirements for most minerals (S, Se, I, and Co are exceptions) are calculated using the factorial approach. Mineral needed for maintenance plus mineral deposited in the growing fetus (gestation requirement) and body (growth requirement) plus mineral secreted in milk (lactation requirement) were summed to generate the requirement for absorbed mineral in either gram or milligrams/day. Because requirements were calculated on an absorbed mineral basis, absorption coefficients (**AC**) for all the minerals had to be generated and multiplied by mineral concentrations to calculate the concentration of absorbed mineral in the diet.

The factorial system has been used for decades to determine requirements for energy and protein and more recently for minerals. Requirements are based on research that measures responses. In other words, the lactation requirement for protein was determined by feeding different amounts of protein and measuring the marginal response in milk production. That marginal response (X grams of protein consumed per pound increase in milk) equaled the lactation requirement. However, conceptually, separating requirements into maintenance, gestation, growth, and lactation components is flawed, and because of their biological functions, the factorial approach may be extremely flawed for many minerals. A major problem is defining maintenance. For example, if extra Cu is needed by the immune system to prevent mastitis, is that a maintenance function

or a lactation function? If extra Se is needed to prevent retained placenta, is that a maintenance function or a reproduction function? The problem with partitioning mineral requirements into various functions is not simply an academic exercise, it can result in erroneous estimates of mineral requirements.

'Maintenance' Requirement

For minerals, the maintenance requirement is equal to the amount of mineral that would be excreted in feces and urine (and maybe skin sloughing) if the animal was fed a diet void of the mineral (i.e., inevitable losses). Depending on the mineral, the current (NRC, 2001) maintenance requirement ranges from 0 (e.g., Fe) to more than 70% of the total requirement (for most minerals maintenance is 30 to 40% of total requirement). Experimentally, measuring the inevitable losses of minerals is very difficult, which can lead to errors in estimating the maintenance requirement. More importantly, mineral status of the animal can affect the inevitable loss of minerals. Gut cells and other cells that contribute to the inevitable loss probably contain less Zn if a cow was fed a diet barely adequate in Zn compared with a cow in good Zn status. Another question is whether cows in different physiological states (for example, lactating vs. dry) have the same inevitable losses of mineral. Much of the research conducted to determine maintenance requirements (most of which was conducted years or decades ago) used non-lactating cows. Intake is much higher for a lactating cow than for a non-lactating cow and inevitable loss of mineral is probably positively correlated with DMI (more digesta is flowing through the system, causing increased secretion and cell losses in the digestive tract).

‘Productive’ (gestation, growth, and lactation) Requirements

By definition, mineral requirement for gestation, growth, and lactation is the amount of mineral that is deposited into the conceptus, body tissue, and milk, respectively. This approach mirrors the net energy approach. For example, the net energy requirement for lactation equals the amount of energy secreted in milk. However for net energy, an efficiency value is used. For example, it requires 1 Mcal of absorbed energy (called metabolizable energy) to secrete 0.64 Mcal into milk. For minerals, the efficiency of putting a mineral into fetal tissue, body tissue, or milk is 100%. This means that there is no mineral cost (or requirement) to make milk or synthesize body tissue. Oxidative metabolism increases in direct proportion to energy use (a high producing cow uses a lot more oxygen than a low producing cow). Many trace minerals are components of antioxidant enzymes and the more oxygen a cell uses, the more free radicals produced which should increase the need for antioxidant enzymes. If this increases the need for Cu to make the enzyme superoxide dismutase, this increased requirement is not considered in the current system.

At least conceptually, the current system could underestimate the requirements for many minerals under standard conditions. In addition, certain disease states, such as a severe infection, increase loss of certain minerals via feces and urine. This may mean that an immune or health requirement needs to be considered, and if necessary, included in the factorial system.

Mineral Supply

A major change that occurred in NRC (2001) was that requirements were calculated for absorbed mineral rather than total mineral. This was a major advance because we know minerals

from some sources are more absorbable than minerals from other sources. However, the use of absorbable mineral has limitations:

- Measuring absorption of some minerals is extremely difficult.
- Actual absorption data and AC are limited. Many values are estimates.
- Absorption is affected by physiological state of the animal and by numerous dietary factors (many of which have not been quantified).
- For many of the trace minerals, the AC is extremely small, and because it is in the denominator (i.e., dietary mineral required = absorbed requirement/AC), a small numerical change in the AC can have a huge effect on dietary requirement (see text box).

Concentrations of Minerals in Basal Ingredients

For most minerals of nutritional interest, good analytical methods that can be conducted on a commercial scale at reasonable costs are available for feeds. Assuming the feed sample is representative, a standard feed analysis (using wet chemistry methods for minerals) should provide accurate concentration data for Ca, P, Mg, K, Na, Cu, Fe, Mn, and Zn. Labs can also routinely measure S and Cl, but often these are separate tests. Although Cr, Co, and Se are of nutritional importance, most labs do not routinely measure these because the concentrations commonly found in feeds are lower than what commercial labs can reliably measure (i.e., inadequate analytical sensitivity) or because of contamination caused by routine sample processing, such as using a steel feed grinder (a major concern for Cr). Although we can get accurate total mineral concentration data for basal ingredients, you must be careful when evaluating and using the data. Concentrations of minerals in feeds, even most macrominerals, are low. For example, 1 ton of average corn silage

(35% dry matter) only contains about 1.7 lb of Ca and 2.5 g of Cu (to put this in perspective a penny weighs about 2.5 g).

Example of the impact of a change in absorption coefficient (AC) on Cu supplementation.

Assumptions:

1. Dry matter intake = 50 lb/day
2. Cow requires 12 mg/day of absorbed Cu/day
3. Basal ingredients provide 220 mg/day of total Cu
4. AC for Cu from copper sulfate (25% Cu) = 5% (NRC, 2001)

If AC for basal diets was 0.03, the diet would provide $220 * 0.03 = 6.6$ mg of absorbed Cu, then the cow would need to be supplemented with $12 - 6.6 = 5.4$ mg of absorbable Cu = **108 mg of Cu from Cu sulfate (5.4/0.05)**

If AC for basal ingredients was 0.05, the diet would provide $220 * 0.05 = 11$ mg of absorbed Cu, then the cow would need to be supplemented with $12 - 11 = 1$ mg of absorbable Cu = **20 mg of Cu from Cu sulfate (1/0.05)**

A change in AC of basal diets from 0.03 to 0.05 (the AC from NRC = 0.04) would increase the amount of supplemental Cu needed by almost 5 X

Sampling error is a problem for most nutrients, and when concentrations are low, sampling error is usually larger. From a survey we conducted on forages, sampling variation for trace minerals was greater than true variation. This means that mineral concentration data from a single sample should be viewed very suspiciously. The mineral concentration of soils is a major factor affecting the concentrations

of most minerals in forages. Therefore, means of samples taken from a farm over time (up to a few years) or from a group of farms within a small geographic area (e.g., a few counties) should be a truer estimate of the actual mineral concentration of a forage than a single sample.

Besides sampling issues, the concentrations of many minerals in feeds are not normally distributed (a normal distribution is the classic bell shaped curve). In a normal distribution, about half the samples have less than the mean or average concentration, about half the samples have more than the average, and about 95% of the samples are within ± 2 standard deviation (**SD**) unit of average. This means that if you know the average concentration and the SD, you have a good description of the population. This information helps with risk assessment. If a feed has an average concentration of Mg of 0.4% and an SD of 0.01% and the distribution is normal, about 95% of the samples of that feed should have between 0.38 and 0.42% Mg. With that information, you should probably conclude it is not worth analyzing that feed for Mg, because even if your sample is 2 or 3 SD units from the mean, it will have no effect on the diet or the animal. However, when distributions are skewed, the average and the SD may not be good descriptors of the population, and for many minerals, concentrations within feeds are not normally distributed (Figures 1 and 2). Often the distributions have long tails because concentrations cannot be less than 0 but can be extremely high for various reasons. Some samples have high concentrations of certain minerals because of contamination with soil or other substances. such as mineral supplements. The more skewed the data, the less valuable the average and SD become in describing the feed. The median is the concentration where half of the samples have a lower mineral concentration and half of the samples have more mineral, and in a normal distribution, the

mean and the median are essentially equal. For concentrations of trace minerals and some macro minerals, the median is usually less than the average because their distributions are skewed. What this means is that for most situations, using the average trace mineral concentration (e.g., feed table data), overestimates the trace mineral concentration in the majority of samples. For skewed populations, the median is a better descriptor of the population than the mean; however, simply replacing average concentration with median concentration does not fix all the problems associated with a skewed distribution.

As a distribution becomes more skewed, the risk that a specific feed will contain excess mineral increases. The Mn data shown in Figure 2 is a good example. The data have an average of 55 ppm and an SD of 23. Assuming a normal distribution, one would expect about 2.5% of the samples to have more than about 100 ppm ($55 + 2 \text{ SD unit}$) and about 2.5% of the samples to have less than about 9 ppm. However, no samples had less than 9 ppm and 5.2 % had more than 100 ppm. If your particular sample of mixed mostly legume silage was in the 5 out of every 100 samples with a very high Mn concentration, your diet would contain substantially more Mn than expected. Excess dietary Mn is rarely a problem for cows, but excess dietary Cu can be (discussed below). Corn silage in Figure 1 had a mean Cu concentration of 6 ppm with a SD of 1.8. With a normal distribution, about 2.5% of the samples should have more than about 10 ppm Cu. However, about 5% of samples have more than 10 ppm Cu (i.e., twice the risk). If you formulate a diet assuming the corn silage is 6 ppm Cu, but it really has 12 ppm and corn silage comprises a significant portion of the diet, over the long term (many months), excess dietary Cu could become a problem.

The bottom line is that averages for trace mineral concentrations in forages (and perhaps other feeds) found in tables should be used with caution. Because of substantial sampling variation, data from a single sample should not be used. The best advice is to generate mean values for trace minerals for forages grown within a limited geographical area.

Do the trace minerals in basal feeds have nutritional value?

Essentially every feedstuff used in dairy diets contains some minerals. The question is, are those minerals biologically available to cows? Although survey data of nutritionists are lacking, but based on personal experience, it is not uncommon for field nutritionists to set trace mineral concentrations in basal ingredients or at least forages, at 0. This approach would be valid if the trace minerals in feedstuffs were not biologically available to cows. Although substantial uncertainty exists regarding the AC for most minerals in most feeds (this includes mineral supplements), a portion of the trace minerals found in most (all?) feedstuffs is clearly available to cows. Tissues from wild ruminants, such as deer (Wolfe et al., 2010) and grazing beef cattle (Sprinkle et al., 2006) that have not received supplemental minerals contain trace minerals, indicating that some absorption of basal minerals occur.

The NRC (2001) estimates that Cu, Mn, and Zn from basal ingredients are 4, 0.75, and 15% absorbable, respectively. The AC assigned to basal ingredients are usually lower than AC for the sulfate form of trace minerals, even though most of the trace minerals contained within plant cells would be in an organic form. The lower AC for trace minerals in basal ingredients may reflect an adjustment for soil contamination. Some of the trace minerals in basal feeds, especially forages, are in the soil

that is attached to the feed and those minerals are often in the oxide form (i.e., low availability). This suggests that feeds with substantially higher ash and trace mineral concentration than typical (i.e., the data tails discussed above) likely have AC that are lower than the NRC values for trace minerals. Concentrations of trace minerals substantially greater than median value should be discounted, but an exact discount cannot be calculated at this time. However, those feeds would still contain some available mineral.

As discussed above, determining the AC for trace minerals is extremely difficult. At least into the foreseeable future, lab tests will not be available to estimate AC for trace minerals from feedstuffs; therefore, we are limited to using the constants provided by sources such as the NRC (2001). On average (and remember the issues with using averages), unsupplemented diets for lactating cows in Ohio based mostly on corn silage, alfalfa, corn grain and soybean meal contain 7 to 9 ppm Cu, 25 to 35 ppm Mn, and 30 to 40 ppm Zn (specific farms may differ greatly from these ranges). For an average Holstein cow (75 lb/day of milk and 53 lb of DMI) using NRC requirements, basal ingredients supply about 80, 235, and 75% of requirements for Cu, Mn, and Zn, respectively. Ignoring minerals supplied by basal ingredients can result in substantial over formulation for trace minerals.

Evaluating Trace Mineral Status

The primary indicators of trace mineral status (either inadequate or excess) are often sick or poor producing animals. For both research purposes and practical diet formulation, more sensitive indicators or markers of mineral status are clearly needed. These would improve our ability to evaluate requirements, mineral sources, and diet adequacy. No biological measures are known which accurately reflect Zn, Mn, and Cr status in cattle. Plasma (or serum)

Zn may be able to discern severe or clinical Zn deficiency, but too many other factors influence serum concentrations to make it sensitive marker of Zn status. Stress and infections reduced plasma Zn in beef cattle (Nockels et al., 1993) and parturition and clinical milk fever has reduced plasma Zn in dairy cows (Goff and Stabel, 1990). Mastitis may also reduce plasma Zn concentrations.

Cleft palate and other calf deformations at birth (Hansen et al., 2006) are specific indicators of clinical Mn deficiency, but markers of marginal deficiencies have not been identified. New, enhanced analytical methods (mass spectroscopy) has greatly increased our ability to accurately measure plasma Mn, and with additional research, plasma and liver Mn concentrations may have value as a status indicator. The glucose tolerance test has been able to differentiate between different dietary supplies of Cr; however, this test is not practical under field conditions.

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

important criteria of a good marker of mineral status. However, liver biopsies are costly and invasive, and generally not practical on a large scale basis. Other Cu measures (e.g., enzyme activity, ceruloplasmin, and Cu concentration in blood fractions) have been suggested as indicators of Cu status. However, liver Cu is mobilized during depletion to support cellular function and changes in enzyme activity or ceruloplasmin and Cu blood concentrations do not reflect status until the liver is depleted of the majority of its Cu stores.

Cobalt has no known nutritional function other than as a component of vitamin B₁₂, so when we refer to Co status, we really mean vitamin B₁₂ status. Liver B₁₂ concentrations reflect Co intake. Assumed adequate hepatic B₁₂ concentrations are between 200 to 400 nmol/kg on a wet weight basis (Stangl et al., 2000). Similar to Cu, liver biopsies to determine B₁₂ concentrations and subsequent Co status are invasive and not practical on a large scale (vitamin B₁₂ is also difficult to measure). Dramatic increases in plasma concentrations of methylmalonic acid and homocysteine are able to indicate Co deficiency in cattle, but these metabolites are not sensitive enough to detect optimal Co status of cattle. (Stangl et al., 2000).

Selenium status of cattle can be evaluated by assaying Se concentrations in blood fractions. Based on the effects of Se supplementation on immune function, reproduction, and mastitis, adequate serum (Weiss and Hogan, 2005) and whole blood (Kommissrud et al., 2005) Se concentrations are around 0.06 and 0.15 µg/mL, respectively. Approximately 60% of the Se in whole blood is in the erythrocytes, which have a half-life of almost 100 days in cattle. Therefore, whole blood Se is a more accurate long-term indicator of Se status compared to plasma or serum, which reflects short-term changes in Se intake more accurately. Whole

blood glutathione peroxidase activity is often assayed to determine relative bioavailability of Se sources. However, glutathione peroxidase activity is somewhat dependent on the lab, so adequacy must be evaluated compared with lab reference values. Selenium supplementation has been shown to increase Se concentrations in milk, but the relationship is highly dependent on Se source (Weiss, 2005). Concentrations also are usually lower than those found in plasma and can be difficult to measure accurately.

Concentrations of Fe in serum and liver can be used to confirm Fe deficiency in cattle. Adequate Fe serum and liver concentrations are 1.3 µg/mL and 65 mg/kg of wet weight, respectively (Kincaid, 2000). Other assays which can assist in evaluating Fe status include serum iron binding capacity or saturation (Weiss et al., 2010), red blood cell count, packed cell volume, serum hemoglobin concentration, and ferritin concentration (Smith, 1989). Assayed serum Fe concentration can provide false results if hemolysis occurs in the serum or plasma due to Fe content of erythrocytes. To avoid erythrocyte Fe contamination, an assay specific for non-heme iron is conducted. Minimum adequate reference values for many of the Fe status markers are unknown due to the almost non-existent occurrence of Fe deficiency in cattle in the US. Many studies (e.g. Weiss et al., 2010) have reported values that, represent animals in adequate Fe status, and those values can be evaluated as a reference if needed.

Recommendations

The primary trace minerals of interest in dairy nutrition are chromium (**Cr**), cobalt (**Co**), copper (**Cu**), iodine (**I**), iron (**Fe**), manganese (**Mn**), selenium (**Se**), and zinc (**Zn**). The NRC (2001) did not establish a requirement for Cr, but for the other trace minerals, the NRC should be the starting point. Iron will not be discussed

because basal diets almost always contain adequate Fe. Iodine also will not be discussed because of limited new information.

Chromium

Feeding diets with more than 0.5 ppm of supplemental Cr or from sources other than Cr propionate is not legal in the U.S. Chromium is a required nutrient; however, the NRC (2001) did not provide a quantitative recommendation. Cr is needed to transport glucose into cells that are sensitive to insulin. Because of analytical difficulties (e.g., normal grinding of feeds prior to chemical analysis can contaminate them with Cr), we do not have good data on Cr concentrations in feedstuffs. Some studies with cattle have shown that supplemental Cr (usually fed at 0.4 to 0.5 ppm of diet DM) reduced the insulin response to a glucose tolerance test (Hayirli et al., 2001; Sumner et al., 2007; Spears et al., 2012). Elevated insulin reduces glucose production by the liver and enhances glucose uptake by skeletal muscle and adipose tissue. These actions reduce the amount of glucose available to the mammary gland for lactose synthesis, and this may be one mode of action for the increased milk yield often observed when Cr is supplemented. Most of the production studies evaluating Cr supplementation (studies used Cr propionate, Cr-methionine, Cr-picolinate, and Cr yeast) started supplementation a few weeks before calving and most ended by about 42 DIM (but a few went later into lactation). Supplementation rates varied but most were 6 to 10 mg/day (approximately 0.3 to 0.5 mg Cr/kg of diet DM). The median milk response from 30 treatments from 14 experiments (treatments that fed supplemental Cr well in excess of the permitted 0.5 ppm were excluded) was +4.1 lb/day (the SD among responses was 3.5 lb/day). About 75% of the treatment comparison yielded an increase in milk of more than 2 lb/day. Although a comprehensive meta-analysis

is needed, based on this preliminary analysis of studies, increased milk yield of at least 2 lb/day is highly probable when approximately 0.5 ppm Cr is supplemented to early lactation cows. Whether this response would be observed throughout lactation is not known. When Cr is supplemented, intake usually increases as expected based on increased milk yield (approximately 0.65 to 0.75 lb increased DMI/lb of increased milk). The potential return on investment from milk can be calculated by using the value of milk and cost of feed plus the cost of the supplement and assuming a median response of about 4 lb of milk, with an expected increase in DMI of about 2.8 lb. At this time, a milk response should only be assumed to occur up to about 42 DIM.

In addition to increased milk yield, supplemental Cr may enhance certain aspects of the immune system and may help reduce morbidity in stressed cattle. Positive effects of Cr on morbidity have mainly been observed in beef cattle (Mowat et al., 1993). Supplemental Cr has usually enhanced cytotoxic T-lymphocyte function in cattle (perhaps via reduced cortisol) but has had variable or no effects on other types of immune function (Weiss and Spears, 2005).

Cobalt

The current NRC requirement for Co is expressed on a dietary concentration basis (i.e., 0.11 ppm in diet DM) rather than on a mg/day of absorbable Co basis. This was done because Co is mostly (perhaps only) required by ruminal bacteria and the amount they need is a function of how much energy (i.e., feed) is available to them. Although Co concentration data for feeds is very limited, the NRC requirement is for total Co, and in many cases, basal ingredients would provide adequate Co. However, because Co concentrations in feeds are often quite low, feed Co data may be questionable. In studies

conducted in WA, basal diets typically contained 0.2 to 0.4 ppm Co (Kincaid et al., 2003; Kincaid and Socha, 2007). In a study conducted in WI, the control diets (no added Co) contained between 1 and 2 ppm Co (Akins et al., 2013). Based on older research (<1970), diets with 0.11 ppm Co maintained adequate concentrations of vitamin B-12 in the liver of cows. Bacteria in a ruminal in vitro system increased B-12 production as supplemental Co was increased up to 1 ppm in the incubation media (Tiffany et al., 2006). However, the response was not linear. The greatest response was found when Co was increased from 0 to 0.1 ppm (B-12 concentration increased about 60%). The increase in B-12 when Co was increased ten-fold (0.1 to 1.0 ppm) was only an additional 40%. Data using growing beef animals (Stangl et al., 2000) found that liver B-12 was maximal when diets contain 0.22 ppm Co (approximately twice as high as current recommendation). With dairy cows, liver B-12 concentrations continued to increase as supplemental Co (from Co glucoheptonate) increased up to 3.6 ppm (Akins et al., 2013). In that study, elevated liver B-12 did not translate into any health or production benefits, indicating that maximal liver B-12 may not be necessary. Milk production responses to increased Co supplementation have been variable. One study (Kincaid et al., 2003) reported a linear increase in milk yield in multiparous cows but no effect in first lactation animals when supplemental Co increased from 0 to about 1 ppm (from Co glucoheptonate). Older cows tend to have lower concentrations of B-12 in their livers, which could explain the parity effect. Based on current data, the NRC (2001) requirement does not result in maximal liver B-12 concentrations in dairy cows. Liver B-12 concentrations generally increase with increasing dietary Co, whereas milk yield responses have been much more variable. Across studies, when total dietary Co (basal plus supplemental) was about 1 to 1.3 ppm, maximum milk responses were observed.

In some locations, basal ingredients may provide that much Co.

Copper

The NRC (2001) requirement for Cu is expressed on a mg/day of absorbable Cu basis and over a wide range of milk yields (40 to 150 lb), with requirements ranging from about 7 to 15 mg/day of absorbed Cu under normal conditions. Because Cu is secreted in milk, as milk yield increases, the NRC requirement for Cu increases. However, because basal ingredients contain Cu and because DMI usually increases as milk yield increases, the dietary concentration of Cu needed to meet the requirement may not change as milk yield increases (Table 1). Contrary to popular practice, diets for pens of high producing cows often do not need to contain higher concentrations of many trace minerals than diets for lower producing cows. Whereas fresh cows, because of low DMI, often need to be fed diets with increased concentrations of trace minerals.

All trace minerals have antagonists that reduce absorption, but often these do not occur in real situations. All trace minerals are toxic, but for most of the minerals, the intakes needed to produce toxicity are usually quite high. Copper, however, is unique among nutritionally important trace minerals in that it is toxic at relatively low intakes (~3 to 4 times requirement), which should dictate caution regarding over supplementation. On the other hand, Cu has numerous real world antagonists which mandate the need to over supplement in several situations. The NRC requirement assumes no antagonism (i.e, dietary S at 0.2% of DM); however, several situations commonly exist which result in reduced Cu absorption including:

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

Most of these antagonisms have not been quantitatively modeled, and specific recommendations cannot be provided. Cows that consume substantial pasture (~50% of the diet) may need to be fed about 2X the NRC requirement when Cu sulfate is used. When dietary sulfur equivalent (this includes S provided by the diet and the drinking water) is >0.25 to 0.3%, additional absorbable Cu should be fed. At higher concentrations of dietary equivalent S (0.4 to 0.5%), cows may need to be fed 2 to 3 X NRC requirement when Cu sulfate is used. We have developed a simple spreadsheet that will calculate dietary sulfur equivalent concentration. Inputs include milk yield, DM intake, dietary S concentration, water S concentration, minimum daily temperature, and dietary Na concentration (<http://dairy.osu.edu/resource/OSUdairypubs.html#computer>; click on “Water Mineral Intake with DCAD”). As an approximation, for an average Holstein cow, for every 100 mg/L (ppm) of S in water, add 0.05 percentage units to the S concentration in the diet to estimate dietary equivalent S. For example, if your diet has 0.26% S and your water has 400 mg/L of S, dietary equivalent S = $0.26 + (4 \times 0.05) = 0.46\%$. Note that some labs report concentrations of sulfate, not S. If your lab reports sulfate, multiply that value by 0.333 to obtain concentration of S.

In most situations, dietary S will be <0.25% of the DM. Diets with high inclusion rates of distillers grains and diets that contain forages that have been fertilized heavily with ammonium sulfate can have substantially higher

concentrations of S. Water S concentration is dependent on source. Water should be sampled and assayed on a regular basis (at least annually) to determine whether water is adding to the S load in the diet.

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

If antagonists are present, the NRC model will overestimate absorbed Cu supply and adjustments should be made to the AC. For an average Holstein cow fed a diet with substantial antagonists, total dietary Cu may need to be 20 to 30 ppm to provide 12 to 15 mg/day of absorbed Cu (when Cu sulfate is fed). Some specialty Cu supplements have been shown to be much less affected by antagonism (Spears, 2003), and if those products are used, total Cu concentration should reflect the higher bioavailability of those products (see example below).

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

Example of Cu fortification needed when antagonists are present.

Assumptions

1. Absorbed Cu requirement is 10 mg/day, but a safety factor of 1.5 is used; desired absorbed Cu requirement = 15 mg/day
2. Basal diet (DMI = 52 lb = 23.6 kg) is 8 ppm Cu with a normal AC = 0.04; however, with antagonists, the AC = 0.02.
3. Cu sulfate has an AC = 0.05 but with antagonist, AC = 0.025
4. A specialty Cu product has been shown to have a relative AC of 2X Cu sulfate when antagonists are present; therefore, its AC = $0.025 \times 2 = 0.05$

Calculations

Basal diet provides 3.8 mg of absorbed Cu/day (23.6 kg/day x 8 x 0.02).

Absorbed Cu needed from specialty source = 15 mg/day desired – 3.8 basal = 11.2 mg/day.

Supplemental Cu needed = 11.2 mg/day/ 0.05 = 224 mg/day.

Total dietary Cu concentration = 8 ppm basal + (224/23.6) = 17 to 18 ppm.

If Cu sulfate was used rather than the specialty mineral, twice as much supplemental Cu would be needed so that total dietary Cu = 27 ppm.

In non-lactating cows that were in good (or excess) Cu status based on liver Cu concentrations and fed diets with approximately 20 ppm total Cu, liver Cu accumulated at an average rate of 0.8 mg/kg DM per day (Balemi et al., 2010). Although milk contains Cu, because of differences in DMI (and subsequent Cu intake), this accumulation of liver Cu is likely similar for a lactating cow fed a diet with 20 ppm Cu. Over a 305-day lactation, a cow fed a diet with ~20 ppm Cu (without antagonists) could accumulate ~250 mg/kg DM in the liver. Over 2 or 3 lactations, liver Cu concentrations would become extremely high. Classic toxicity is thought to occur when liver Cu concentrations are >2000 mg/kg DM. Beef cattle are tolerant to extremely high liver Cu concentrations (Felix et al., 2012), and many of the studies used to establish the upper limit for liver Cu used beef cattle. However, beef cattle usually have short lifespans and may not be good models for dairy cows. Chronic Cu poisoning is subclinical and can cause liver degeneration, which is evident based on liver enzyme aspartate transaminase (**AST**) and gamma-glutamyl transpeptidase (**GGT**) activities in plasma (Bidewell et al., 2012). Accumulating evidence suggests problems may start occurring at much lower concentrations (500 or 600 mg/kg DM). Elevated activity of AST and GGT can indicate liver dysfunction, and activity of those enzymes were significantly greater in heifers and bulls that had average liver Cu concentrations of 640 mg/kg DM compared with animals with average liver Cu of 175 mg/kg DM (Gummow, 1996). What may be considered acceptable overfeeding of Cu (e.g., ~15 or 20 ppm supplemental Cu) may result in problems because of the duration of the overfeeding.

Manganese

The 2001 NRC greatly reduced the requirement for Mn compared with the earlier

NRC. Based on NRC (2001), most lactating cows need between 2 and 3 mg/day of absorbable Mn, which based on typical DMI, translates to 14 to 16 ppm of total Mn in the diet. Recent research with pregnant beef heifers strongly suggests that the current NRC recommendation is not adequate (Hansen et al., 2006). In that study, beef heifers were fed a diet with about 16 ppm Mn for the last 6 month of gestation and 70% of the calves borne from those heifers had clinical defects directly related to Mn deficiency. Because of intake, a lactating cow will consume substantially more Mn per day than a gestating heifer and milk is not a major draw on Mn; therefore, the dietary concentration that elicited clinical deficiency in heifers may not cause clinical deficiency in lactating cows. Using Mn balance studies in lactating cows (Weiss and Socha, 2005), we estimated that lactating cows (average milk yield in the experiment = 84 lb/day) needed to consume 580 mg of Mn to be in Mn balance. Based on the DMI in that experiment, that translated into a dietary concentration of 28 ppm for total dietary Mn. We think that is a more accurate estimate of Mn requirement than the NRC (2001) requirement. One reason for the discrepancy is that lactating dairy cows have high requirements for Ca and P, and those minerals, especially P, can reduce absorption of Mn. As discussed above, uncertainty exists and reasonable safety factors (i.e., 1.2 to 1.5 X) should be applied. For Mn, the starting point is 28 ppm and after the safety factor is applied, diets for lactating cows should have 33 to 42 ppm total Mn.

Selenium

Per US FDA regulations, the amount of supplemental Se in dairy cow diets cannot exceed 0.3 ppm. Fortunately, in the vast majority of situations, diets with 0.3 to 0.4 ppm total Se (basal at 0.1 + 0.3 supplemental) is adequate. Excess S (from water and diet) reduces the

absorption of Se substantially (Ivancic and Weiss, 2001); however, the only legal option to overcome that problem is to use a high quality Se-yeast product rather than selenite or selenate. Under normal conditions, inorganic Se provides adequate available Se to the cow. However, Se from Se yeast results in substantially greater concentrations of Se in milk and colostrum and in the newborn calf if the dam was fed Se yeast during the dry period (Weiss, 2005). Clinical measures, such as mastitis prevalence or immune function, have not shown any consistent differences when inorganic Se or Se yeast was fed. Because of increased transfer of Se to the fetus and into colostrum, feeding a portion of Se as Se-yeast is a good idea. Using Se-yeast in situations with excess S should also be considered.

Zinc

The Zn requirement in NRC (2001) is on a mg/day of absorbed Zn basis, and for lactating cows, it ranges from about 110 to 260 mg/day (dependent on milk yield). Assuming typical AC and DMI, diets with 40 to 50 ppm total Zn should be adequate. No new data are available contradicting the current NRC recommendation. Real world antagonists for Zn are not a major concern; therefore, the current requirement plus a modest safety factor for risk management is adequate. For an average Holstein cow (75 lb of milk), the absorbed Zn requirement is 165 mg/day and with a safety factor of 1.2 to 1.5 X, that cow should be fed a diet that provides 200 to 250 mg/day of absorbed Zn. As with Cu, if you are using a form of Zn with greater bioavailability, dietary concentrations should be less than if diets are based on Zn sulfate. Suppliers of those minerals should have data on relative (usually relative to Zn sulfate) bioavailability of their products.

Conclusions

Adequate supply of trace minerals improves the health and productivity of dairy cows; excess or inadequate trace minerals have the opposite effect. The 2001 NRC requirements (or the FDA regulation) for Cu, Zn, and Se are adequate in most situations and only a modest safety factor should be applied for risk management. Because of regulations, no safety factor can be applied to Se. For most minerals, diets should be formulated for total absorbable minerals and the minerals provided by basal ingredients must be included. This also means that diets that include sources of supplemental mineral that have higher bioavailability should have lower total concentrations of trace minerals than diets based on trace mineral sulfates. For Cu, numerous antagonists exist, and in those cases, diets need to provide substantially more Cu than recommended by NRC. Although many situations dictate higher concentrations of dietary Cu, be aware of excessive Cu supplementation. Overfeeding Cu for months or years can result in high liver Cu concentrations that may be negatively affecting cow health. The bottom line is to feed slightly more than adequate, but not excessive, amounts of trace minerals.

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Table 1. Effect of intake and milk production on requirements (NRC, 2001) of certain trace minerals.¹

	Early lactation cow 75 lb milk; 35 lb DMI		High producing cow 120 lb milk; 67 lb DMI		Average cow 75 lb milk; 53 lb DMI	
	Absorbed requirement (mg/day)	Dietary requirement (mg/kg of diet DM)	Absorbed requirement (mg/day)	Dietary requirement (mg/kg of diet DM)	Absorbed requirement (mg/day)	Dietary requirement (mg/kg of diet DM)
Cu	9.7	14	12.8	10	9.7	10
Fe	34.0	22	54.4	18	34.0	14
Mn	2.3	19	2.9	13	2.3	13
Zn	165	61	247	49	165	43

¹Basal diets were assumed to contain 8, 225, 30, and 35 ppm of Cu, Fe, Mn, and Zn, respectively. Basal absorption coefficients were 0.04, 0.10, 0.0075, and 0.15 for Cu, Fe, Mn, and Zn, respectively. If supplemental minerals were needed, absorption coefficients for sulfate forms were used.

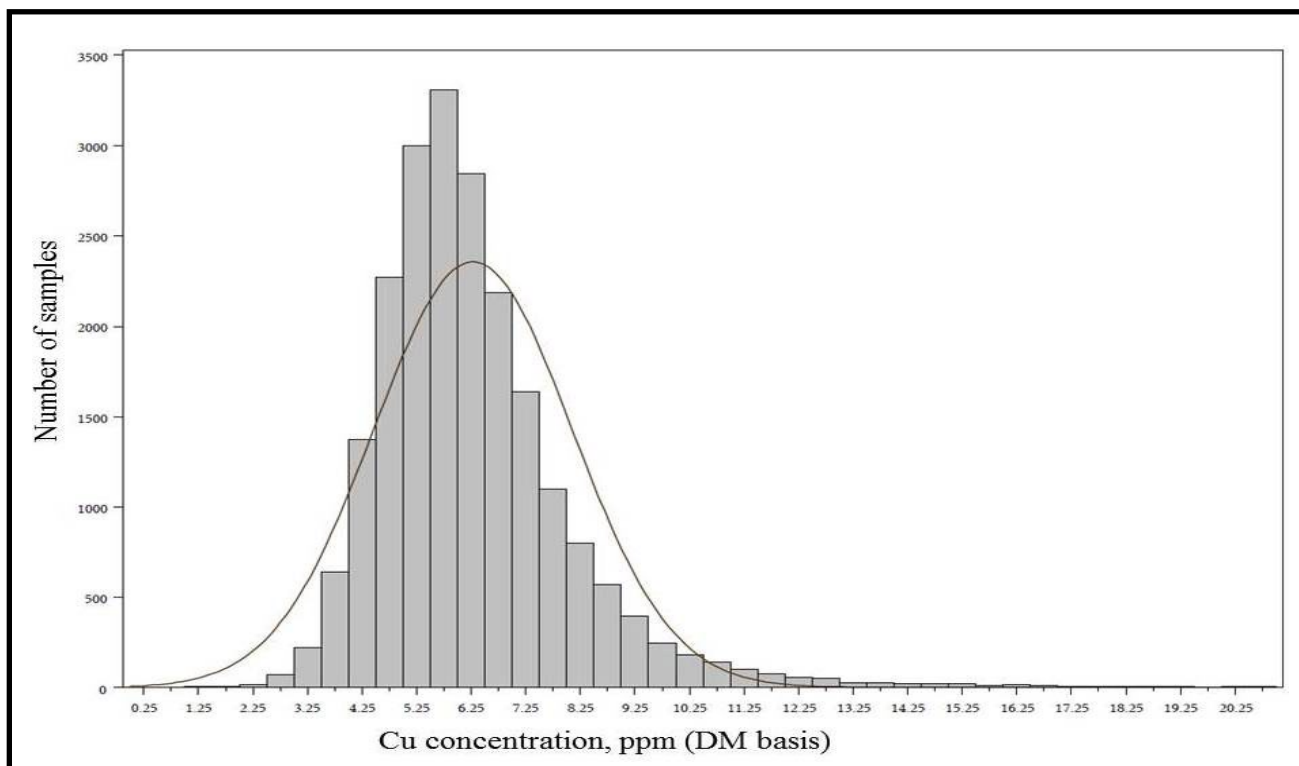


Figure 1. Distribution of Cu concentrations in corn silage grown throughout the U.S. The smooth line indicates a normal distribution, while the bars indicate the actual distribution. Figure courtesy of J. Knapp (Knapp et al., 2015).

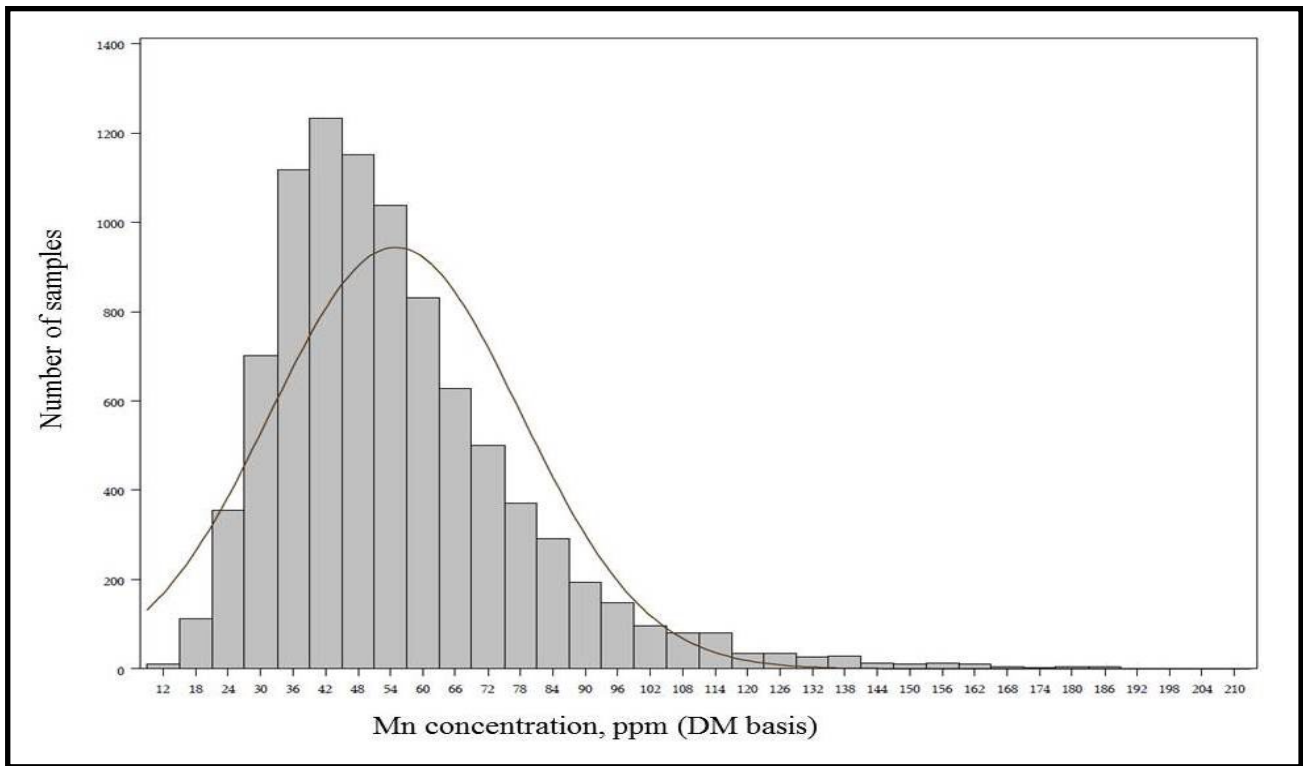


Figure 2. Distribution of Mn concentrations in mixed, mostly legume silage grown throughout the U.S. The smooth line indicates a normal distribution, while the bars indicate the actual distribution. Figure courtesy of J. Knapp (Knapp et al., 2015).

Grouping Strategies for Dry and Fresh Cows to Optimize Health and Performance

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Summary

Management of periparturient dairy cows has been identified as critical for health and performance. Ultimately, housing and management of periparturient cows must provide an environment that is free of stress, is conducive to natural behavior, and optimizes water and feed intake. Negative interactions of the cow with its environment, herdmates, and herdsmen may elicit behavioral changes, such as avoidance, separation/isolation, and reduced resting time and feeding time. If such behavioral changes are severe and prolonged, they may translate into impairment of immune and metabolic statuses, increased incidence of health disorders, and compromised reproductive and productive performances. In this presentation, physiological changes that occur during the peripartum that are associated with impaired immune function will be discussed. Furthermore, experiments that have evaluated how housing and grouping strategies affect behavior, immune and metabolic status, and performance will be presented.

Introduction

In 1983, Albright described the issues concerning public perception of animal wellbeing as: "People are calling for what are more "humane practices" in the treatment of animals ... these concerned individuals are of

nonfarm background and generally have been exposed to only a few farms (Albright, 1983)". This statement is still very relevant today. Growing pressure from consumers has resulted in legislators interfering on livestock husbandry practices. An example of such interference is the approval in 2008 of "California Proposition 2 (Standards for Confining Farm Animals)". This prompted the American Veterinary Medical Association to issue the following statement: "Proposition 2 is admirable in its goal to improve the welfare of production farm animals; however, it ignores critical aspects of animal welfare that ultimately would threaten the well-being of the very animals it strives to protect." "Proposition 2 may have negative impacts on animals, consumers, and the industry. More attention needs to be paid to the behavioral and social needs of food animals, ..., but the standards in this ballot initiative fall short in improving animal welfare because they fail to adequately consider other factors. Animal welfare is a complex issue and demands that decisions be based on science ...". Our ability to assure consumers that dairy products meet the highest standards of safety, quality, and animal welfare is vital to the sustainability of the US dairy industry. Thus, understanding management practices that affect animal wellbeing is crucial to produce the best practice guidelines to be adopted by dairy farmers and to demonstrate to the public that the utmost care is taken to provide dairy animals with

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a safe, comfortable, and health conducive environment. Nonetheless, we must not lose focus that dairy farms are profit driven as any other business.

Moberg (2000) described stress as being part of life and not inherently harmful to animals. As in humans, dairy animals have developed mechanisms to cope with stress and only in severe cases of stress do they present abnormal responses that may lead to disease and poor performance. The recognition that some management strategies may produce excessive stress or chronic stress and lead to disease has sensitized us for the importance of stress to dairy cow well-being. Once a stressor is identified, an organism may respond through neuroendocrine (pituitary-hypothalamic-adrenal axis, e.g., cortisol secretion), immune (innate or adaptive responses), autonomic (e.g., “fight or flight”), and behavioral (e.g., stress avoidance) changes. Each and every step of the response to stressors is important. Evaluating one response only may not be sufficient to understand the overall consequence of stressors to the animal.

Cows are social animals and as such are highly susceptible to social interactions and hierarchical order. Once housed within a group, dominant cows display physical and non-physical aggressive behavior towards submissive cows. Situations that exacerbate these deleterious interactions among dominant and submissive cows (e.g., lack of feed or water, limited feed bunk space, and limited resting space) have the potential to affect health and performance. Although group performance is the most common used parameter to evaluate management and protocols, often evaluation of averages masks the poor performance of subordinate cows in particular. Therefore, management should be focused to provide all cows with sufficient feed, water, and resting

space to minimize the expression of subordinate behaviors. Although much focus has been placed on behavioral responses to stressors, it is important to note that often behavioral responses are short lived and have minor implications to overall well-being, health, and performance. A holistic approach to understanding how cows respond to stressors and the consequences to health and performance may generate a more precise understanding of the relationship between these stressors and animal well-being.

Prepartum Grouping Management and Transition Cow Health

Regrouping of dairy cows is used in dairy operations to maintain homogenous groups in terms of gestation stage to optimize nutritional management. Thus, in many dairy operations, cows are housed as a group from approximately 230 to 250 days of gestation in so called “dry cow pens” and as another group from 251 days of gestation to parturition in so called “close-up cow pens”. Every week, cows from the dry-cow pen are moved to the close-up cow pen, which results in weekly disruption of social interactions, and for many cows, disruption of social interactions in the last days before parturition. Constant regrouping of cows changes the hierarchical order among them, forcing cows to re-establish social relationships through physical and nonphysical interactions and exacerbating aggressive and submissive behaviors (von Keyserlingk et al., 2008). Furthermore, because dry-cows and close-up cows are not producing milk, their management is often taken for granted, resulting in overstocked pens, insufficient water and feed availability, and exposure to adverse weather conditions (i.e., heat stress). These managerial inadequacies that increase and prolong the negative energy balance during the peripartum period transform the normal homeorhetic changes into metabolic diseases (i.e., excessively

elevated fat mobilization, hepatic lipidosis, and ketosis), further suppressing immune function of dairy cows and predisposing them to health disorders and compromised productive, reproductive, and economic performances.

Separation of prepartum heifers and cows

Smaller cows are in general more submissive than larger cows. Consequently, when prepartum heifers are housed together with mature cows, they are more likely to express submissive behavior. In a study in which prepartum heifers were housed with mature cows during the prepartum or were housed alone, heifers housed with mature cows had reduced feed intake and reduced resting time during the prepartum and reduced milk yield compared with heifers housed alone (Table 1). Therefore, we recommend that primiparous cows be housed separately from mature cows from at least 21 days before to 21 days after calving. If this is not possible, prepartum and postpartum pens should have a stocking density of < 80%.

Stocking density prepartum and its effects on behavior, feed intake, and immune function

Situations of limited space or access to feed exacerbate aggressive and submissive behaviors. Two small but elegant studies conducted in research facilities at the University of British Columbia in Canada demonstrated the effects of overstocking of prepartum cows on behavior and feed intake. According to one of these studies, cows housed in pens in which the ratio of cows to feeding bin was 2:1 had altered behavior compared with cows housed in pens with cow to feeding bin ratio of 1:1 (Hosseinkhani et al., 2008). Similarly, the second study demonstrated that cows housed in pens with 12²/cow of feed bunk space had altered behavior compared with cows housed in pens

with 24²/cow of feed bunk space (Proudfoot et al., 2009). These altered behaviors included increased rate of feed intake, fewer meals per day, increased feed sorting, decreased overall feed intake, increased standing time, and increased rate of displacement from the feeding area (Hosseinkhani et al., 2008; Proudfoot et al., 2009). The consequences of stocking density for dominant and submissive cows are likely to be distinct. Dominant cows are likely predisposed to ruminal acidosis when they have increased rate of feed intake, fewer meals per day, and increased feed sorting. On the other hand, submissive cows are likely predisposed to metabolic diseases, such as hepatic lipidosis and ketosis because of reduced feed intake and lameness because of increased standing time and displacement rate. Therefore, overstocking of pens of prepartum cows, a common problem in dairy operations of all sizes, predisposes all cows to inadequate nutrient intake prepartum and consequently compromised immune function. Because cows have allelomimetic behavior, characterized by cows doing the same activity at the same time, it is fundamental to assure that space is available for all cows to eat at the same time without the expression of aggressive and submissive behaviors during the prepartum period.

A study conducted in Italy evaluated the humoral immunity and productive performance of dairy ewes that were housed in high or low stocking density conditions from late gestation to mid-lactation (Carporese et al., 2009). Ewes that were housed in high stocking density conditions had reduced anti-ovalbumin IgG concentration in response to an ovalbumin challenge compared with ewes housed in low stocking density conditions (Carporese et al., 2009). Further, ewes that were housed in high stocking density conditions tended to have a greater number of aggressive interactions and had reduced milk yield and increased milk somatic cell count (Carporese et al., 2009).

In a recent experiment (Silva et al., 2014), prepartum Jersey cows were housed to attain 100% stocking density of headlocks (109% stocking density of stalls; **100SD**) or 80% stocking density of headlocks (87% stocking density of stalls; **80SD**). Although new cows entered the prepartum pen twice weekly in order to try to maintain a stocking density close to 80 and 100%, the average headlock stocking densities were 74.1 ± 0.4 and $94.5 \pm 0.3\%$ for 80SD and 100SD, respectively ($P < 0.01$; Figure 1). The stall stocking densities were 80.8 ± 0.4 and $103.1 \pm 0.4\%$ for 80SD and 100SD, respectively ($P < 0.01$). Increased stocking density in the prepartum pen resulted in increased daily average displacement from the feed bunk ($P < 0.01$; Figure 2) but had minimal effect on average daily lying (Figure 3) and feeding (Figure 4) times. Metabolic profile of prepartum dairy cattle exposed to 80 and 100% stocking density was generally not different (Silva et al., 2014). Similarly, innate and adaptive immune functions were not compromised by 100% stocking density (data not shown).

Not surprisingly, there was no effect of stocking density on incidence of periparturient diseases, removal from the herd within 60 days postpartum (Table 2), and yield of energy corrected milk (80SD = 75.2 ± 1.1 vs. 100SD = 74.4 ± 1.1 lb/day; $P = 0.56$).

A recent experiment conducted in Canada evaluated the metabolic responses of cows housed at 80% stall stocking density and 35" of feedbunk space per cow ($n = 24$) and cows housed at 120% stall stocking density and 18" of feedbunk space per cow ($n = 24$) (Miltenburg et al., 2014). Group sizes were 6 and 10 cows per pen and the cows were enrolled in the experiment 21 days before expected calving date. Although cows housed in overstocked pens had greater albumin and

bilirubin concentrations, they also had reduced β -hydroxy butyrate (**BHBA**) and non-esterified fatty acids (**NEFA**) concentrations compared with understocked cows. Stocking density had no effect on neutrophil function (oxidative burst). Number of cows in this experiment was small, but no differences between treatments were observed in incidence of uterine diseases.

Recently, our group conducted an experiment to evaluate the rumination, activity, and lying behavior pattern of periparturient dairy animals. During the experiment, stocking density of the pens, based on feedbunk space, was monitored, but it was not manipulated purposely to compare effects of stocking density on rumination, activity, and lying behavior. Evaluating the data retrospectively, however, we observed that different stocking densities in the last 7 days prepartum (range: parous = 63 to 103%, nulliparous = 90 to 120%) was not correlated with average rumination (min/d; Figure 5A and 5B) and lying time (min/d; Figure 6A and 6B) during the last 7 days prepartum (Chebel, personal communication).

Current recommendations indicate that stocking density during the prepartum should be 80% of headlock and at least 30" of linear feed bunk space per animal, depending on breed. In the experiment by Silva et al. (2014) and Lobeck-Luchterhand et al. (2014), we demonstrated that when parous and nulliparous animals are housed separately, when water is readily available, when the length of the "close-up" prepartum period is > 21 days, and when feed bunk management is appropriate, target stocking density on the day of regrouping may be as high as 100% of headlocks.

An issue that is often overlooked is the amount of water and access to water available to prepartum and postpartum cows. In general, it is recommended that a minimum 4 to 5" of

linear water trough space per cow and 1 water trough per 20 cows to assure that cows have sufficient access to water.

Effects of regrouping frequency on behavior, feed intake, and milk yield

Another situation commonly observed in dairy operations that may pose a risk to the health of peripartum cows is frequent regrouping during the prepartum period. Regrouping of dairy cows is used in dairy operations to maintain homogenous groups in terms of gestation stage to optimize nutritional management. Thus, in many dairy operations cows are housed as a group from approximately 230 to 250 days of gestation in so called “dry cow pens” and as another group from 251 days of gestation to parturition in so called “close-up cow pens”. Every week, cows from the dry-cow pen are moved to the close-up cow pen, which results in weekly disruption of social interactions and for many cows disruption of social interactions in the last days before parturition. The effects of regrouping frequency of cows on behavior, feed intake, and health have been less studied and have yielded more contradictory results. In small studies also conducted in Canada, cows were demonstrated to have reduced feeding time, greater rate of displacement from the feed bunk and stalls, and reduced milk yield within a few hours after regrouping (von Keyserlingk et al., 2008). Although the question has not yet been definitively answered, cows may require 3 to 14 days after regrouping to re-establish social stability to pre-regrouping levels (Grant and Albright, 1995). This could be a significant problem for close-up cows because weekly entry of new cows in the close-up pen could result in social disruption and stress on the last days of gestation, compromising further dry matter intake (DMI) and immune parameters.

Coonen et al. (2011) evaluated dry matter intake, plasma NEFA concentration, and 30-day milk yield of close-up cows (14 to 28 days before expected calving date) that were housed in stable (no new cows entering the close-up pen) or dynamic pen (new cows entering the close-up pen twice weekly). The pens were relatively small (10 cows per pen) and the total number of cows used in the experiment was 85. In this small study, no differences between ‘stable’ and ‘dynamic’ grouping systems in feed bunk displacement rate, DMI ($P = 0.53$), NEFA concentrations during the peripartum ($P > 0.32$), and milk yield ($P = 0.32$) in the first 30 DIM were observed. The observations that DMI, NEFA concentration, and milk yield did not differ are novel and suggest that larger experiments are necessary.

In a recent study (Silva et al., 2013a and 2013b; Lobeck-Luchterhand et al., 2014), the hypothesis that constant disturbance of social order prepartum by weekly introducing new cows in a close-up pen was tested in a large dairy herd (6,400 lactating cows). Cows (254 ± 7 days of gestation) were paired by gestation length and assigned randomly to an All-In-All-Out (AIAO) or control treatments. In the AIAO ($n = 259$) treatment, groups of 44 cows were moved into a pen where they remained for 5 wk, whereas in the control treatment ($n = 308$), approximately 10 cows were moved into a pen weekly to maintain a stocking density of 100% and 92% relative to stalls and headlocks, respectively, 7.9 m²/cow. At the completion of 5 wk, cows in the AIAO treatment that had not calved by 5 wk were moved to a new pen and a new replicate was initiated. The data referent to these AIAO cows that had to be regrouped at the end of the 5 wk replicate were used for statistical analysis. Pens were identical in size (44 stalls and 48 headlocks) and design and each of the pens received each treatment a total of 3 times, totaling 6 replicates.

Video recording cameras were placed above the feed lane for determination of feed bunk displacement activity (Lobeck-Luchterhand et al., 2014). Displacement from the feed bunk was measured, in both pens, during 3 h on the day cows were moved to the close-up pen (-30 days before expected calving date) at 13:00 \pm 1:00 and following fresh feed delivery (05:00 \pm 1:00) 1, 2, 3 and 7 days after cows were moved to the control close-up pen. The average stocking density of the control pen was 87% (69.5 to 100%), whereas in the AIAO pen, the average stocking density was 73% (7.3 to 100%; Figure 7; Silva et al., 2013a). A greater number of displacements (Figure 8) and a greater displacement rate (Figure 9) were observed in the control treatment than in the AIAO treatment (Lobeck-Luchterhand et al., 2014). Minimal changes in feeding time, however, were observed during the 5 weeks preceding calving (Figure 10; Lobeck-Luchterhand et al., 2014). Percentage of cows at the feed bunk at different times of the day were similar between AIAO and control treatments (Figure 11; Lobeck-Luchterhand et al., 2014). Despite these changes in behavior, no changes in immune (innate and adaptive; Silva et al., 2013b) and metabolic parameters were observed (Silva et al., 2013a). Consequently, no differences in incidences of disease (Table 3) and yield of energy corrected milk (Figure 12) were observed.

There were 18 AIAO cows that did not calve within 5 wk and had to be mixed with other cows. The average interval between mixing of these cows and calving was 4.1 \pm 0.6 days (Silva et al., 2013a). When compared with AIAO that calved within the 5 wk replicate and were not regrouped, AIAO cows that had to be regrouped at the end of the 5 wk replicate had greater milk yield, greater yields of fat and protein, and greater yield of energy corrected milk (Table 4; Silva et al., 2013a).

Weekly entry of new cows in a close-up pen is expected to cause more agonistic interactions in the feed bunk than the stable pen. The increased rate of displacement from the feed bunk did not affect innate immune function, metabolic parameters, incidence of diseases, and reproductive and productive performances. It is interesting that even AIAO cows that underwent group change within 4.1 \pm 0.6 days prepartum had no significant increase in incidence of disease or reduction in reproductive performance.

In a recent experiment conducted by researchers in Canada, however, the behavioral response to regrouping was dependent on stocking density, such that increased stocking density (100% of headlocks) resulted in more frequent antagonistic behavior in the feedbunk compared with reduced stocking density (50 or 25% of headlocks; Talebi et al., 2014). It remains, however, that behavioral changes are 1 of the 4 biological responses to stress, with neuroendocrine, immune, and autonomic being the other 3. Stressors that only cause a transient change in behavior but have no effects on other responses seem to have little importance to biological function of cows.

Grouping strategy during the postpartum period

Similar to the concerns described for the prepartum period, during the postpartum period cows must be offered the best environment possible. Although controversy exists regarding whether or not prepartum feed intake should be maximized, during the postpartum period cows should increase feed intake at a very fast rate to reduce the extent of negative energy balance. However, because of the difficulty in applying different management strategies to milking cows, limited experiments have compared housing and grouping strategies during the postpartum period.

In an observational study of 24 Canadian herds (66 to 570 lactating cows, mean = 161.8 ± 120 lactating cows), researchers evaluated risk factors for improved performance (Sova et al., 2013). In these herds, average feedbunk space was 21" (14 to 39"), but no description of grouping strategy (e.g., separation of primiparous and multiparous) was given. Nonetheless, factors associated with DMI were milking frequency and feeding frequency, such that 3x milking vs 2x milking increased feed intake by 3.12 lb/day and 2x feeding vs 1x feeding increased feed intake by 2.62 lb/day. On the other hand, managerial factors associated with milk yield were milking frequency, feeding frequency, and linear water space. Increasing milking frequency from 2x to 3x increased milk yield by 13.0 lb/day, increasing feeding frequency from 1x to 2x increased milk yield by 4.42 lb/day, and increasing linear water through space by 1 cm increased milk yield by 0.84 lb/day. Although this was not a controlled experiment, the findings of this observational experiment demonstrated that feedbunk and water through space are critical to maximize milk yield. The positive effects of increased milking frequency on DMI and milk yield also are very important; however, milking >4x/day may pose challenges to cow budget time, such that resting and feeding time may be compromised. Suggested cow budget time is 3 to 5 hr/day of feeding, 10 to 14 hr/day of lying in a freestall, and 7 to 10 hr/day of ruminating (Grant and Albright, 2001). Krawczel et al. (2012) evaluated behavior and production of cows subjected to 100, 113, 131, and 142% stocking density based on number of stalls and headlocks. Each cow was subjected to the different stocking densities for 14 days. Lying time was reduced as stocking density increased (100% = 12.9 hr/day, 113% = 12.8 hr/day, 131% = 12.2 hr/day, and 142% = 12.3 hr/day). Overall daily feeding and rumination time were not affected by stocking density, but greater

stocking density was associated with reduced rumination while in a stall (100% = 95.1, 113% = 93.7, 131% = 89.6 and 142% = 97.3% of time in stall). There was a slight worsening of leg hygiene score after 14 days of exposure to high stocking density. There was a linear increase in displacement from the feedbunk as stocking density increased ($y = 0.27x - 18.5$; $R^2 = 0.60$; $P < 0.01$); however, stocking density did not affect cortisol concentrations or milk yield. Finally, regrouping of lactating cows produced several alterations in feeding behavior (feeding time – 15 fewer minutes in the first hour after regrouping; displacements from the feed bunk – increased 2.5x in the first day after regrouping), reduced resting time (3 hr fewer of resting time in the first day after regrouping; von Keyserlingk et al., 2008). Furthermore, regrouping caused a reduction in milk yield (8.8 lb) on the day of regrouping. Importantly, however, the consequences of regrouping were very short lived. Furthermore in this experiment, 1 cow was moved to a group of 11 cows. This is hardly the scenario observed in commercial herds where larger groups of cows are moved to larger pens, which means cows may have more means to avoid confrontation and may benefit from familiarization with herdmates before regrouping.

Conclusions

Transition cows are predisposed to immunosuppression because of changes in endocrine and metabolic parameters during the periparturient period. Prepartum cows and heifers should be housed separately when possible to reduce agonistic interactions and to assure that submissive animals (usually heifers) have proper access to water, feed, and resting space. A recently proposed system to reduce regrouping of prepartum cows (AIAO system) has not resulted in improvements in metabolic, immune, health, or productive parameters,

even though it reduced the rate of agonistic interaction in the feed bunk. This indicates that regrouping of prepartum cows results in transient disruption of social interactions, but it is likely insufficient to alter neuroendocrine and immune functions sufficiently to compromise biological functions. Although we recently demonstrated that managing prepartum cows/heifers to achieve 100% stocking density on the day of regrouping does not compromise immune function, health, and performance compared with a target stocking density of 80%, more studies are necessary to evaluate the ideal stocking density in the prepartum pens in different circumstances.

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Table 1. Performance of primiparous cows when grouped separately from multiparous cows.¹

Item	Multiparous + Primiparous	Primiparous Only
Eating time, min/day	184	205
Eating bouts/day	5.9	6.4
Concentrate intake, lb/day	22.2	25.5
Silage intake, lb/day	16.9	18.9
Lying time, min/day	424	461
Resting periods/day	5.3	6.3
Milk yield, lb/130 day	5,243	5,698
Milk fat, %	3.92	3.97

¹Adapted from Grant and Albright (1995)

Table 2. Effects of prepartum stocking density (80SD vs. 100SD) on incidence of postpartum health disorders, lameness, and removal from the herd within 60 days postpartum (Silva et al., 2014).

Items	80SD, %	100SD, %	<i>P</i> – value
Retained fetal membranes	5.1	7.8	0.19
Metritis	21.2	16.7	0.11
Acute metritis	9.9	9.4	0.64
Vaginal purulent discharge at 35 ± 3 DIM	5.8	7.9	0.35
Mastitis up to 60 DIM	2.9	4.6	0.18
Displacement of abomasum up to 60 DIM	1.0	0.7	0.78
Locomotion score > 2 at 1 ± 1 DIM	0.6	0.0	0.27
Locomotion score > 2 at 35 ± 3 DIM	3.8	2.6	0.37
Locomotion score > 2 at 56 ± 3 DIM	3.5	2.1	0.44
Removed within 60 DIM	6.1	5.1	0.63

Table 3. Effects of prepartum grouping strategy (TRD vs AIAO)¹ on incidence of postpartum health disorders, lameness, and removal from the herd within 60 days postpartum (Silva et al., 2013a).

Items	TRD ¹ , %	AIAO ¹ , %	<i>P</i> – value
Retained fetal membranes	10.9	11.6	0.82
Metritis	16.7	19.8	0.37
Acute metritis	1.7	3.6	0.22
Sub-clinical endometritis at 30 days postpartum ²	20.7	24.1	0.42
Endometritis at 35 days postpartum ²	10.3	10.3	0.96
Displacement of abomasum	3.2	1.7	0.38
Mastitis within 60 days postpartum	13.8	11.3	0.45
Lame at 1 ± 1 DIM	4.3	4.8	0.82
Lame at 28 ± 3 DIM	10.0	7.5	0.45
Lame at 56 ± 3 DIM	9.1	6.0	0.25
Removal from the herd within 60 d postpartum	9.1	8.9	0.94

¹TRD (traditional prepartum grouping strategy) – weekly entry of new cows into the prepartum pen; and AIAO (All-In-All-Out prepartum grouping strategy) – no entry of new cows in the prepartum pen. Target stocking density was 100% of stalls and 91.6% of headlocks and 7.9 m²/cow (26 ft²/cow).

Table 4. Comparison of productive parameters and milk quality of All-In-All-Out (AIAO) cows that calved within their replicate and AIAO cows that had to be moved to a different pen (Silva et al., 2013a).

Items	AIAO that calved within their replicate	AIAO moved to a different pen	<i>P</i> – value
Milk yield, lb/day	61.6 ± 1.0	3.5 ± 3.4	< 0.01
Fat yield, lb/day	2.75 ± 0.04	3.28 ± 0.15	< 0.01
Protein yield, lb/day	2.31 ± 0.04	2.75 ± 0.11	< 0.01
3.5% fat corrected milk yield, lb/day	78.7 ± 1.28	93.7 ± 4.20	< 0.01
Energy corrected milk yield, lb/day	73.5 ± 1.17	87.3 ± 3.8	< 0.01
Linear somatic cell count	2.94 ± 0.08	3.18 ± 0.28	0.41

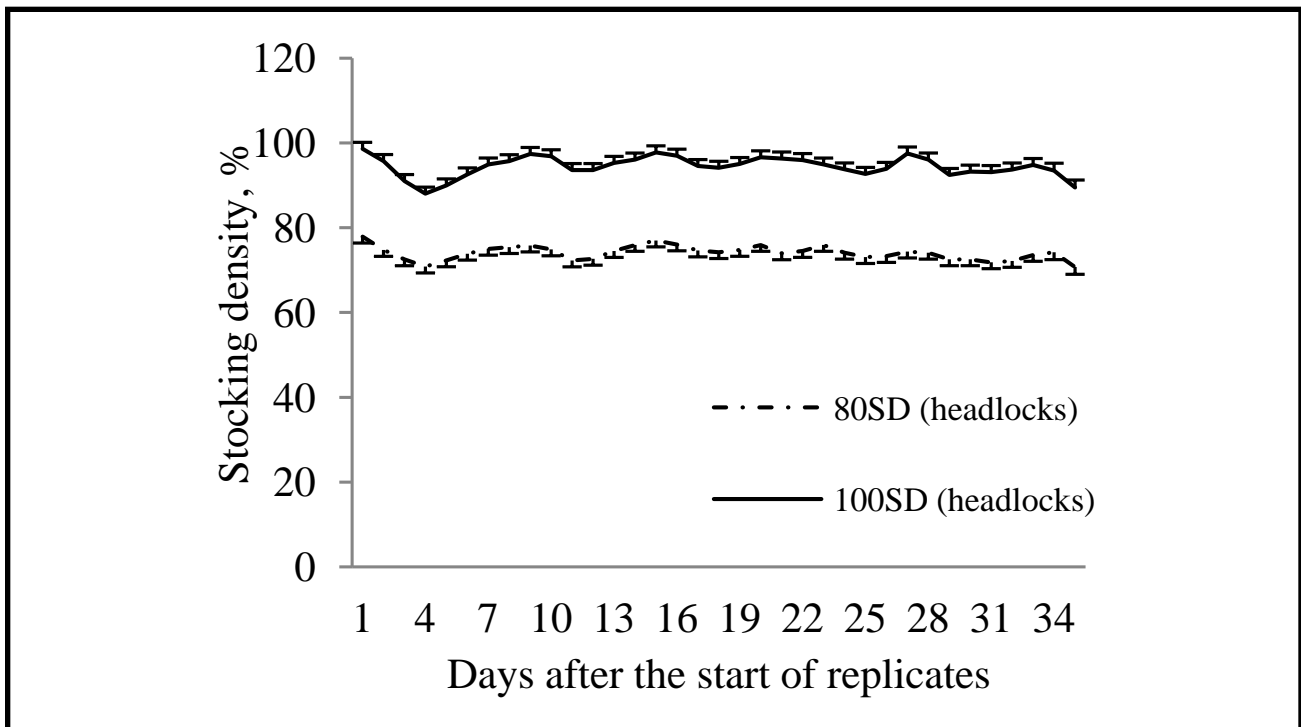


Figure 1. Headlock stocking density of heifers and cows submitted to the 80 and 100% stocking density treatments (Silva et al., 2014).

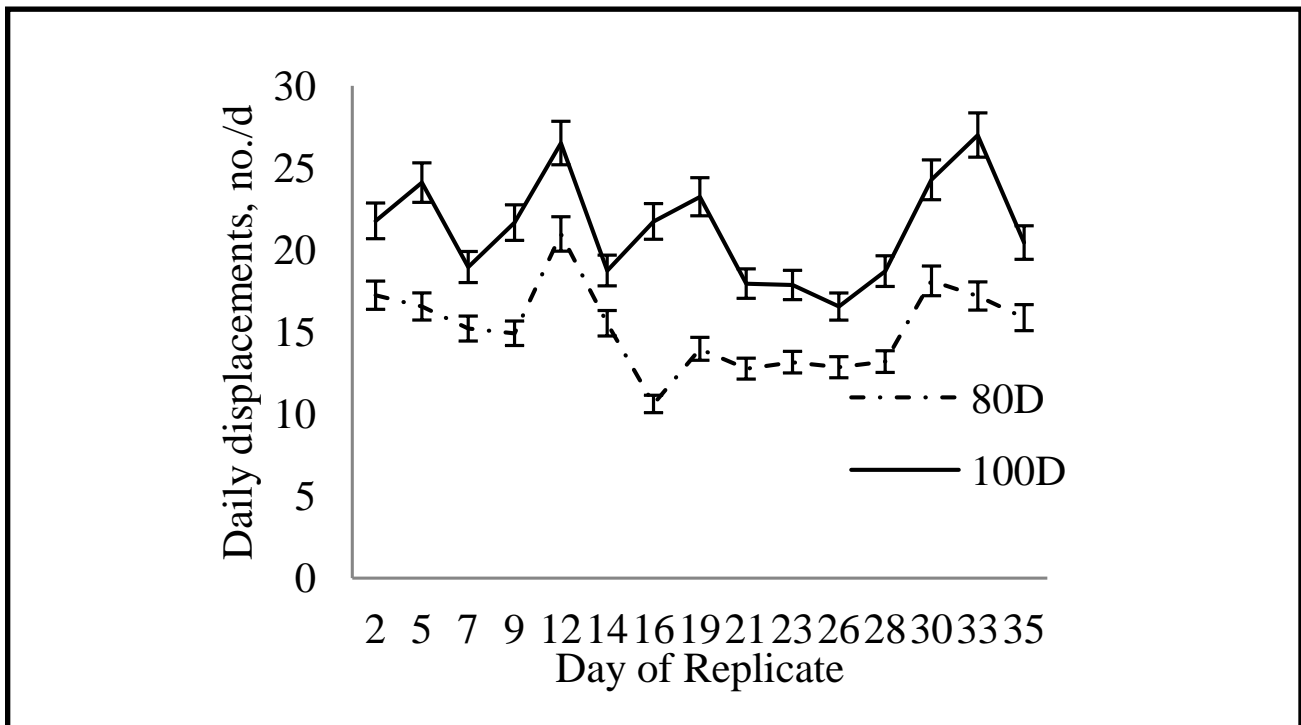


Figure 2. Effects of prepartum stocking density (80 or 100%) on daily number of displacements (Lobeck-Luchterhand et al., 2014).

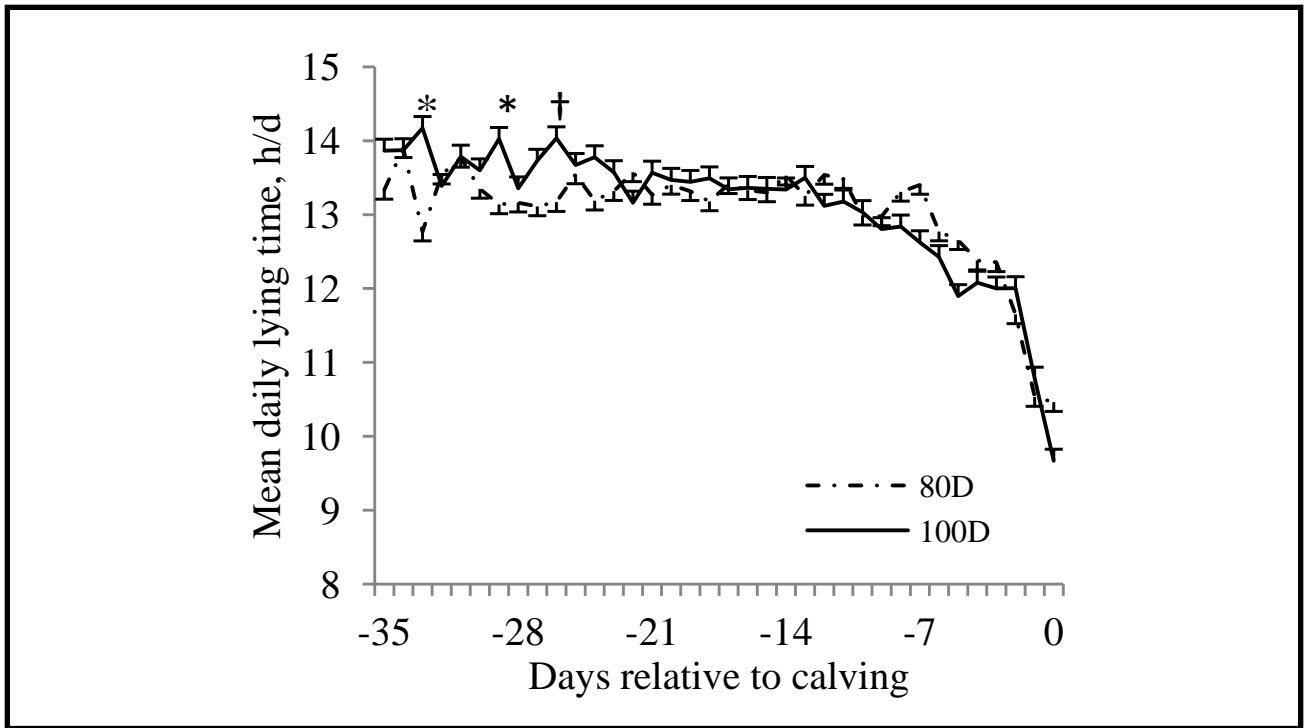


Figure 3. Effect of prepartum stocking density (80 to 100%) on daily lying time (Lobeck-Luchterhand et al., 2014).

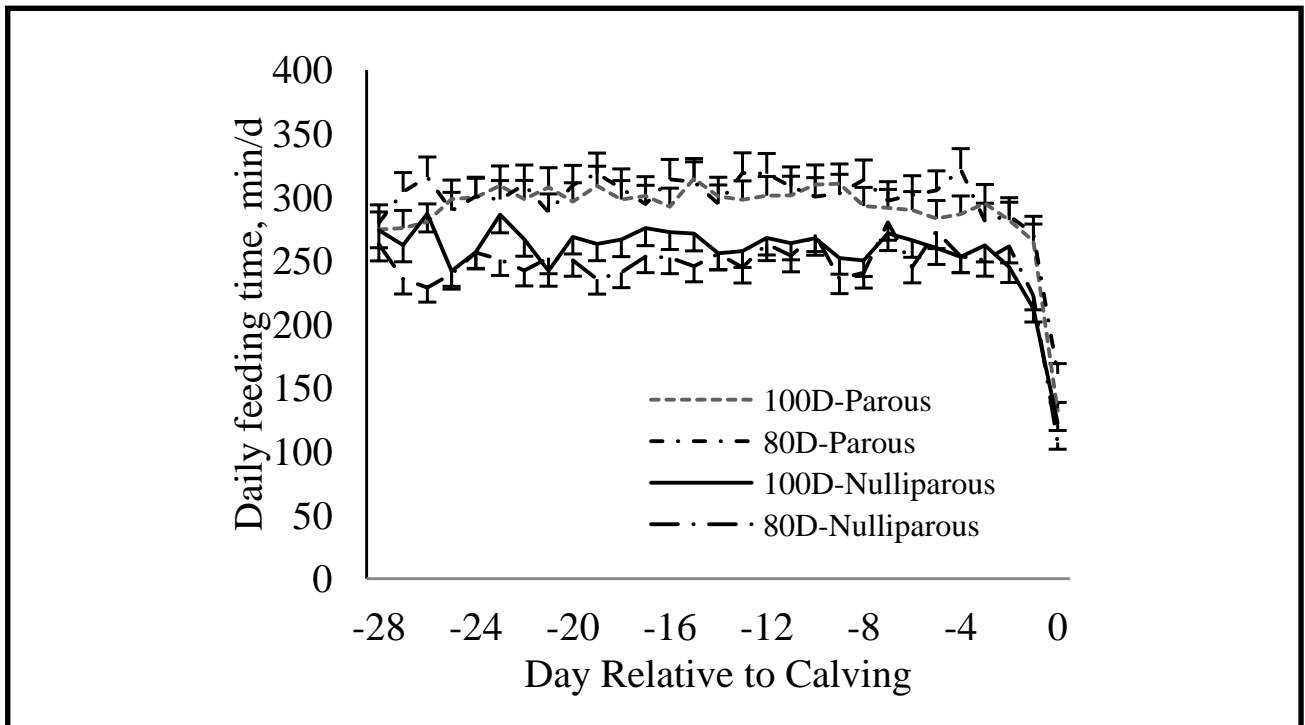


Figure 4. Effect of prepartum stocking density (80 to 100%) on daily average feeding time (Lobeck-Luchterhand et al., 2014).

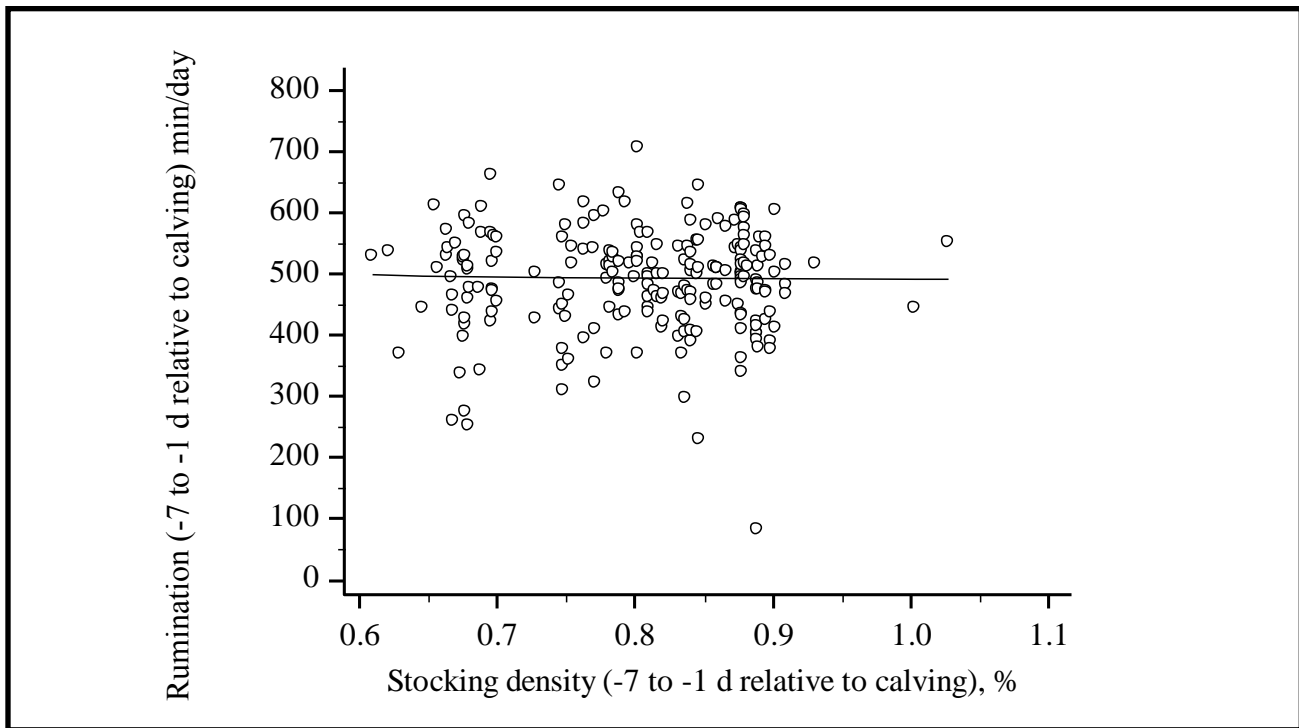


Figure 5A. Correlation between average stocking density (percentage feed bunk space) and rumination (min/d) during the last 7 days prepartum among parous animals ($n = 219$; $P = 0.83$, $r = 0.01$).

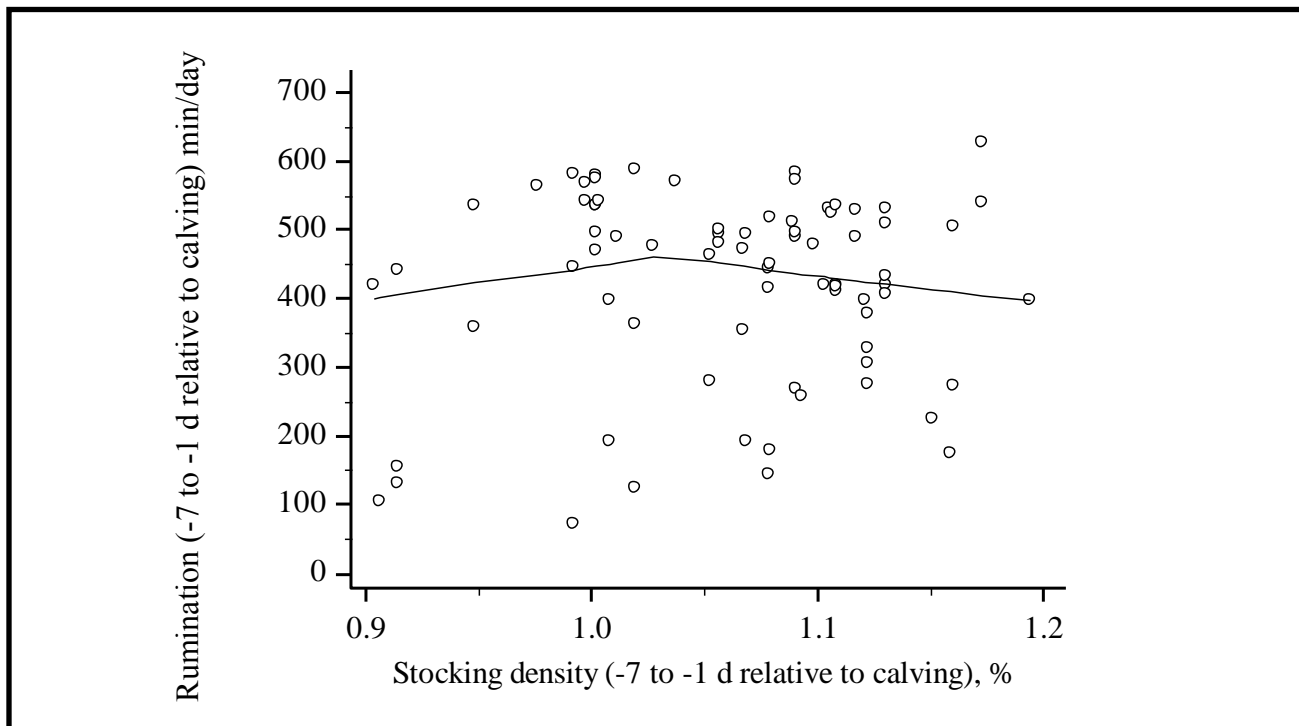


Figure 5B. Correlation between average stocking density (percentage feed bunk space) and rumination (min/d) during the last 7 days prepartum among nulliparous animals ($n = 77$; $P = 0.42$, $r = 0.09$).

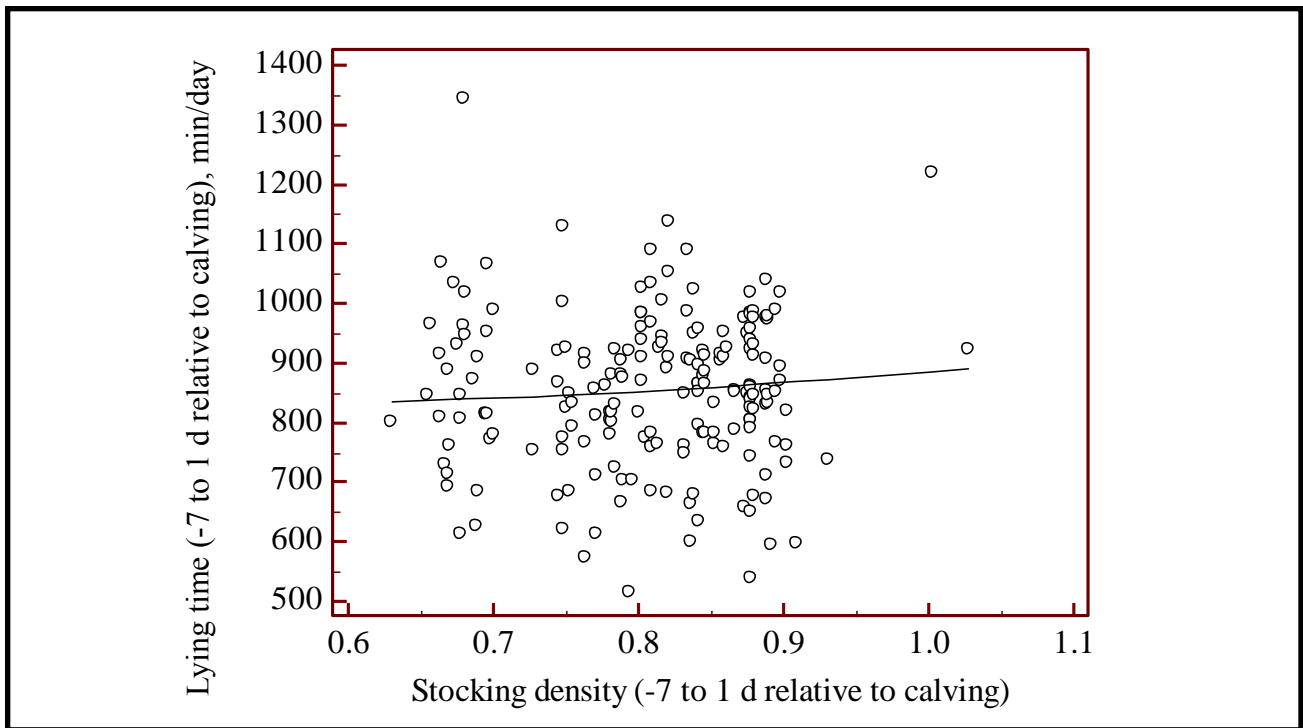


Figure 6A. Correlation between average stocking density (percentage feed bunk space) and lying time (min/d) during the last 7 days prepartum among parous animals ($n = 219$; $P = 0.67$, $r = 0.03$).

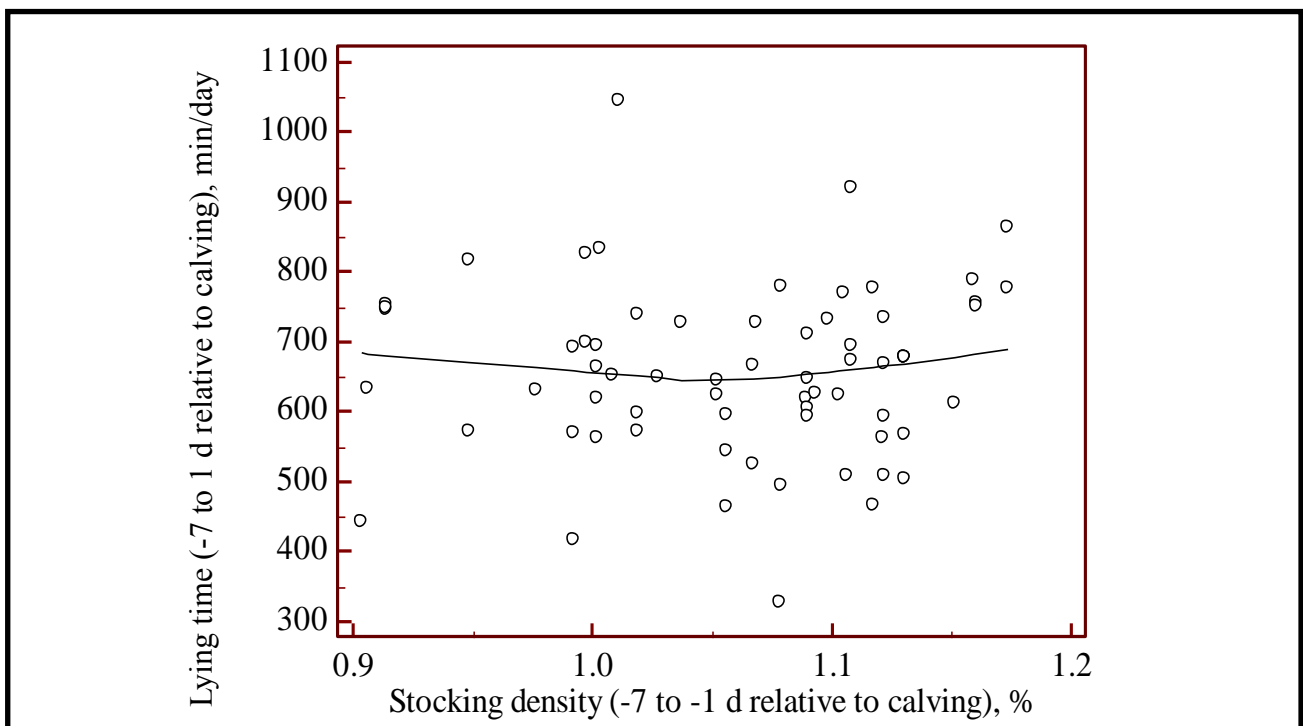


Figure 6B. Correlation between average stocking density (percentage feed bunk space) and lying time (min/d) during the last 7 days prepartum among nulliparous animals ($n = 219$; $P = 0.83$; $r = 0.03$).

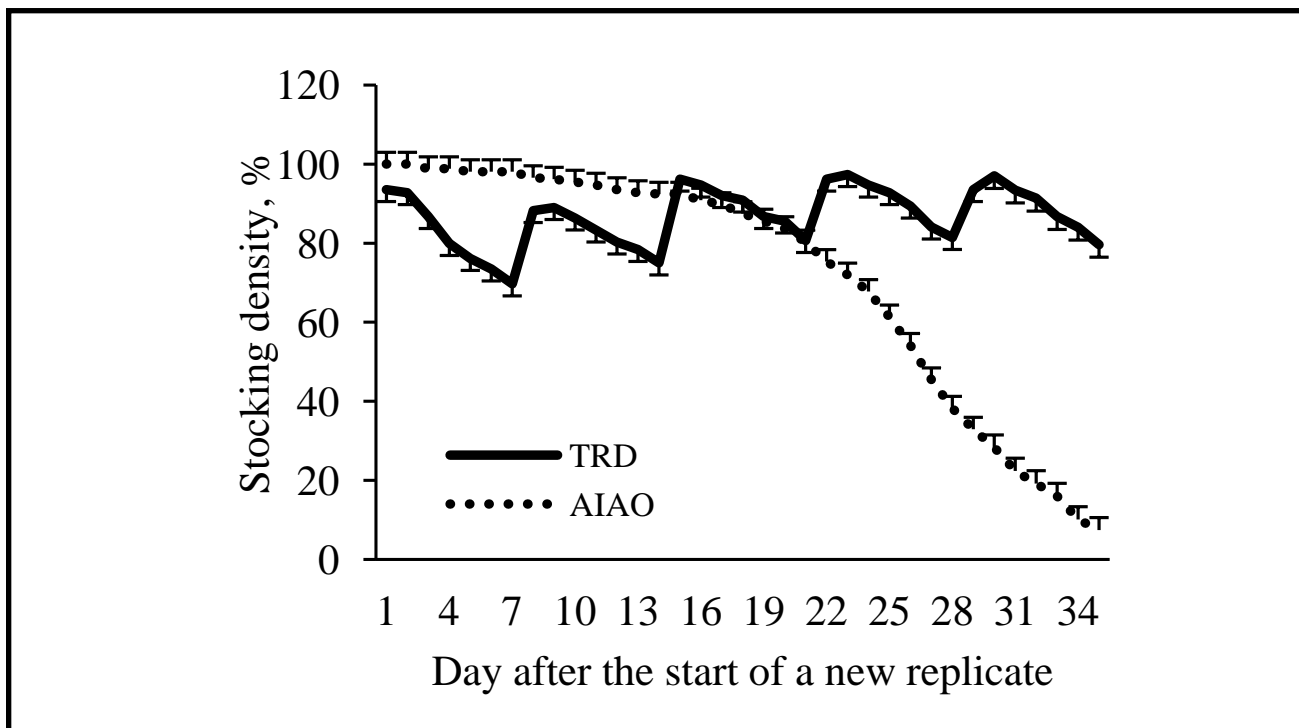


Figure 7. Effect of prepartum grouping strategy on stocking density of prepartum pens (TRD = traditional prepartum grouping strategy; AIAO = All-In-All-Out prepartum grouping strategy) (Silva et al., 2013A).

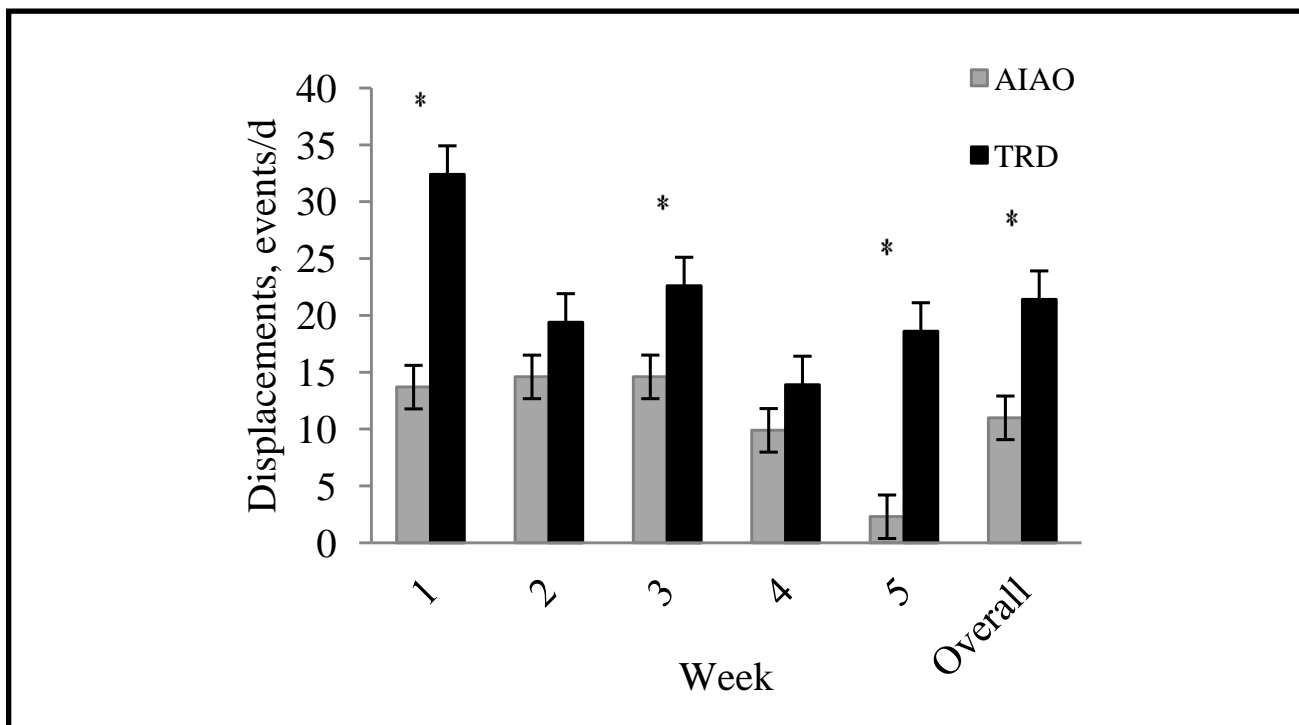


Figure 8. Effect of grouping strategy on average number of displacements during the prepartum period (TRD = traditional prepartum grouping strategy; AIAO = All-In-All-Out prepartum grouping strategy) (Lobeck-Luchterhand et al., 2014).

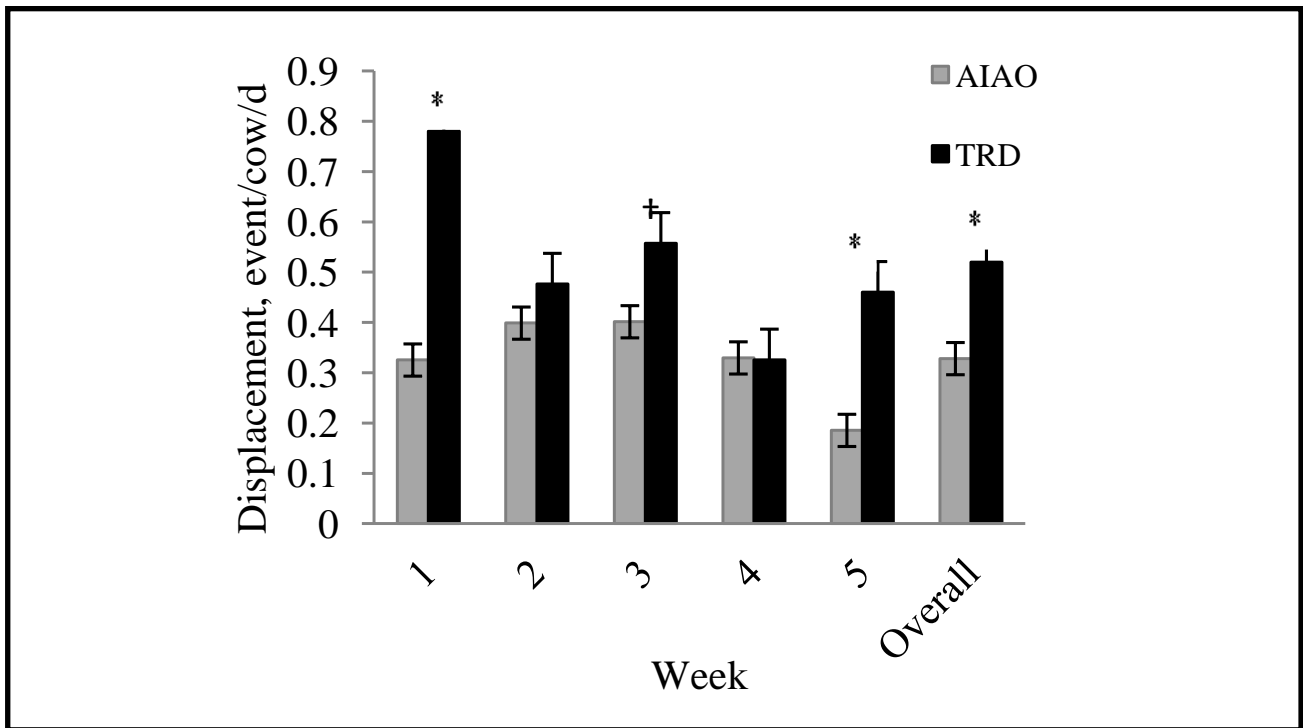


Figure 9. Effect of grouping strategy on average rate of displacement during the prepartum period (TRD = traditional prepartum grouping strategy; AIAO = All-In-All-Out prepartum grouping strategy) (Lobeck-Luchterhand et al., 2014).

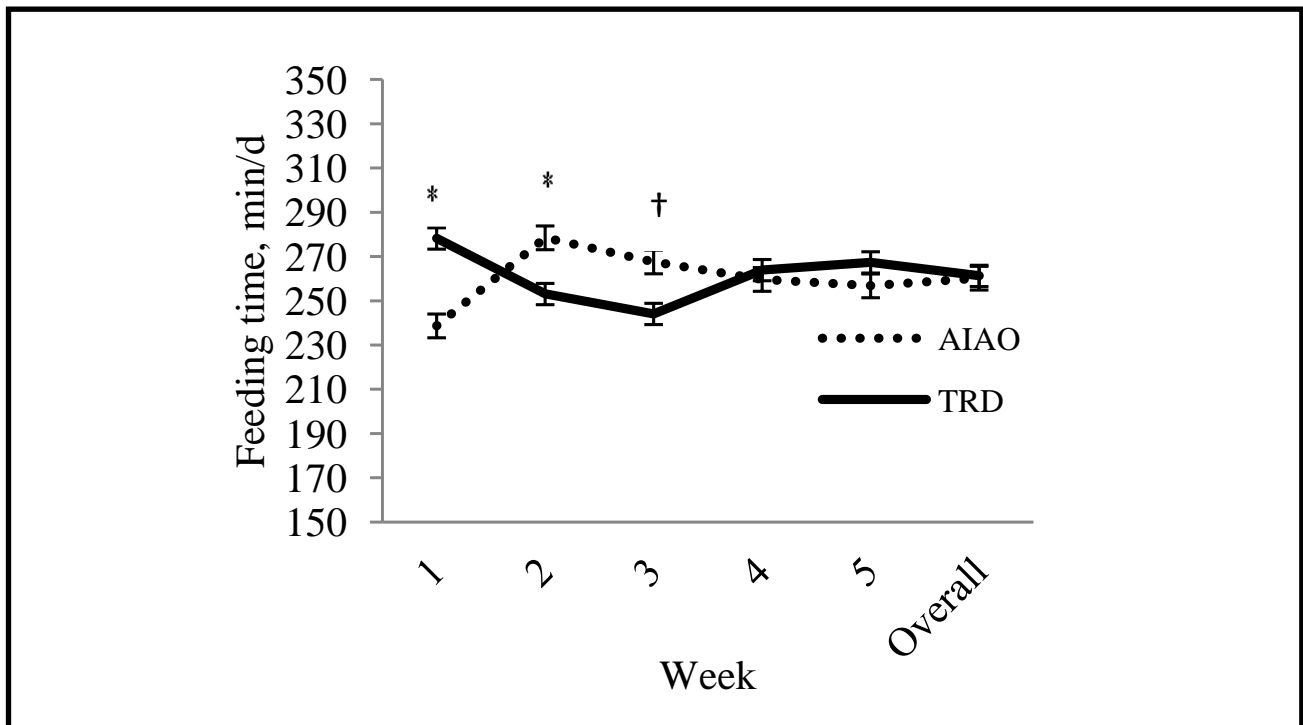


Figure 10. Effect of grouping strategy on average daily feeding time during the prepartum period (TRD = traditional prepartum grouping strategy; AIAO = All-In-All-Out prepartum grouping strategy) (Lobeck-Luchterhand et al., 2014).

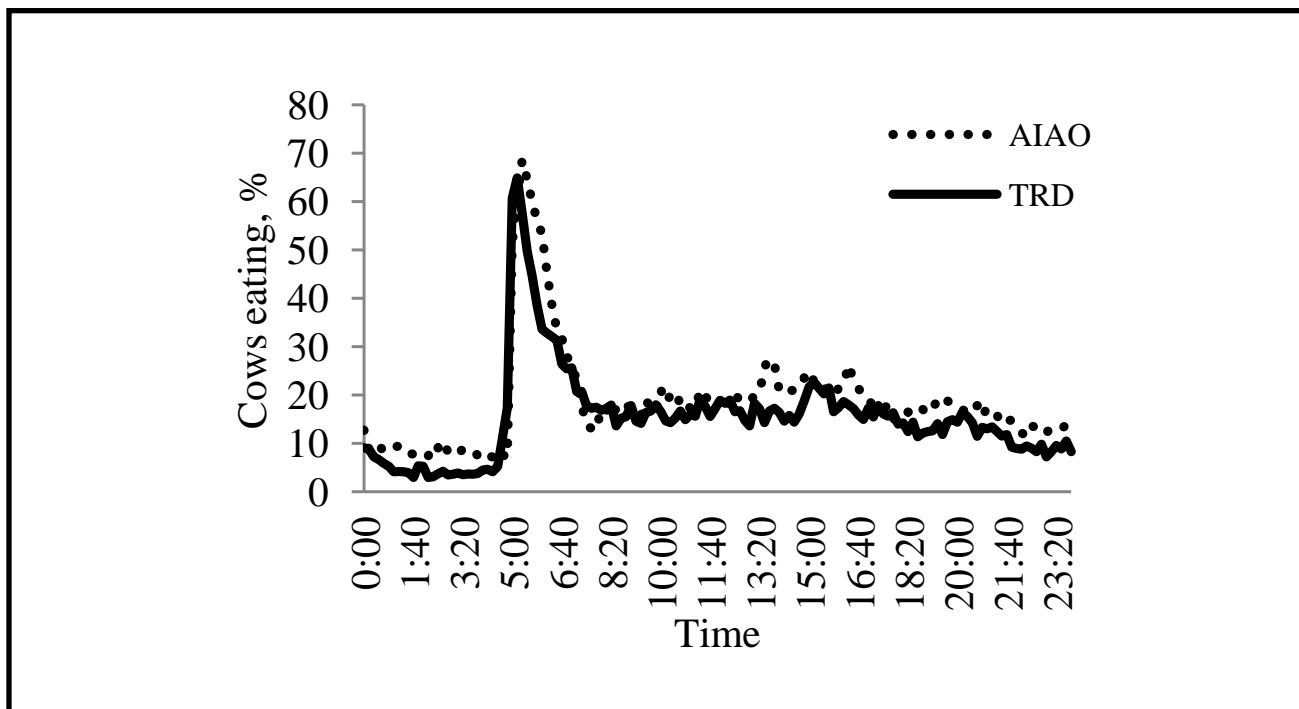


Figure 11. Average percentage of cows at the feed bunk during the prepartum period (TRD = traditional prepartum grouping strategy – weekly entry of new cows into the prepartum pen; AIAO = All-In-All-Out prepartum grouping strategy – no entry of new cows in the prepartum pen) (Lobeck-Luchterhand et al., 2014).

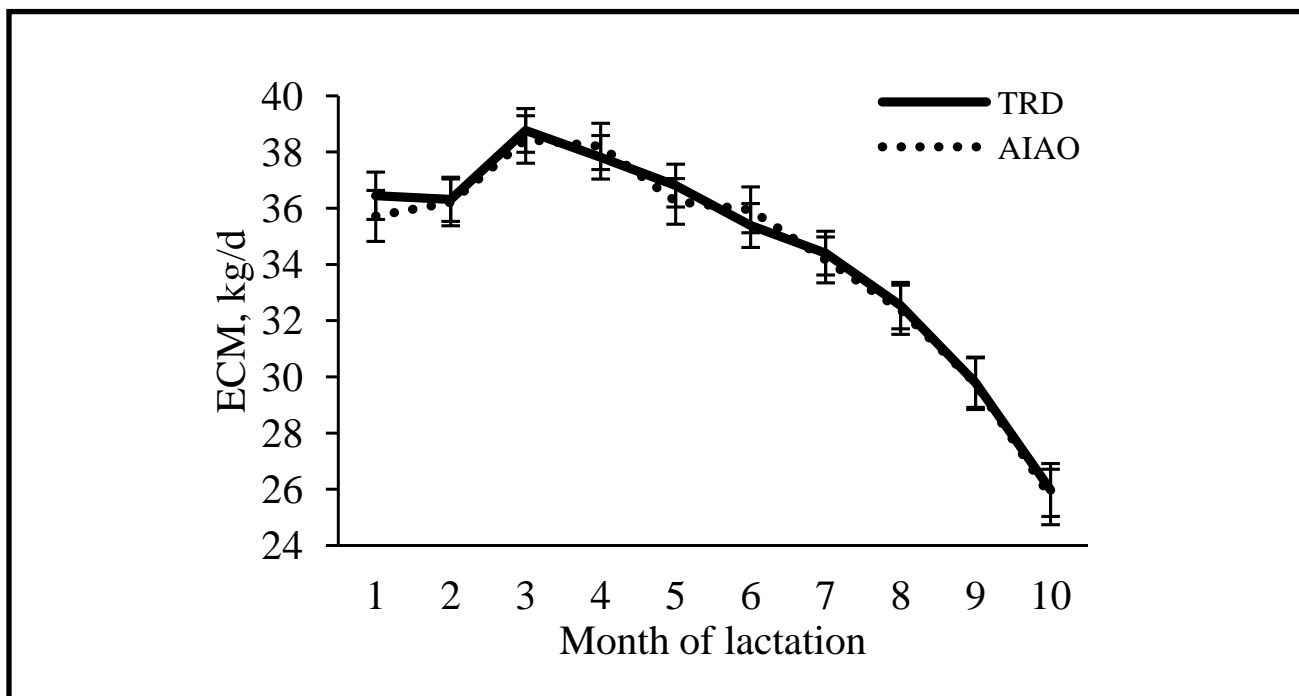


Figure 12. Yield of energy corrected milk (ECM) according to prepartum grouping strategy (TRD vs AIAO; TRD = traditional prepartum grouping strategy – weekly entry of new cows into the prepartum pen, and AIAO = All-In-All-Out prepartum grouping strategy – no entry of new cows in the prepartum pen) (Silva et al., 2013a).

Use of Milk Urea Nitrogen to Improve Nitrogen Efficiency and Reduce Environmental Impact of Dairy Cows

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The global human population is projected to increase from its current estimate of 7.1 to 9.4 billion by the year 2050 (U.S. Census Bureau, 2008). These projections are alarming since food production will have to increase between 40 and 65% (Hubert et al., 2010) and meeting such demand will be a challenge as arable land and other resources for food production are limited (Rockstrom et al., 2009; Hertel, 2011). Meeting this demand will require increased efficiency of production in all facets of the system. Additionally, gains in productivity cannot come at the expense of environmental health or the gains will not be sustainable. Nitrogen export to the environment can result in eutrophication of aquatic ecosystems; increased atmospheric particulates; decreased stratospheric ozone concentration; greenhouse warming; increased acidity of soil, precipitation, and surface water; coastal hypoxia; and methemoglobinemia in infants (Wolfe and Patz, 2002). The use of management tools, such as milk urea nitrogen (MUN), can help improve the efficiency of milk production, reduce feed costs, and reduce environmental problems associated with dairy production (Jonker et al., 2002b).

The Impact of Nitrogen Excretion on the Environment

Excess nitrogen fed to dairy cattle and other animals is excreted as urea in manure

(Lobley et al., 2000; Lapierre and Lobley, 2001; Reynolds and Kristensen, 2008), much of which is converted into ammonia and volatilized into the atmosphere (James et al., 1999; Li et al., 2009). Ammonia emissions to the atmosphere are a concern as they can form particles less than 2.5 microns in size (**PM_{2.5}**), which cause haze and contribute to lung and asthma problems in humans (WHO, 2005). Excess soil nitrogen can result in high levels of nitrate in drinking water or the leeching of nitrogen into surface water (Dinnes et al., 2002). Consumption of water with nitrates causes severe health problems in infants (methemoglobinemia), while nitrogen in surface water results in eutrophication and other serious environmental problems (Wolfe and Patz, 2002). Thus, the use of management practices that improve nitrogen efficiency of lactating dairy cattle may aid in the reduction of environmental and health risks.

Use of MUN to Achieve Optimum Return

High producing dairy cows have an overall average nitrogen efficiency between 25 and 28% (Jonker et al., 2002a; Hristov et al., 2004), which is less than half the post-absorptive efficiencies of precision-fed growing pigs and poultry (Baker, 1991, 1996; Nahm, 2002). Higher efficiencies can be achieved in poultry and pigs since they are fed diets that perfectly match their amino acid requirements (precision feeding). Unfortunately, we do not

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currently possess the same level of knowledge of amino acid requirements in ruminants. However, because nitrogen efficiency is related to blood and milk urea nitrogen concentrations in dairy cattle (Jonker et al., 1998), we can use MUN values as a management tool to monitor and improve nitrogen efficiency.

Milk urea is a product of nitrogen breakdown in the body and is highly correlated to dietary nitrogen and nitrogen balance of a cow (Oltner and Wiktorsson, 1983; Broderick and Clayton, 1997). Dietary protein is the major determinant of MUN concentrations (Jonker et al., 1998). If protein in the diet is deficient relative to the cow's requirements, MUN concentrations will be low. Conversely, if protein in the diet is in excess of the cow's requirements, MUN concentrations will be high (Figure 1). If this were the whole story, using MUN would be simple. Unfortunately, there are several additional factors that influence MUN concentrations, although they do not negate the relationship. These include time of milk sampling, season of the year, BW, DIM, breed, level of production, and other nutritional factors (DePeters and Cant, 1992; Broderick and Clayton, 1997; Kauffman and St-Pierre, 2001). Dehydration results in increased blood urea nitrogen and MUN (Weeth and Lesperance, 1965; Steiger Burgos et al., 2001), while high salt content in the diet causes reduced MUN (Spek et al., 2012).

Starch is commonly suggested as one of the nutritional factors controlling MUN, and indeed, it will alter MUN, but only if it impacts milk protein production (Kauffman and St-Pierre, 2001). If we take a hypothetical cow being fed 50 lb/day of DM with 17% CP and a moderate level of dietary starch, nitrogen intake will be 1.36 lb/day. If she is producing 80 lb/day of milk at 3.0% protein, she is secreting 2.4 lb/day of milk protein or 0.38 lb/day of milk

nitrogen. Much of the remaining 0.98 lb/day of nitrogen that was consumed but not converted to milk protein will be converted to urea and eventually excreted. If we increase the dietary starch content by addition of finely ground starch so that it is ruminally available, it very likely will stimulate microbial growth in the rumen (Aldrich et al., 1993), which will use more of the waste nitrogen generated in the rumen, and thus less ammonia will be absorbed and converted to urea by the cow. However, the resulting extra microbial protein flows to the small intestine, where it is mostly digested and absorbed as amino acids. If those amino acids are not used to make more milk protein, the cow will simply degrade them and convert the nitrogen to urea. So the defining event is an increase in milk protein production. In the absence of a milk protein production response, nothing has been gained and the blood and milk urea nitrogen contents will be the same for both levels of dietary starch.

The target level for MUN across herds is generally 12 mg/dl (Jonker et al., 1999). If MUN is greater than that, the herd is likely fed protein in excess of needs. If MUN is below 12 mg/dl, the herd may be experiencing a protein deficiency. However, there are differences among herds and among cows within a herd after all of the above factors have been considered, suggesting that the genetic makeup of the herd may play a role in determining herd and cow MUN concentrations (Aguilar et al., 2012). This is confirmed by the observation that MUN is genetically heritable (Wood et al., 2003). Thus, 2 hypothetical herds with the same breed and BW of cows fed the same diet, with the same milk production, and at the same stage of lactation could have different MUN concentrations. Therefore, reducing MUN concentrations below 12 mg/dl without losing milk production may not be possible for all herds. Conversely, some herds may be able

to achieve concentrations below 12 mg/dl and are wasting nitrogen at 12 mg/dl. To achieve maximum nitrogen efficiency and minimize ration costs, herds should establish their own specific targets for MUN.

Establishing a Herd Specific MUN Target

Use the following strategy to establish a target MUN concentration for your herd. If you have a one-group TMR, then it is quite straightforward. If you feed multiple rations to lactating cows, then it is a bit more tedious as the following process will have to be repeated for each group:

1. Balance the diet to just meet (NRC, 2001) requirements for energy, rumen degradable protein (**RDP**), metabolizable protein (**MP**), and sodium chloride, and ensure that the animals have adequate access to water. Feed the diet for 2 weeks and record the herd or group MUN value (1 bulk tank sample).
2. As dietary rumen undegradable protein (**RUP**) is generally more expensive than RDP, start with RUP, although either way works. Reduce RUP content by 0.25% units while holding energy, RDP, and salt content constant. Feed the diet for 2 weeks and record the ending milk production, DM intake, and MUN concentration.
3. Repeat step 2 until the cows decrease milk production or DM intake.
4. The step immediately before the cows lose milk production or DM intake is the requirement for RUP for your herd.
5. If there is a loss in production on the very first reduction in RUP, it is possible that the cows were already being fed a deficient diet. In this case, try adding 0.25% units of RUP to the first ration to see if you get an increase in milk production.
6. Once a herd specific RUP level is determined, repeat the same process for RDP content using 0.5% unit reductions while holding energy constant and RUP at the threshold level established above until a loss in milk production or DM intake is experienced.
7. The last RDP reduction step before a loss in milk production or DM intake was observed is your herd specific RDP requirement.
8. The final values for RDP, RUP, MP, and MUN are your herd's target levels.

Feeding to meet but not exceed your herd specific target RDP and RUP levels will result in the maximum achievable nitrogen efficiency under current feeding conditions and knowledge, and herd MUN values can be compared to target MUN concentrations to determine if the feeding program is staying on target. If MUN increases above the target, the cows are being fed more protein than needed and nitrogen efficiency has declined. If MUN drops below the target, it is likely that a loss in milk production has occurred or will in the near future, and corrective measures should be taken. In either case, MUN does not provide information regarding the source of the problem. It simply indicates that the animals are deficient in nitrogen or have an excess of nitrogen, and you will have to determine whether it is a problem with RDP, RUP, other dietary factors, feed formulation, or animal health. It is also important to recognize that all of the safety margin associated with overfeeding protein has been removed, and thus managing the feeding program to maintain consistency is critical to avoid a loss in production. Silages should be frequently assessed for DM content, and nutrient analyses of dietary ingredients

should be monitored to maintain diet consistency. The frequency of nutrient analyses assessment is a function of herd size and cost of the analyses. St-Pierre and Cobanov (2007) provide suggested monitoring frequencies for herds of varying size at different analytical costs that can be used to establish a monitoring program.

The target MUN value should be valid for several years unless you dramatically change your facilities, import different cattle, change salt feeding practices, or water availability becomes restricted. However, keep in mind that the diet required to obtain the target MUN value may change across several years. Eventually, it may drift due to genetic selection in your herd and should probably be reassessed in 5 years. Thus, you can monitor your herd's milk urea nitrogen to keep a handle on your nitrogen feeding program and improve animal nitrogen efficiency, while simultaneously reducing feeding costs and nitrogen excretion to the environment.

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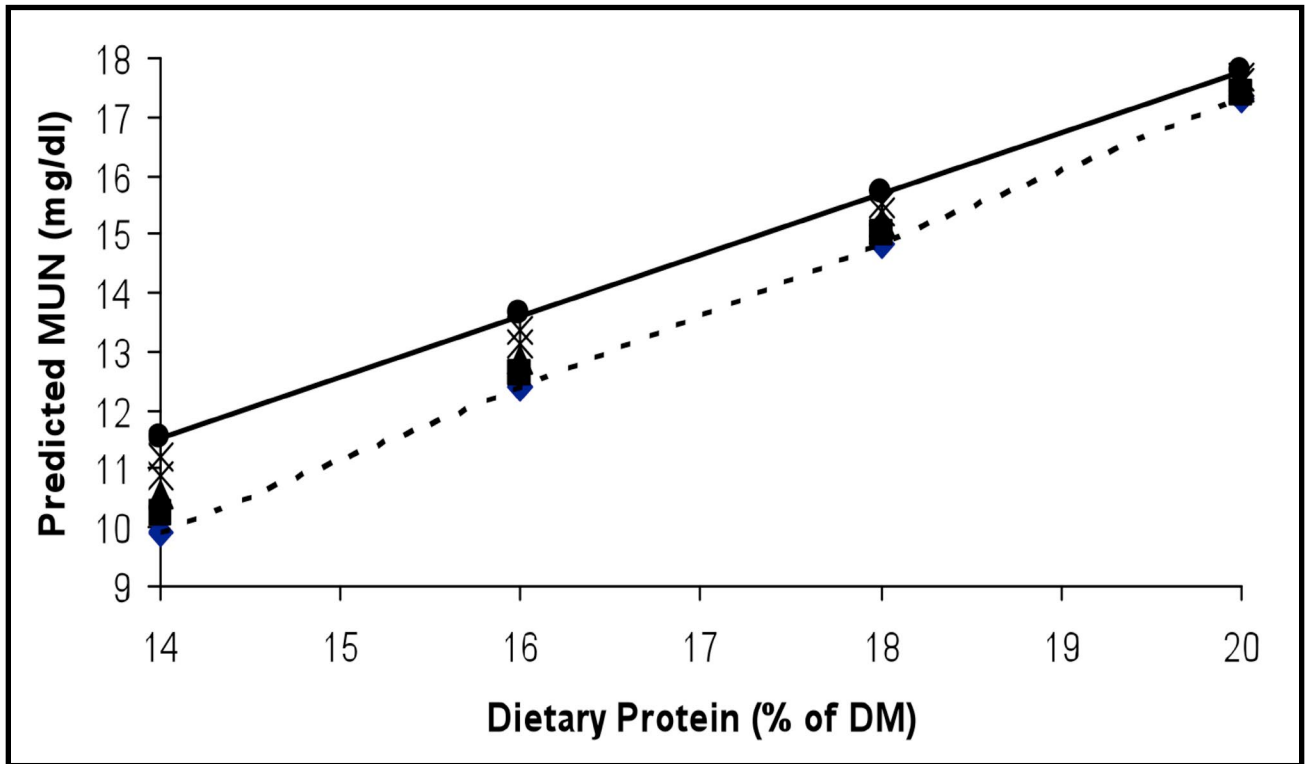


Figure 1. Least squares mean estimates for MUN versus dietary CP predicted from a statistical model with varying milk yield and the observed mean inputs for milk protein content, dietary NDF content, and days in milk set to mean values for the study [\bullet = 6.6 lb/day milk, \blacksquare = 70.4 lb/day milk, \blacktriangle = 74.8 lb/day milk, \times = 79.2 lb/day milk, $*$ = 83.6 lb/day milk, \bullet = 88.0 lb/day milk, solid line = 88.0 lb/day milk regression ($y = 1.04 \text{ CP} - 3.0$), dashed line = 66.0 lb/day milk regression ($y = 1.23 \text{ CP} - 7.34$); Aguilar et al., 2012].

Understanding the Effects of Drought Stress on Corn Silage Yield and Quality

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Summary

Uncontrolled environmental factors can affect DM yield and composition of corn whole-plant for silage. The spring and summer drought of 2012 reduced corn yields substantially when compared to 2011. In Virginia, drought stress affected DM yields and composition differently, depending on the region. The extremely low DM yield observed for the Southern Piedmont region in 2012 (2.0 ton DM/acre) could be attributed to the severe drought suffered that year. However, from the perspective of water status, the Southern Piedmont region had similar water status at the same phenological state than the Shenandoah Valley region, suggesting that factors other than drought stress also affected DM yield in the Southern Piedmont in 2012. Analysis of maximum temperatures showed that heat stress had a major effect on kernel development in the Southern Piedmont but not in the Shenandoah Valley. Therefore, in the Southern Piedmont region, heat stress exacerbated the effects of drought, reducing substantially DM yields and kernel development. Crop management practices, such as hybrid selection and planting date, should be considered to avoid high temperature stress during silking and kernel development.

Introduction

Whole-plant corn silage is a major ingredient in diets for dairy cattle. Therefore, producing high yielding and good quality forage is critical for minimizing production costs in dairy farming systems. Different management practices or genotype selections can affect yield and quality of corn whole-plant for silage. Whole-plant DM yields can be increased with higher planting densities (Cusicanqui and Lauer, 1999; Ferreira et al., 2014) or nitrogen fertilization rates (Roth et al., 2013). Increasing corn plant density likely increases fiber concentration and decreases in vitro DM digestibility of corn whole-plant (Cusicanqui and Lauer, 1999) due to a lower grain to stover ratio (Roth et al., 2013). Delaying harvesting time also increases DM yields and reduces fiber concentration of corn whole-plant (Bal et al., 1997; Ma et al., 2006), although nutrient utilization can be diminished if kernel processors are not utilized when chopping at late maturity stages (Ferreira and Mertens, 2006). Increasing cutting height at harvesting reduces fiber and lignin concentrations of corn whole-plant (Kung et al., 2008), although this reduces DM yields by 7.4 to 16.7% (Wu and Roth, 2003; Kung et al., 2008). With regard to genotype selection, planting corn hybrids with the brown midrib 3 mutation results in whole-plant corn silages with greater in vitro NDF digestibility (Oba and Allen, 2000; Taylor and Allen, 2005), although

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DM yield is typically inferior for these hybrids (Lee and Brewbaker, 1984; Bal et al., 2000).

Despite these multiple controlled factors, uncontrolled environmental factors can affect DM yield and composition of corn whole-plant for silage (NeSmith and Ritchie, 1992; Çakir, 2004; Ferreira et al. 2014). This paper will discuss how abiotic stresses affect yield and composition of corn for silage.

“2012...One of the Worst Agricultural Calamities in the U.S.”

The spring and summer drought of 2012 will be remembered as one of the “worst agricultural calamities” in the United States (USDA, 2013). The drought of 2012 reduced the national corn grain and silage yields by 16.2 and 16.3%, respectively, when compared to 2011 (USDA, 2013).

Corn hybrid performance trials completed at different locations across the state of Virginia (Behl et al., 2011; Behl et al., 2012) showed that climate affected DM yields differently. Indeed, whole-plant DM yields from the same corn hybrids ranged from 1.9 to 8.0 ton/acre in 2012 and from 5.1 to 8.1 ton/acre in 2011 (Table 1). Based on rainfalls (Table 2), we would have not expected the second lowest DM yield (5.6 ton/acre) in the Southern Piedmont region for 2011, the site-year with the greatest amount of rainfalls (Table 2). Rainfalls in the Shenandoah Valley region were not abundant in either year. Therefore, lower DM yield in the Southern Piedmont region may reflect inferior soil quality or fertility compared to the Shenandoah Valley region. The Virginia Agricultural Land Use Evaluation System (Donohue et al., 1994) recognizes this fact and estimates the yield potential of the soil at the Shenandoah Valley region to approximately 15% higher than the yield potential at the Southern

Piedmont. Therefore, the extremely low DM yield observed for the Southern Piedmont region in 2012 (2.0 ton/acre) should be attributed to the severe drought suffered that year. However, precipitations in the Shenandoah Valley region were not much more abundant than for the Southern Piedmont region that year [262 and 228 mm (10.5 and 9.1 inches), respectively; Table 2). This observation suggests that factors other than drought stress also affected DM yield in the Southern Piedmont region in 2012.

Corn Composition

Dry matter concentration of the corn silage varied substantially among site-years (Table 3). The high variation for DM concentration among site-years is attributed to the low DM concentration (25.3%) observed for the Southern Piedmont region in 2012, likely due to the a reduced proportion of grain component in the whole plant. Similarly to DM concentration, CP concentration varied substantially among site-years (Table 3). The high variation for CP concentration among site-years is attributed to the high CP concentration (10.9% CP) observed for the Southern Piedmont region in 2012. In agreement with the observed DM concentration, a greater proportion of vegetative tissues in the whole plant, due to a reduced grain component, can explain the observed high concentration of CP for the Southern Piedmont region in 2012.

Neutral detergent fiber also varied substantially among site-years (Table 3). The NDF concentration in 2012 was substantially lower for the Shenandoah Valley region (43.0%) than for the Southern Piedmont region (56.6%), indicating that corn crops were affected differently despite summer drought. Fiber concentration in whole-plant corn silage is highly and negatively correlated to starch concentration (Ferreira and Mertens, 2005). Unfortunately, starch concentrations were not

reported in these hybrid tests, but it is likely that kernel development explains the difference in NDF concentrations between these regions for 2012. An inferior kernel development for the Southern Piedmont region during 2012 is also supported by the low DM concentration (25.3%) and the relatively high CP concentration (10.9%) of the whole-plant (Table 3).

Timing of Rainfalls

After obtaining climate data, cumulated rainfalls were plotted against growing-degree days (Figure 1). Surprisingly, the Southern Piedmont region had greater cumulative rainfalls than the Shenandoah Valley region for the same stage of development of the crop. From the perspective of water status, these observations suggest that the Southern Piedmont site had similar water status at similar fenological state than the Shenandoah Valley site. These observations suggest that differences in NDF concentration between the Southern Piedmont and Shenandoah Valley regions should be attributed to factors beyond water status.

Heat Stress and Kernel Development

Heat stress during kernel development can greatly affect corn grain yield (Hanft and Jones, 1986; Cheikh and Jones, 1994). Kernel development is divided by a lag phase with little kernel growth and a linear growing phase with major accumulation of DM. The lag phase, which starts immediately after pollination and lasts 10 to 12 days after pollination, is critical for kernel development (Cheikh and Jones, 1994). The endosperm is the structure of the corn kernel that contains starch granules. Cell division of the endosperm cells during the lag phase determines the capacity of the endosperm to accumulate starch within the grain (Cheikh and Jones, 1994). Cheikh and Jones (1994) cultured corn kernels *in vitro* at different temperatures and observed

that heat stressed kernels [i.e., kernels cultured at 35°C (95°F)] accumulated 18 to 75% less DM than non-stressed kernels [i.e., kernels cultured at 25°C (77°F)]. Reduced DM accumulation can be related to reductions in starch synthesis within the endosperm when kernels are subjected to temperatures greater than 35°C (95°F) (Hanft and Jones, 1986). In addition to reduced kernel growth, Cheikh and Jones (1994) reported 23 to 97% kernel abortion when subjected to heat stress.

The date at which pollination occurred was estimated (Figure 2) under the assumption that silking occurred at 1400 growing-degree days (Neild and Newman, 1987). In 2011, maximum temperatures were below 35°C (95°F) throughout the whole critical period of kernel development for the Southern Piedmont region (Figure 2A). In the Shenandoah Valley region, maximum temperatures were above 35°C (95°F) for only a few days during the critical period of kernel development (Figure 2B). Based on these observations, heat stress would have not affected kernel development. In 2012, however, the Southern Piedmont region had maximum daily temperatures above 35°C (95°F) for an extended period (11 days) right after silking (Figure 2C), whereas maximum daily temperatures were $7.1 \pm 2.3^\circ\text{C}$ lower in the Shenandoah Valley region around silking (Figure 2D). It is therefore likely that heat stress had a major effect on kernel development in the Southern Piedmont region but not in the Shenandoah Valley region. Therefore, in the Southern Piedmont region, heat stress exacerbated the effects of drought, reducing substantially DM yields and kernel development.

Implications

The observations from this study have major practical implications. In the first instance, heat stress may affect the nutritional composition

of corn silage, even in crops with adequate water status. Similar to the data reported in this study, Ferreira (unpublished data) observed concentrations of 28.1% DM, 11.6% CP, and 59.9% NDF for corn silage originated from an irrigated corn field suffering heat stress immediately after pollination, suggesting that silage quality is not ensured exclusively by water status.

Dairy farmers, agronomists, and dairy consultants should also not overlook the regional temperatures when planning a strategy to ensure forage stocks for dairy farms. In regions with high summer temperatures, choosing early maturity corn hybrids or delaying planting date should be considered to avoid high temperature stress during silking and kernel development. With regard to harvesting management, monitoring daily temperatures might help to better decide whether harvesting and chopping should be anticipated when drought occurs. High temperatures around pollination might be considered as an indicator that silage yield or quality would not increase or improve substantially after a relieving rain.

Finally, planting alternative forages, such as Sorghum species, should also be considered to minimize the risk associated to growing corn in regions with high summer temperatures (Aydin et al., 1999; Amer et al., 2011). Sorghum species are characterized for having greater resistance to drought stress than corn. Compared to corn, Sorghum species usually require a delayed planting date, therefore escaping the high summer temperatures during kernel development.

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Table 1. Dry matter yield (ton/acre) of silage from 8 corn hybrids tested at the Southern Piedmont and Shenandoah Valley regions in the State of Virginia.

Hybrid	Southern Piedmont		Shenandoah Valley	
	2011	2012	2011	2012
A	5.5	2.3	7.6	6.1
B	5.9	2.1	5.1	8.0
C	5.5	2.0	6.6	7.7
D	5.9	1.9	7.2	5.9
E	5.6	2.2	5.9	5.2
F	5.9	2.0	8.1	4.2
G	5.1	1.9	7.0	5.2

Table 2. Planting and silage harvesting dates, and rainfalls of experimental corn plots at the Southern Piedmont and Shenandoah Valley regions in the State of Virginia during 2011 and 2012.

	Southern Piedmont		Shenandoah Valley	
	2011	2012	2011	2012
Planting date	April 18	April 10	May 6	May 21
Harvesting date	August 31	July 17	August 24	September 12
Growing period, days	136	119	111	125
Rainfalls, mm ¹	501	228	280	262
April	12.7	71.9	0	0
May	103.4	65.8	86.1	61.2
June	92.2	27.2	82.6	37.1
July	138.9	62.7	34.0	65.8
August	153.7	0	77.7	78.5
September	0	0	0	19.8

¹1 mm = 0.04 inches.

Table 3. Composition of 8 corn hybrids harvested as silage and tested at the Southern Piedmont and Shenandoah Valley regions in the State of Virginia during 2011 and 2012.

Hybrid	Dry Matter, %				Crude Protein, % of DM				Neutral Detergent Fiber, % of DM			
	Southern Piedmont		Shenandoah Valley		Southern Piedmont		Shenandoah Valley		Southern Piedmont		Shenandoah Valley	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
A	39.6	28.2	32.2	37.4	8.6	10.3	8.0	7.4	51.2	58.4	52.8	42.3
B	34.8	26.9	33.5	34.5	8.8	10.5	8.1	7.5	49.9	55.7	50.5	44.9
C	33.1	23.8	30.2	34.2	8.6	11.5	7.9	6.7	52.5	55.4	54.7	41.8
D	38.7	24.9	31.4	28.2	8.2	10.7	7.2	7.2	47.6	58.8	55.5	42.6
E	34.2	21.1	30.6	28.1	9.6	11.5	7.8	6.9	50.1	55.6	54.5	45.3
F	40.5	27.5	36.1	48.8	8.4	10.9	7.7	6.9	57.7	55.9	51.4	40.3
G	38.2	27.4	35.3	39.7	8.4	10.2	7.0	6.8	51.4	57.6	50.6	42.4
H	36.8	22.5	31.1	32.4	9.1	11.4	8.0	7.2	51.2	55.5	52.1	44.5
Average	37.0	25.3	32.6	35.4	8.7	10.9	7.7	7.1	51.5	56.6	52.8	43.0

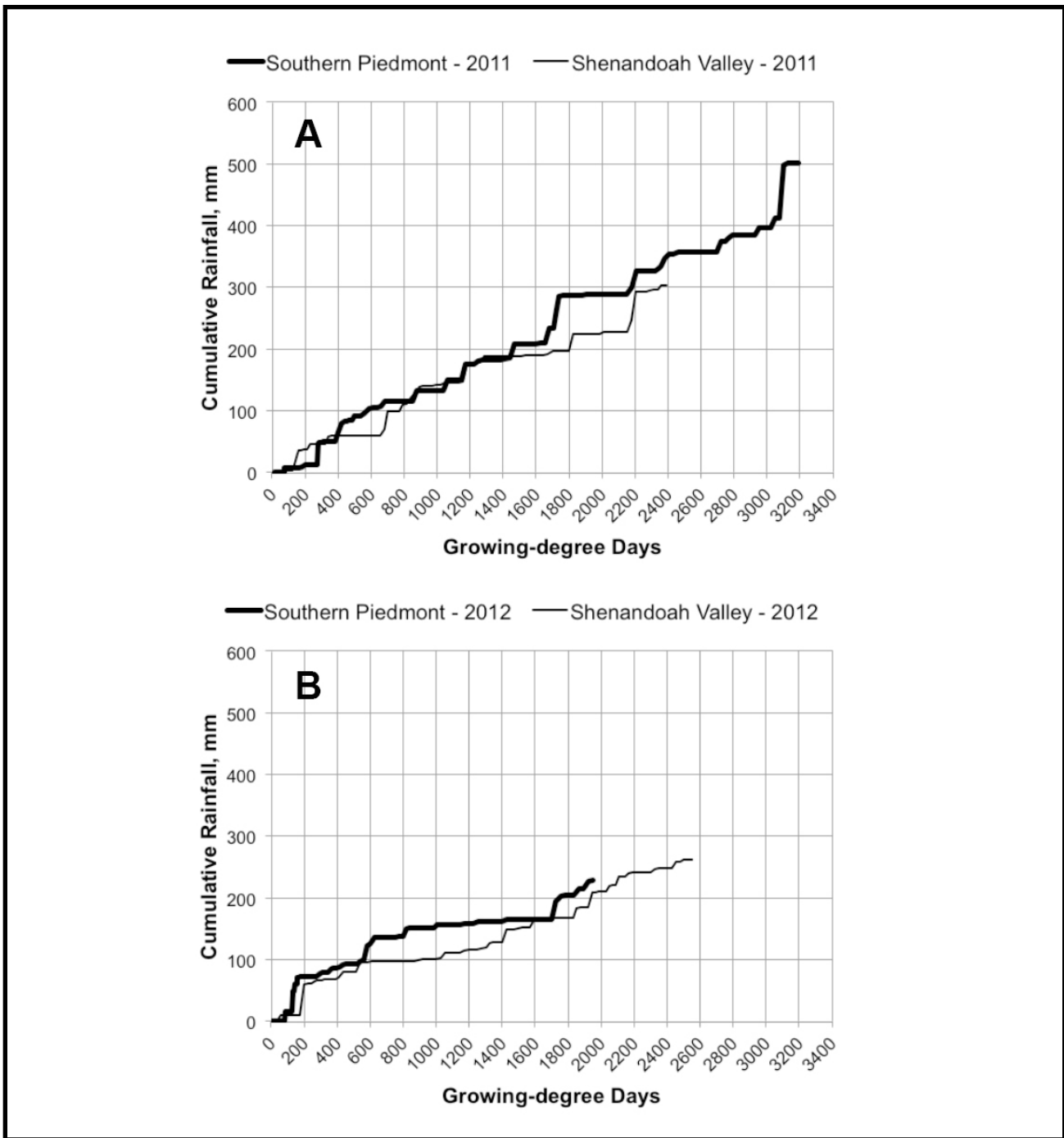


Figure 1. Cumulative rainfalls (1 mm = 0.04 inches) at different growing-degree days of corn crops grown at 2 regions during 2011 (A) and 2012 (B) in the State of Virginia. Thick and thin lines represent the cumulative precipitations for the Southern Piedmont and Shenandoah Valley regions, respectively.

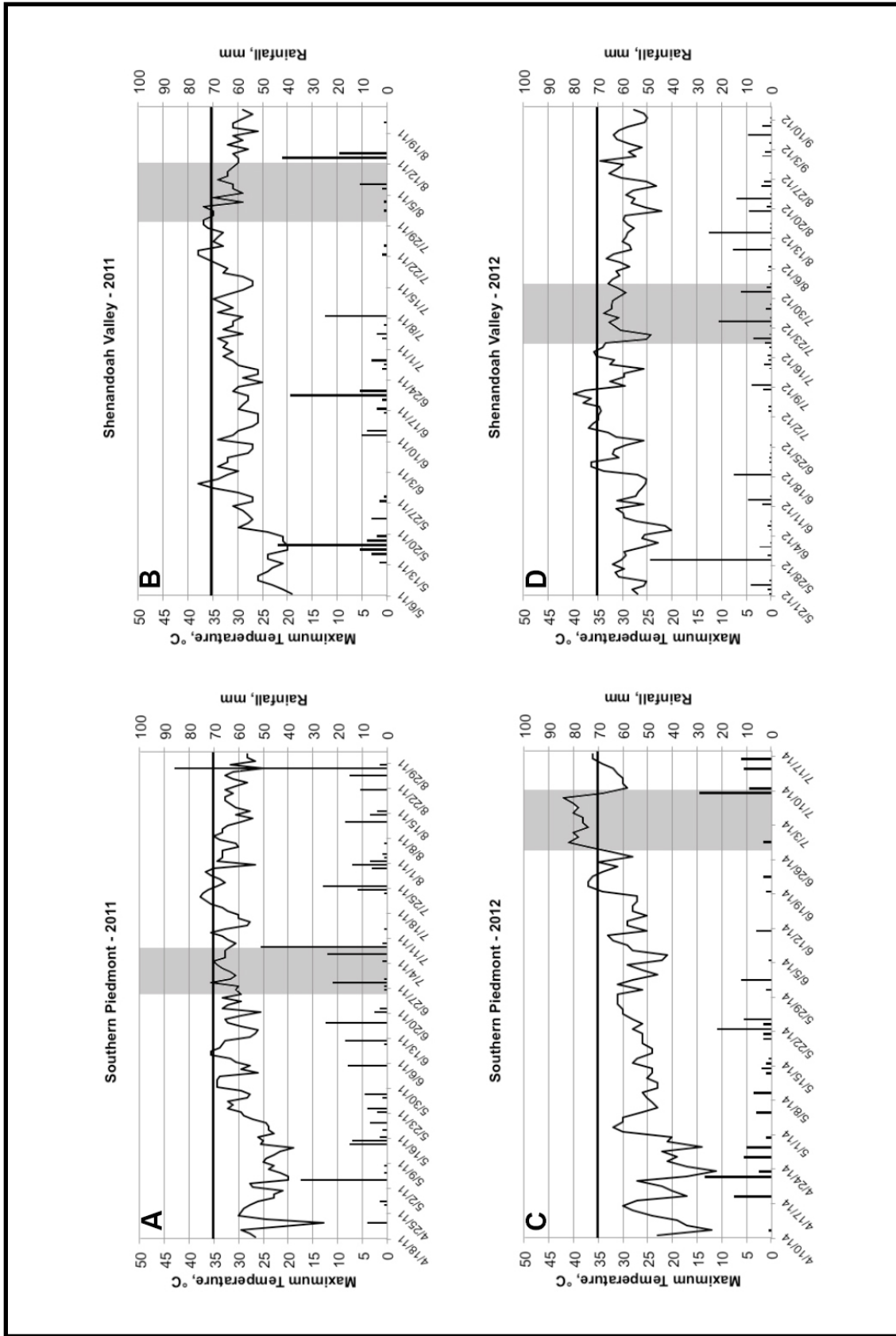


Figure 2. Daily maximum temperatures (line) and rainfalls (columns) during the crop cycle at 2 regions during 2011 and 2012 in the state of Virginia. The shaded region represents the critical stage for kernel development. The thick horizontal line represents the threshold temperature for heat stress (>35°C; 95°F). Prolonged heat stress after silking occurred only in the Southern Piedmont region during 2012 but not in other site-years.



Relationship of NDF Digestibility to Animal Performance

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Summary

In order to optimize the utilization of forages, an accurate laboratory measure of fiber digestibility is essential. The measure should mimic in vivo digestion and should be consistent across forage types. A new in vitro lab assay has been developed that predicts total tract NDF digestion (**TTNDFD**) in ruminants. The test is based on a patented and licensed in vitro assay and model of fiber digestion. The in vitro TTNDFD assay is available through commercial labs and has been calibrated to NIR analysis. The TTNDFD model predicts fiber digestion of alfalfa, corn silage, and grass forages in cattle and has been validated against directly measured NDF digestibility in lactating dairy cattle.

Introduction

The digestibility of NDF is more variable than the digestibility of any other feed component and can profoundly affect intake and milk production. In high producing dairy cows, the variation in total tract fiber digestion can account for enough energy to support as much as 8 to 10 lb of potential milk yield. Fiber digestion is affected both by characteristics of the plant material and by the animal consuming the fiber. To accurately predict how fiber will be utilized, laboratory measures that predict the rate of fiber digestion and the proportion

of total fiber that is potentially digestible are needed. The rate and potential extent of NDF digestion are heavily influenced by the genetics and growing environment of the plant. Fiber digestion is also affected by the rate of passage of the potentially digestible fiber through the animal's rumen and hindgut, and therefore, prediction of fiber utilization must also account for animal factors.

Predicting Fiber Digestion with Laboratory Tests and Modeling

There are at least 4 critical factors that affect fiber digestion and performance in ruminants:

1. *The proportion of feed fiber that is potentially digestible.* Forage NDF consists of 2 components, potentially digestible (**pdNDF**) and indigestible NDF (**iNDF**). The proportion of NDF in the pdNDF fraction varies due to feed type and the growing environment. On average, the pdNDF fraction of alfalfa is about 60 to 65% of total NDF. The proportion of potentially digestible fiber in corn silage is typically greater than in alfalfa NDF; 75 to 85% of corn silage NDF is potentially digestible. The proportion of NDF that is indigestible is typically estimated from long term incubations of fiber in the rumens of cattle or long term in vitro digestions.

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The NDF residue remaining after 240 h of incubation (uNDF_{240}), for example, is often used as an estimate of iNDF. The pdNDF is typically determined by subtracting the uNDF fraction from total NDF. The iNDF proportion can only be cleared from the digestive tract by passage while the pdNDF fraction disappears by passage and microbial digestion. Since fiber is bulky and one of the slowest digesting components of the diet, clearance of fiber from the rumen is an important factor limiting feed intake.

2. *The rate of digestion of potentially digestible fiber (kd).* The rate of fiber digestion also differs due to forage type and the growing environment. The potentially digestible fiber in alfalfa digests nearly twice as fast (4 to 6%/hour) as the potentially digestible NDF in corn silage (2 to 3%/hour). Even though fiber digestion rates for forages are slow, differences in rate of fiber digestion have a big impact on how much of the potentially digestible fiber will digest. The total-tract NDF digestibility of alfalfa and corn silage are similar, but the process of NDF digestion is quite different. In corn silage, there is a larger fraction of digestible fiber that digests slowly. In alfalfa, there is a smaller proportion of digestible fiber, but the faster rate of digestion of the potentially digestible fraction compensates for the bigger pool of iNDF.
3. *The rate of passage of potentially digestible NDF through the cow (kp).* Both cow size and feed intake affect the passage rates of pdNDF and iNDF. Passage of fiber is much slower than the passage of forage DM. The passage rates of iNDF and pdNDF are not the same. Passage of the pdNDF fraction is slower than passage of the iNDF fraction (Lund et al., 2007). As intake goes up, the rate of passage of both fractions also increases, and as a result, NDF digestibility declines.
4. *Ruminal and hindgut fiber digestion.* Approximately 90 to 95% of fiber digestion occurs in the rumen (Huhtanen et al., 2010), but digestion beyond the rumen must be accounted for if one is to accurately predict the amount of energy derived from NDF. When both ruminal and hindgut digestion are accounted for, a total-tract NDF digestion (TTNDFD) measurement can be calculated and this digestion coefficient can be directly validated with dairy cattle.

An accurate assessment of fiber digestion requires that the 4 factors be integrated into a single measurement. The rumen fiber digestion process can be described mathematically as:

$$\text{Proportion of fiber digested} = \text{pdNDF} \times \left(\frac{\text{kd}}{\text{kd} + \text{kp}} \right);$$

where, pdNDF is the fraction or amount of potentially digestible NDF, kd is the rate of digestion of potentially digestible fiber, and kp is the rate of passage of potentially digestible NDF (Mertens, 1993). One way of accounting for hindgut digestion is to divide the proportion of NDF digested in the rumen by the proportion of total fiber digested in the hindgut.

Challenges with Assessing Forage Quality with uNDF_{240} , kd, NDFD30, or NDFD48

Nutritionists currently use many different tests to assess fiber digestibility or to compare forages. Using assays that predict iNDF (such as uNDF_{240}), or in vitro digestion of fiber after a fixed time (NDFD30 or NDFD48), as stand-alone measures of forage quality have limitations. Using only the pdNDF (or inversely the uNDF_{240}) value or kd value is not an accurate assessment of fiber quality because forages can differ in both pdNDF content and kd. A simple analogy demonstrates this point. Fiber quality is an estimate of the

amount of digestible energy generated from a given quantity of forage NDF and is somewhat analogous to predicting how far you can drive a car before it runs out of gas. You need to know how much gas is in the tank and the fuel efficiency of the car to predict the distance that the car will travel. Forage quality is conceptually similar. The amount of digestible energy from fiber (i.e., how far you can drive a car) depends on the amount of fiber that is digestible (i.e., the amount of gas) and the efficiency of fiber digestion (i.e., the fuel efficiency). Knowing you have 10 gallons of fuel may be somewhat useful, but you can't accurately determine how far you can go unless you also know the fuel efficiency. The driver would also have some bearing on the distance traveled. If the driver has a 'lead foot', the distance traveled will be less than for someone who is a more conservative driver. This is a bit like the effect of rate of passage on fiber digestion. Integrating pdNDF, kd, and kp into a single term is a more comprehensive measure of fiber quality than any of the individual terms that are used to determine fiber utilization.

In vitro NDF digestibility measured after 30 h (NDFD30) or 48 h (NDFD48) is widely used to index forage fiber digestibility. Oba and Allen (1999) reviewed several feeding studies with dairy cattle and concluded that a 1% change in vitro or in situ NDF digestibility (NDFD30 or NDFD48) was correlated with a 0.37 lb increase in voluntary DMI, and 0.55 lb increase in 4% fat corrected milk yield. The change in situ or in vitro fiber digestibility within a study was correlated with intake and milk production, but there was no significant correlation between the absolute measures of fiber digestion and intake or milk yield across studies. For field nutritionists, this suggests that in vitro methods differ enough from lab to lab to make it impractical to compare results between labs.

There is also another challenge with using values like NDFD30 to assess forage quality. The NDF residue remaining after a given time in a flask of rumen fluid is simply *undigested* NDF. That residue consists of truly *indigestible* NDF and the portion of the *potentially digestible* NDF that has not yet been digested. There is no way of knowing or estimating the rate of fiber digestion or the fraction of indigestible NDF from this measurement alone. If we go back to the car and gas analogy, the NDFD30 value is like reading the gas gauge of the car. The gauge may indicate a half tank of fuel, but this doesn't tell you how big the tank is or the fuel efficiency of the car and so you can't accurately determine how far you can drive. The iNDF fractions and rates of fiber degradation can vary considerably within forage type. In forages measured in our lab, the iNDF fractions in alfalfa and grasses vary from less than 5% to over 55% of NDF, while corn silage iNDF values range from less than 10% to over 40% of NDF (unpublished data). Krizsan et al. (2010) reported that iNDF values in a database of 172 feeds ranged from 2.4 to 17.4% of feed DM. In addition, the estimated rates of degradation of pdNDF vary from about 1 to over 10%/hour when measured by using multiple incubation time points and fitting the disappearance of pdNDF to first order kinetics.

***In vivo* Measurement of Fiber Digestion**

Total tract apparent NDF digestibility values for diets fed to dairy cows are readily available and are a valuable tool for field nutritionists. Goeser (2008) summarized total tract NDF digestibility measurements that were reported from 25 corn silage feeding trials (81 treatment comparisons) and in 20 trials in which legumes and grasses (64 treatment comparisons) were the primary forages fed to high producing ruminants. Summary statistics suggest that in vivo NDF digestibility coefficients can vary by 30 to 35% units among legumes, grasses, and

corn silages. The TTNDFD of corn silage based diets, for example, average about 42% of NDF but range from 20 to nearly 60% of NDF. A more recent survey of corn silage based feeding trials (Ferraretto and Shaver, 2012) reported that the treatment means for TTNDFD averaged 44.3 ± 2.5 % in 106 treatment observations from 24 dairy feeding trials that were published in peer-reviewed journals between 2001 and 2011. Diets for high producing dairy cows are typically formulated to contain between 28 and 35% total NDF. For cows that are expected to produce over 90 lb/day, a 30-unit change in TTNDFD is equivalent to the digestible energy needed to support more than 10 lb of milk production.

Measuring the Fiber Digestion Process *in vivo* with the Rumen Evacuation Method

Measuring the process of ruminal and hindgut fiber digestion *in vivo* is laborious and expensive, but it is the 'gold standard' to which other estimates of fiber digestion should be compared. Comprehensive evaluations of *in vivo* fiber digestion are most commonly measured by the 'rumen evacuation' technique (Huhtanen et al., 1997; Ivan et al., 2005; Taylor and Allen, 2005). With this method, the critical dynamic components that contribute to the digestion of fiber are directly measured in rumen-cannulated animals. Rumen pools of digestible and indigestible fiber are measured by total rumen evacuation. Rates of digestion of potentially digestible NDF and rates of passage of pdNDF and indigestible NDF are also measured as well as total tract NDF digestion.

Despite the cost and labor, a large number of rumen evacuation studies have been published from studies done in the US and Northern Europe with dairy cattle. Krizsan et al. (2010) compared ruminal passage rates of iNDF as measured by the rumen evacuation

technique to empirical estimates of particulate passage rate in cattle. Their database included 49 studies in which 172 treatment means were measured. From this database, they published predictive equations for passage of iNDF in lactating cow fed diets based on corn silage, grass silage, alfalfa, and pasture-based grass diets. Huhtanen et al., (2010) also published a meta-analysis of the NDF digestion process using the rumen evacuation method. Thirty-two studies and 122 diets were included in this analysis. Most of the published studies are with lactating dairy cattle fed grass, alfalfa, or corn silage based diets. The fiber digestion module of the recently published Nordic Feed Evaluation system (NorFor) is based on fiber kinetic parameters estimated by the rumen evacuation technique (NorFor, 2011).

The rates of pdNDF degradation of diets when measured by the rumen evacuation method typically range from approximately 2 to 6%/hour (Greenfield et al., 2001; Ivan et al., 2005; Taylor and Allen, 2005; and Volker Linton and Allen, 2008). Corn silage based diets typically have slower rates of pdNDF degradation than alfalfa. The NDF in diets based on temperate grasses tends to have a similar proportion of pdNDF as corn silage, but grass fiber degrades faster than corn silage fiber, and slower than alfalfa fiber.

The University of Wisconsin *in vitro* TTNDFD Assay

University of Wisconsin researchers have recently developed an *in vitro* lab assay and model for predicting NDF digestion in dairy cattle that can be used by field nutritionists. The outcome is TTNDFD. The TTNDFD value is benchmarked to fiber digestibility values that have been obtained from feeding studies where NDF digestion has been directly measured. Total tract fiber digestibility is reported because

this value can be used not only to predict *in vivo* fiber utilization but also to predict forage digestible energy (DE), net energy (NE), or total digestible nutrient (TDN) values.

The TTNDFD assay accounts for pdNDF, kd, kp, and hindgut digestion of NDF (Figure 1). Measurement of the pdNDF fraction and the kd of pdNDF are based on a modified Goering and Van Soest (1970) *in vitro* procedure (Goesser and Combs, 2009). The pdNDF fraction is estimated from long term (120 or 240 h) *in vitro* incubations. Multiple measurements of *in vitro* NDF digestibility are used to calculate a rate of ruminal pdNDF digestion. The approach accounts for ruminal and post-ruminal fiber digestion and can be adjusted for changes in fiber passage as size or intake of the animal changes. Rates of fiber passage are estimated from regressions that have been derived from *in vivo* studies (Lund et al., 2007; Krizsan et al., 2010). In this model, the diet TTNDFD can be calculated by summing the amount of digestible fiber provided from each feed. The *in vitro* method has been calibrated to near infrared spectroscopy (NIR) so that kd and iNDF fractions in a feed can be predicted quickly and with little additional cost.

Several feeding studies have been conducted with various forages to test the model and to validate that the estimates of digestion and passage that are used in the model are consistent with what is measured in cattle fed diets containing the test forages (Verbeten et al., 2011; Lopes et al., 2013; Lopes et al., 2015a; Lopes et al., 2015b). In addition, our lab group has been monitoring commercial lab derived TTNDFD for corn silages, alfalfa, and grass forages and comparing these values to the digestibility coefficients for the respective forages that have been published in peer-reviewed feeding studies.

Field Observations with TTNDFD

We have been monitoring the TTNDFD values of corn silages, alfalfa, and grasses that have been submitted to a commercial forage-testing lab for routine analysis. The TTNDFD values for corn silage, alfalfa, and grasses are summarized in Table 1. The average values represent over 7000 samples each of corn silage or alfalfa and over 1200 grass forage samples.

The means, standard deviations (SD), and ranges in TTNDFD values coincide with *in vivo* measures of TTNDFD that have been reported in dozens of controlled feeding studies published in peer reviewed journals. For consultants, we recommend that tested forages be compared these mean TTNDFD values. When comparing 2 forages with similar total NDF, a forage that is more than one SD below the mean TTNDFD value would be among the lowest 15% of forages sampled and a 6 to 7 unit difference from the mean TTNDFD value would indicate that their forage fiber would reduce the DE value of the forage by enough to reduce potential milk yield by 2 to 3 lb. A forage which is one SD above the mean TTNDFD value would be higher in fiber digestibility than 85% of the forages tested and would contain enough additional DE to potentially support 2 to 3 lb more milk production. Experiences with this test in the field suggests that diets that incorporate large amounts of low TTNDFD forage support less milk and cows consume less feed DM than expected. Cows fed these types of diets respond well to additions of extra starch, or addition of sources of more highly digestible fiber, such as soy hulls.

Validation with Controlled Feeding Studies

The laboratory prediction of TTNDFD of forages and diets has been validated to fiber digestibility values that have been directly

measured in feeding studies. One study (Lopes et al., 2015a) was designed to compare estimates of ruminal fiber digestion predicted from *in vitro* NDFD analysis of feeds to the ruminal fiber digestion measured in cattle fed the same feeds. The feeding study was conducted with lactating dairy cows fed either low fiber digestibility corn silage or to higher fiber digestibility corn silage as the main source of dietary NDF (Table 2). The fiber characteristics of the low fiber digestibility corn silage (34.4% NDF, pdNDF 58.6% of NDF, and kd 3.2%/h) and the higher fiber digestibility corn silage (38.4% NDF, pdNDF 74.3% of NDF, and kd 3.3%/h) were determined by our *in vitro* TTNDFD method prior to the feeding experiment. The fiber characteristics of the 2 silages and the other feeds used in the diets were then used to predict TTNDFD digestibility of the treatment rations. The predictions for each diet were then compared to the observed measures of fiber digestion in dairy cows fed the same feeds. The *in vitro* method predicted that the higher fiber digestibility corn silage was higher in TTNDFD than the low fiber digestibility corn silage because it contained a larger proportion of potentially digestible NDF. Rates of pdNDF digestion and passage and the measured pool of pdNDF in the rumens of cows fed the experimental diets were directly measured in cows and compared to the fiber digestion parameters from the TTNDFD assay and model. It is important to note that the fiber digestion parameters measured directly in the cows are independent of the *in vitro* measurements. Results of the study indicate that the *in vitro* TTNDFD were similar to the directly measured *in vivo* total tract NDF digestibility values and provide evidence that supports the concept that *in vivo* fiber digestion can be predicted from *in vitro* fiber kinetics.

The objective of another *in vivo* experiment (Lopes et al., 2013) was to compare estimates of total tract fiber digestion as

predicted by the *in vitro* TTNDFD model to *in vivo* measurements in lactating dairy cows. Cows were fed diets that varied in proportions of corn silage and alfalfa. The *in vitro* fiber digestion parameters for corn silage (NDF = 34.4%, pdNDF kd = 3.2%/h, and pdNDF = 58.6% of NDF) and alfalfa silage (NDF = 34.7%, pdNDF kd = 6.1%/h, and pdNDF = 51.3% of NDF) indicate that fiber in the corn silage contains more pdNDF than alfalfa, but the rate of digestion of alfalfa fiber is nearly twice as fast as corn silage fiber. The feeding experiment measured how cows utilize forages that differ in pdNDF and kd (Table 4). The diets contained approximately 55% forage and the dietary NDF concentration was similar across the 4 treatments.

Feed intake was lower when cows consumed the diets that contained 100% of forage as alfalfa silage than it was when cows were fed diets containing corn silage. The observed (*in vivo*) total tract NDF digestion values were calculated from feed and fecal samples. Cows consuming the diet with alfalfa as the only forage had higher NDF digestibility than cows on the diets that contained corn silage. Milk and FCM yields did not differ due to treatment. The NDF digestibility coefficients predicted by the *in vitro* TTNDFD method were similar to the *in vivo* values. The fiber digestibility coefficients suggest that the faster rate of fiber digestion of alfalfa fiber compensates for content of pdNDF, but as higher proportions of alfalfa forage are fed, the amount of indigestible fiber in the rumen increases and rumen fill becomes a more predominant factor limiting feed intake.

These feeding experiments demonstrate that the *in vitro* TTNDFD analysis can provide important insights into fiber utilization by dairy cattle. The rates of fiber degradation determined from the *in vitro* NDFD assays are consistent with values measured in *in vivo* feeding

studies. The kd, kp, and pdNDF parameters predicted by the TTNDFD model appear to be consistent with *in vivo* measures, and the total tract digestion of NDF as predicted by the TTNDFD model is consistent with observed *in vivo* digestion. A third study (Lopes et al., 2015b) compared 21 diets from seven feeding experiments and showed that TTNDFD of total mixed rations analyzed by the *in vitro* TTNDFD method were highly correlated to the directly measured *in vivo* total tract NDF digestibilities of the same diets in lactating dairy cows.

Conclusions: How to Use the TTNDFD Test

The key to getting the most out of forages is understanding how energy values are affected by NDF and NDF digestibility. This test is intended to be an additional tool to provide a clearer understanding of how forage fiber is utilized in dairy cattle. It is not intended to be the only tool to use to evaluate forage quality or fiber utilization by dairy cattle. Table 5 summarizes important limitations to this assay. In top quality forages, NDF accounts for 35 to 45% of the total DM, and this fiber is the source of 30 to 40% of the digestible energy. A 30% NDF diet with a TTNDFD of 33% would support 7 to 10 lb less milk than a 30% NDF diet with a TTNDFD of 45%, assuming no reduction in feed intake. The average TTNDFD value for most diets formulated with alfalfa and corn silages will be about 42 to 44%, and this should be a target for ration formulations.

The TTNDFD value can also be used as a stand-alone value to index forages. A consultant could compare values from their forage test to the values in Table 1. For example, note in the Table 1 that an average alfalfa will have a TTNDFD value of 43%. An alfalfa with a TTNDFD value one SD below average (less than 36%), would be among the bottom 15% of the alfalfa samples tested. A sample with low

TTNDFD likely will not be utilized as well as 'typical' alfalfa containing similar amounts of total NDF. Our validation studies with corn silages, alfalfa, and temperate grasses indicate that TTNDFD values of feeds can be used in ration formulation and evaluation to 'fine-tune' the amount and overall digestibility of NDF in rations for high producing dairy cattle. The ability to predict fiber digestibility and incorporate this information into rations could improve our ability to optimize forage utilization and milk production.

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Table 1. Typical total tract NDF digestibility (TTNDFD) values of corn silage, alfalfa, or grass.¹

	TTNDFD, % of NDF	SD ²	Range
Corn Silage	42	± 6	20-60
Alfalfa	43	± 7	25-80
Grass	47	± 8	6-80

¹Samples submitted to Rock River Laboratories, Watertown, WI.

²SD = Standard deviation.

Table 2. Effects of source of corn silage on total tract NDF digestion (Lopes et al., 2015a).¹

	LFDCS	HFDCS	SE
Feed, % of TMR DM			
Low fiber digestibility corn silage	47	0	
High fiber digestibility corn silage	0	47	
Alfalfa silage	17	13	
Concentrate mix	36	40	
Diet composition			
NDF, % of DM	27.5	28.3	
pdNDF, % of NDF	68.9	75.9	
Results			
DMI, lb/day	55	56	1.3
4% FCM, lb/day	76	77	1
Observed TTNDFD, in vivo	47	43	2.5
Predicted TTNDFD, in vitro	43	50	0.9

¹LFDCS = Low fiber digestibility corn silage, HFDCS = high fiber digestibility corn silage, pdNDF = potentially digestible NDF, and TTNDFD = total tract NDF digestibility.

Table 3. Comparison of rumen and total tract NDF digestion of diets predicted from TTNDFD model and observed in vivo (Lopes et al. 2015a).¹

Item	Predicted <i>in vitro</i>	Observed <i>in vivo</i>	SEM	<i>P</i> value
Input				
pdNDF kd, %/h	4.1	4.3	0.5	0.72
pdNDF kp, %/h	2.7	2.8	0.3	0.56
Output				
NDF digested in rumen, lb	6.01	5.79	0.48	0.64
NDF digested in hindgut, lb	0.79	1.41	0.42	0.05
NDF digested in total tract, lb	6.80	7.19	0.48	0.42
Total tract NDF digestibility, % of NDF	46.4	49.5	0.07	0.13

¹pdNDF = Potentially digestible NDF, Kd = rate of digestion, and Kp = rate of passage.

Table 4. Effect of changing ratios of corn silage to alfalfa on intake, production, and fiber digestion in dairy cows (Lopes et al., 2013).¹

	100CS	67CS	33CS	0CS	
Corn silage (CS):alfalfa (AS) ratio	0AS	33AS	67AS	100AS	
Corn silage, % of TMR	56	37	18	0	
Alfalfa silage, % of TMR	0	19	37	55	
Concentrate mix, % of TMR	44	44	45	45	
Diet composition					
NDF, % of DM	24.9	25.5	24.6	25.5	
iNDF, % of NDF	31.1	31.6	31.8	32.3	
Results					
					SE
DMI, lb/day	55.4 ^{ab}	55.7 ^a	53.5 ^b	48.2 ^c	1.8
4% FCM, lb/day	79.6	77.9	77.8	78.3	2.0
Observed TTNDFD, in vivo	38.3 ^a	40.9 ^{ab}	39.4 ^{ab}	43.8 ^c	1.9
Predicted TTNDFD, in vitro	38.0	41.0	41.0	45.0	2.1

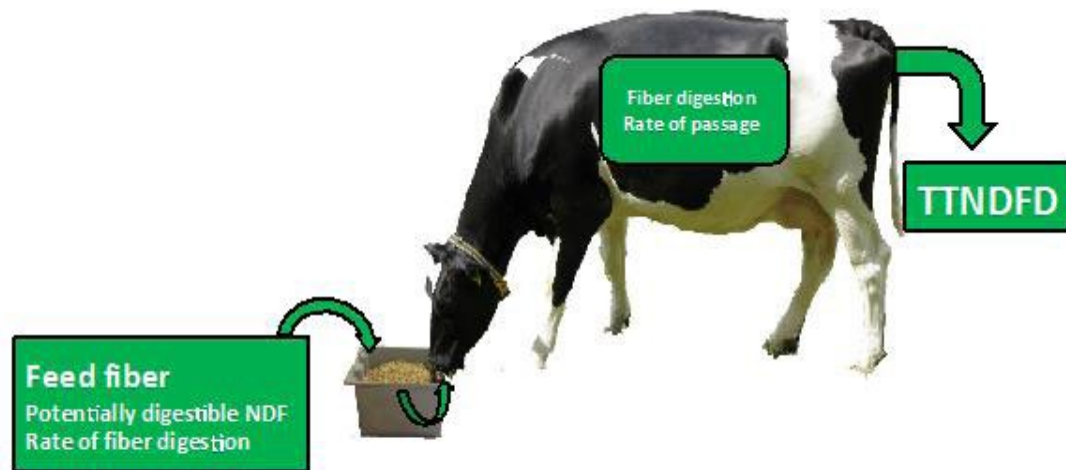
¹iNDF = Indigestible NDF and TTNDFD = total tract NDF digestibility.

Table 5. Guidelines for using total tract NDF digestibility (TTNDFD).

1. The TTNDFD assay is intended to evaluate the digestibility of NDF of feeds and rations in animals fed an otherwise balanced diet. Inadequacies of other nutrients (protein, amino acids, and minerals) or excesses of dietary components other than fiber (i.e., mycotoxins) are not accounted for in this assay.
 2. The TTNDFD can be used to compare fiber utilization across forage or fiber sources. For example, fiber digestibility of corn silage can be compared to fiber digestibility of alfalfa, grass, or co-product feeds.
 3. TTNDFD does not account for differences in physical form (effective fiber) of forages.
 4. TTNDFD estimates total tract digestibility of fiber for a dairy cow consuming about 54 lb/day of DM.
 5. In vitro NDFD values (NDFD24, NDFD30, or NDFD48) should not be used as a single indicator to compare fiber digestibility of forages. These values do not factor in indigestible fiber or NDF concentration of forages. Single time NDFD values are poorly correlated to total tract fiber digestibility.
 6. Total NDF and TTNDFD must be considered when comparing forages for quality.
-

TTNDFD → *Total Tract NDF Digestibility*

Feed and cow factors are combined to true fiber digestion



A 2-3 unit change in ration TTNDFD corresponds to 1 pound change in milk yield.

Figure 1. The TTNDFD model.

Gut Fill Revisited

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Summary

Generally, a dairy cow's daily dry matter intake (DMI) will be under the influence of the physical capacity of the rumen. This is known as gut fill. As demonstrated by several research groups, the undigested NDF pool is related to gut filling effect of a ration. Specifically, it is suggested that NDF_{u30}° , i.e., the undigested NDF pool remaining after 30 hr of in vitro rumen incubation, is an appropriate proxy for gut fill. The proposed gut fill index for a ration is pounds of NDF_{u30}° supplied from forages and other products that are larger than 4 mm. Monitoring a ration's gut fill index will assist in identifying ration changes that may impact DMI. However, rumen environments which change overall fiber digestibility will bias the gut fill index. Under higher acid conditions, fiber digestibility will be compromised, leading to a falsely lower gut fill capacity. Rations with low particle size will result in increased passage rates, leading to a falsely higher gut fill capacity. In general, corn silage will have the lowest NDF_{u30}° pool size of forages, with most grasses having NDF_{u30}° pools size approximately 35% larger. NDF_{u30}° is meant to be an indicator of gut fill that is appropriate for evaluating forages as well as, the gut fill load for a ration's forage base.

Introduction

Gut fill is generally referred to as the physical distention of the rumen resulting in cessation of a meal. This phenomenon has been recognized for decades. Perhaps one of the earliest characterizations of gut fill was presented by Conrad (1966) where several digestion trials were summarized. These trials suggested that as dry matter disappearance (DMD) increases to 66%, that DMI concomitantly increases. It is appropriate to conclude that DMD defines gut fill in this discussion. Beyond 66% DMD, DMI decreases as DMD increases presumably due to factors other than gut fill. Studying the relationship between chemical composition of feeds and DMD, Goering and Van Soest (1970) published the following equation:

$$DMD = NDF * NDFD + 0.98 NDS - 12.9,$$

where NDF = neutral detergent fiber, NDFD = digestibility of the NDF, and NDS = neutral detergent solubles which is defined as 1-NDF. On the surface, this equation seems difficult to interpret. Mertens (2010) mathematically rearranged this equation, revealing the following equation:

$$DMD = 87.1 - (0.98 - NDFD) * NDF$$

This equation suggests that the maximal DMD is 87.1% and will decrease as NDF increases and

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NDFD decreases. It has recently been suggested that the proper interpretation of this equation is that DMD is related to the pool size of undigested NDF defined as $(1 - \text{NDFD}) * \text{NDF}$ (Jones, 2014). Logically, it can be demonstrated that gut fill is related to the pool size of undigested NDF.

The Rise of NDFd and Subsequently NDF_{u30} ©

Even though NDF digestibility has been in the scientific discourse since the 1960's, the classic view is that NDF as a percent of body weight (BW) is a principle influencer of DMI. The following equation was utilized in Merten's (2010) intake model:

$$\text{NDF intake} = 1.25\% * \text{BW}$$

Waldo (1986) suggested that cell wall concentration (i.e., NDF) of forage diets is the best single chemical predictor of DMI by ruminants.

However, it is clear that increasing NDF digestibility increases intake (Oba and Allen, 1999). This understanding led to forages being characterized by NDF digestibility (NDFd; % of NDF). Allen (2000) concluded that "Digestibility of NDF measured in vitro or in situ using a constant incubation time was a significant indicator of the filling effects of NDF ...".

A common convention is to use a 30-hr in vitro incubation to estimate NDF digestibility (NDF_{d30} , % of NDF). Feeds with higher NDF_{d30} (% of NDF) are generally found to promote more intake. However, the effect of the potentially digestible NDF fraction on gut fill was not clear when evaluated by Allen and Mertens (1988).

Jones and Siciliano-Jones (2013) proposed that the proper characterization

of fiber related to intake is the pool size of undigested fiber (NDF_u ; % of DM). Following the convention presented above, the pool size of undigested fiber after a 30-hr *in vitro* incubation was introduced (NDF_{u30} ©; % of DM, Copyright FARME Institute, Inc).

Hall (2013) discussed a debate over which incubation time is most appropriate for estimating NDF digestibility (Figure 1). The NRC (2001) uses a 48 hr incubation time to estimate energy derived from NDF. Our purpose is to estimate "gut fill" which must take into account passage rate. The 30-hr incubation time point seems appropriate given static in vitro fermentation and a standard particle passage rate. If the remaining particles have not been fermented or passed, they contribute to gut fill.

It is important to differentiate between the terms of "undigested" and "indigestible". The former refers to the ability to be digested given a finite time. In this case, 30 hr. The latter refers to the ability to be digested given infinite time. Usually, this is estimated at 240 hr of incubation in rumen fluid.

Examining previous work on the NDF digestibility, expressed as percent of the NDF fraction, we can substitute the measure of NDF_{u30} © expressed as pool size. Previous work that increased NDF digestibility in diets also decreased NDF_{u30} ©, usually without noting it. For example, Allen (2000) notes that "DMI by cows will be less limited by distention in the gastrointestinal tract as NDF digestibility increases." The concept of fiber digestibility impacting gut fill is not new. However, the proper representation and utilization of NDF_{u30} © as a gut fill indicator is new.

NDF_{u30}[©] in Ration Design

In February 2015, the US Patent Office issued a patent which contains claims for the use of undigested NDF and starch digestibility for ration formulation (Weakley, 2015).

NDF_{u30}[©] is proposed as an indicator of gut fill to be used in designing certain dairy cow rations (Jones and Siciliano-Jones, 2014). First, it is only appropriate to discuss NDF_{u30}[©] in rations where DMI is limited by gut fill. This is typical of intakes during peak production (Mertens, 2010). Situations where DMI is limited by low energy requirement or acid load will likely not respond to manipulating NDF_{u30}[©] content.

NDF_{u30}[©] acts as a gut fill factor only when fed particle size is large enough to inhibit passage from the rumen. The threshold particle size allowing passage from the rumen appears to be 2 to 4 mm (Allen and Mertens, 1988). Consequently, undigested NDF in particles below this threshold will not be expected to contribute to gut fill as they are not retained in the rumen. Therefore, we propose calculating the pool size of NDF_{u30}[©] only on feeds that have a particle size above 4 mm. In general, only forages and certain large particle by-products (e.g., whole cotton seed) are included in calculating the gut fill load.

Our basic procedure is to calculate the NDF_{u30}[©] content (i.e., gut fill) in the forage portion of a ration for a high producing group of cows. As a starting point, high producing large Holstein cows appear to eat to gut fill of approximately 6.2 to 6.5 lb/day of NDF_{u30}[©]. However, what is important is how this NDF_{u30}[©] content changes over time relative to DMI (Jones, 2014). If a forage or ration change results in increased gut fill in the proposed ration, there is a high probability that DMI will

decrease such that the group's actual threshold of NDF_{u30}[©] capacity is not exceeded.

Using the above procedure requires 2 assumptions. First, it is assumed that gut fill is the rations most constraining factor. Second, a forage base (including all significant sources of NDF_{u30}[©]) must be the initial component of ration design. Designing a ration with NDF_{u30}[©] starts with a forage base that does not violate a gut fill. This is also intuitive since a ration should be first balanced for the rumen and then for the animal.

It is tempting to discuss NDF_{u30}[©] as a percent of ration dry matter. This has benefits for ration formulation but does not reflect the underlying subject that gut fill is a pool size issue. Let's start with a farm specific assumption that the highest producing cows have not historically consumed more than 6.3 lb of NDF_{u30}[©]. Problems arise when a group is balanced for a DMI which is below that consumed by the highest producing cows. For example, a group ration might be balanced for 53 lb of DMI. However, the highest producing cows might be eating 70 lb of DM to support peak milk production. A typical calculation is to determine the percentage of NDF_{u30}[©] to ensure that the highest producing cows are not challenged with more than 6.3 lb of NDF_{u30}[©] intake. In this case, the base ration needs to be 9% NDF_{u30}[©] (6.3 lb NDF_{u30}[©]/70 lb intake). Conversely if NDF_{u30}[©] percentage is calculated from the group intake (6.3 lb NDF_{u30}[©]/53 lb intake), the NDF_{u30}[©] content will increase such that the highest producing cows will reach their fill capacity at a reduced DMI. Recommendations for NDF_{u30}[©] as a percentage of DM should be avoided for this reason. This calculation is only useful in determining if the base ration for a group will support the highest producing cows.

Common ration design rules can violate the gut fill capacity of cows, resulting in lower milk production. For example, a common ration feature is inclusion of 3 lb of WCS (DM basis) in all diets. Assuming that WCS is 40% NDF_{u30}° , WCS contributes 1.2 lbs of NDF_{u30}° to these diets. In a year when NDF digestibility of corn silage is poor (e.g., NDF_{u30}° increases from 15 to 18%), a ration containing 20 lb of corn silage will see an increase of 0.6 lb of NDF_{u30}° . Without adjusting the WCS or the corn silage inclusion rates, the high producing cows will have DMI limited by gut fill due to excess NDF_{u30}° .

A common consequence of exceeding the gut fill capacity of high producing cows is lower than expected peak production. When DMI is limited by gut fill, the highest producing cows will be impacted the most due to the inability to consume sufficient DMI. When older animals are peaking poorly compared to their younger cohorts, especially when persistency is high, a gut fill problem should be suspected.

Distribution of NDF_{u30}° in Forages

Figure 2 contains the distribution of NDF_{u30}° for both corn silage and hay crop silage in the Cumberland Valley Analytical Services (Hagerstown, MD) database. Corn silage has a mean NDF_{u30}° value of 17.2%. For hay crop silage, the mean is 23.9% NDF_{u30}° . Hay crop silage is about 35% higher in NDF_{u30}° than corn silage.

The variance seen in these distributions suggest fairly large gut fill differences. First, it becomes clear why high corn silage diets generally result in less gut fill. The average corn silage sample has nearly 7 percentage points less NDF_{u30}° . A ration that contains equal amounts of average corn silage and average haylage with

a constraint of 6 lb of NDF_{u30}° will contain 29 lb of forage. Conversely, a diet with 80% average corn silage and 20% average haylage will allow 32 lb of forage.

A common scenario occurs when a growing year results in lower fiber digestibility (i.e., higher NDF_{u30}°). Consider again a 80:20 corn silage:haylage diet when the NDF_{u30}° changes from an excellent corn silage (25% quartile; 14.97% NDF_{u30}°) to a poor corn silage (75% quartile, 19.12% NDF_{u30}°). The NDF_{u30}° content of the diet will increase from 6 to 7.3 lb. If our group was eating 66 lb of DM (9% NDF_{u30}°), the intakes will probably decrease to 54 lb due to increased gut fill.

A related topic is the accuracy of NDF digestibility as measured in the laboratory. One should remember that digestibility testing has been common since the 80's (Nocek and Russell, 1988) and was intended to be a qualitative test for ranking forages since the variability is much higher than typical chemical analyses performed on forages. Hall and Mertens (2012) reported that within a given laboratory, 95% of the digestibility results for a given forage sample fall between $\pm 4.9\%$ NDFD from the mean. If we use a typical forage consisting of 40% NDF and a 50% NDFd, then the NDF_{u30}° will be 20%. If the NDFd measure varies from 45 to 55%, then the NDF_{u30}° will vary from 18 to 22%. This does not take into account the variation inherent in NDF chemical analysis which would further increase the range of values. Using NDF_{u30}° as a gut fill index is consistent with the notion of a qualitative index.

When Does Predicted $\text{NDF}_{u30}^{\circ} \neq$ Actual NDF_{u30}° ?

As forage analysis evolves, it is becoming more biological than chemical in nature. For example, measuring starch content is a simple

chemical analysis. Conversely, estimating starch availability requires mimicking the biology of starch digestion. This is also true for NDF digestibility. To correctly apply NDF_{u30}° in ration design, it is important to explore scenarios where the predicted NDF_{u30}° does not properly estimate the biological NDF_{u30}° .

As an example, consider the haylage sample shown in Figure 3. The NDF_{u30} is 27% of the DM. If our new diet design calls for 2 lb of NDF_{u30}° from haylage, we will limit inclusion in the diet of this haylage to 7.5 lb of DM. In this scenario, the cows will almost certainly increase DMI. Why? The NDF_{u30}° is not really 27%. This analysis demonstrates a classic example of NDF which is not corrected for ash contamination. Looking closer at this sample, there is a 9 point difference between aNDF and aNDFom. Further, the NDF_{d30} and NDF_{u30}° are calculated from aNDF ($13.7\% + 27\% = 40.7\%$). From a typical haylage, the NDF_{u30}° is overestimated by approximately 6 to 7 points. A better estimate is 21% NDF_{u30} which now allows an inclusion of 9.5 lb of haylage in our example diet. When there is high ash content ($> 3\%$) in the NDF fraction, the undigested portion will be overestimated when calculated using aNDF which is not corrected for ash content.

A second scenario which will overpredict the gut fill impact of forages is finely chopped diets. NDF_{u30}° calculated *in vitro* is independent of passage rate. When passage rate increases, the amount of particles remaining in the rumen at a specific time decreases. Consequently, excessive NDF_{u30}° intake can be an indicator of increased NDF passage. Another documented scenario is that passage rate changes with the animal's cold stress. Hence, gut fill capacity may change during periods of cold stress.

Ration characteristics that reduce fiber digestibility constitute a third scenario where

gut fill is higher than predicted. The most common scenario is increased acid load that inhibits fiber digesting bacteria. Low ruminal pH from highly fermentable feeds can decrease rate of fiber digestion and increase the filling effect of the diet (Allen and Mertens, 1988). Recently, ration starch has been a focus as a dietary component that lowers ruminal pH. This focus has ignored the reality that digestible NDF can also be highly fermentable and contribute to acidosis. In a recent popular press summary, Fredin (2014) showed that replacing starch with non-forage fiber sources did not change rumen pH. It should not be surprising that low starch diets combined with other sources of highly fermentable carbohydrate can result in low rumen pH, which will depress fiber digestion. This, in turn, increases actual NDF_{u30}° and the gut fill characteristics of the diet.

Differences in particle retention time for different types of forage NDF can cause predicted NDF_{u30}° to not correspond to actual NDF_{u30}° . In general, NDF in legumes is thought to have less filling effect than NDF in grasses (Oba and Allen, 1999). An example of this effect was seen in a study to examine perennial ryegrass silage compared to alfalfa silage, where the alfalfa silage was found to support greater DMI (Hoffman et al., 1998). Recalculating their data into a gut fill context, the alfalfa silage was 20.9% NDFu while the perennial ryegrass was 16.8% NDFu as a percent of DM. However, in this case, the cows consuming the alfalfa silage ate nearly 5 lb more DM than the perennial ryegrass. The differing gut fill effect of different forage types argues for monitoring the gut fill effects in diet specific scenarios (Jones, 2014).

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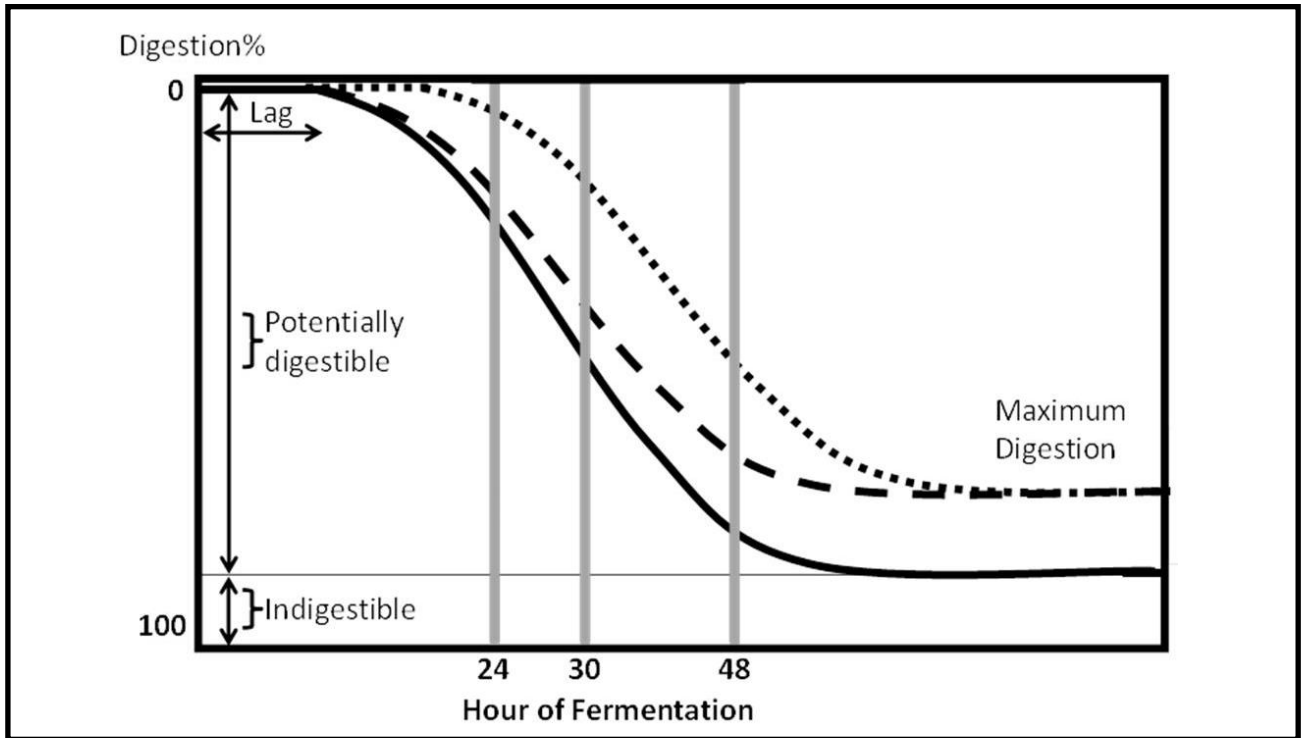


Figure 1. Rate of digestion as seen at different time points given different digestion rates and lag times (Hall, 2013).

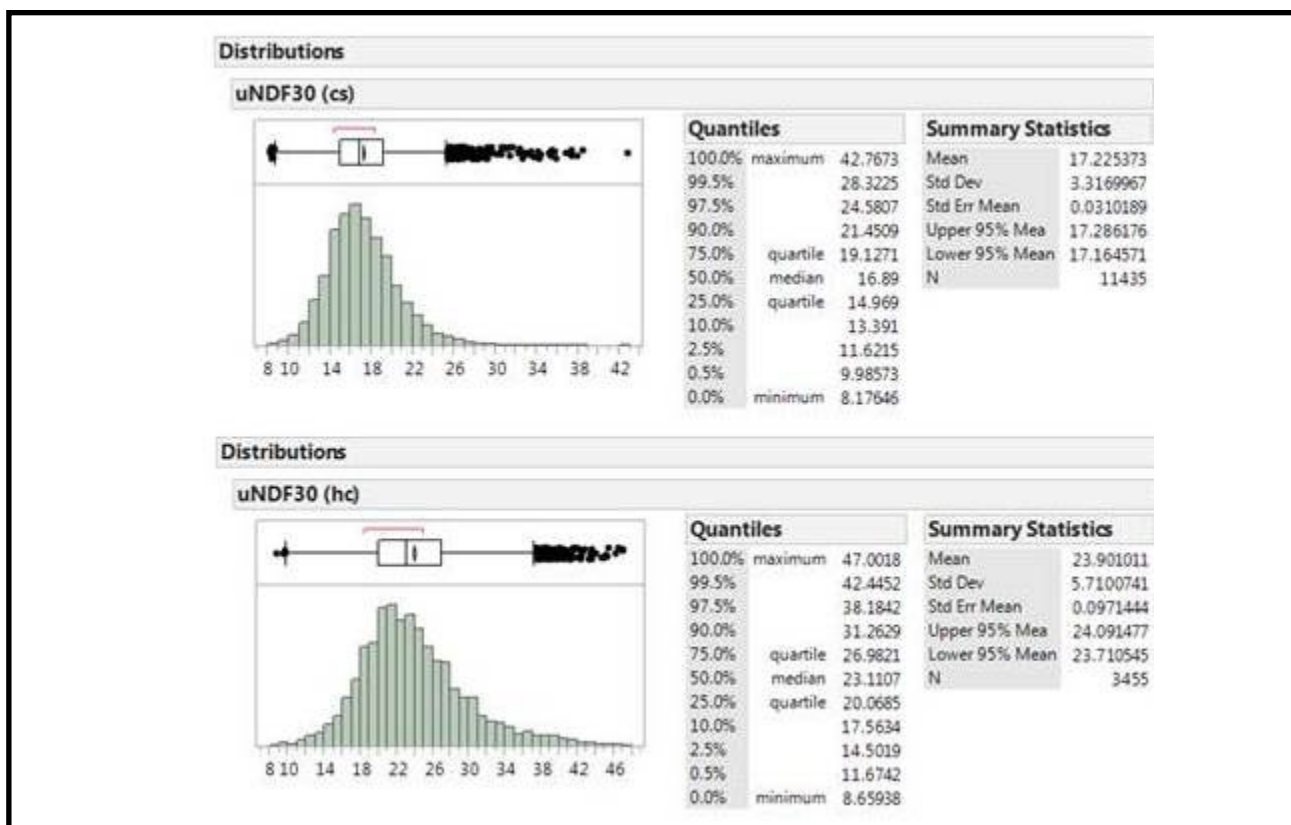


Figure 2. Distribution of NDF_{u30} content for corn silage and haylage observed in the Cumberland Valley Analytical Service database (provided by R. Ward, 2013, Cumberland Valley Analytical Services, Hagerstown, MD).

FIBER	% NDF	% DM
ADF	89.3	33.1
aNDF		40.7
aNDFom		31.8
NDR (NDF w/o sulfite)		
peNDF		
Crude Fiber		
Lignin	18.32	7.46
NDF Digestibility (12 hr)		
NDF Digestibility (24 hr)		
NDF Digestibility (30 hr)	33.8	13.7
NDF Digestibility (48 hr)		
NDF Digestibility (240 hr)	46.5	18.9
uNDF (30 hr)	66.2	27.0
uNDF (240 hr)	53.5	21.8

Figure 3. Example fiber analysis in a forage sample that contains ash contamination in the NDF fraction.

Effective Outcomes of TMR Audits

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Diamond V

Abstract

Total mixed rations (TMR) are formulated to contain a combination of feedstuffs that provide the right balance of nutrients in every bite consumed. Poorly mixed TMR negatively impact animal performance and health. A system has been developed to monitor how well the feedstuffs are blended and delivered to the feed bunk. This system is called the TMR Audit (Oelberg and Stone, 2014). There are 10 factors in the TMR mixing process that can create variation in the TMR before it is delivered to the feed bunk. Additionally, time-lapse cameras can be utilized to evaluate animal access to the TMR and feed push routines. The desired outcomes of a TMR Audit are: 1) reduced variation in feed ingredients and TMR, 2) improved feeding efficiency, 3) reduced feed waste, and 4) improved feed bunk management.

Introduction

Feed costs represent the largest portion of the cost to produce milk. Much effort has been spent on making sure the cow gets the most out of the feed by feeding highly digestible forages, well processed grains, and commodities that provide available levels of amino acids, minerals, and vitamins. Oftentimes the performance of the cows does not match predicted performance from ration formulation software. Reasons for this can

vary but can include improper knowledge of actual dry matter intakes, poor cow comfort leading to excessive maintenance costs not accounted for in the ration software, and finally, improper mixing of the TMR. Sova et al. (2014) showed a negative association between fed ration coefficient of variation (CV) in NEL and average test-day milk yield. The data were collected from 22 farms for 7 consecutive days during summer and winter months. They also showed a negative association between the CV of long forage particles and average test-day milk yield. As the CV of these components increased (more variation), the average test-day milk decreased. Various methods of testing mixer efficiency have been developed using salt (Harner et al., 1995; Groesbeck et al., 2004) or a drug such as Rumensin® (Biermann, 2008). Others, have used these methods to test the effects of mix time after the last added ingredient (Harner et al., 1995; Groesbeck et al., 2004; Biermann, 2008), or loading sequence (Groesbeck et al., 2004; Biermann, 2008). However, these methods require collecting and sending the samples to a lab for analysis and then one must wait for the results. A faster and lower cost method was needed to do an on-farm evaluation of TMR consistency.

TMR Audit

The TMR Audit evaluates feed out management of forages so that variation in moisture and

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nutrients are reduced prior to loading into the TMR (Oelberg and Stone, 2014). The audit also evaluates feed center organization, use of on-farm premixes and TMR loading sequences and timing. Making slight changes in the TMR loading and mixing routine can lead to significant improvements in fuel and labor efficiency, mixer performance, and reduced feed waste. Much attention is paid to how and when feed is delivered to the pens. Finally, the TMR Audit also uses time-lapse cameras positioned over the feed bunks to evaluate cows' access to feed and feed push up schedules. This manuscript will focus on the 10 mixing factors that cause variation in TMR particle size.

The Ten Factors Causing TMR Variation

A key goal of the TMR Audit is to help reduce variation of the major ingredients. The next part of the audit is to evaluate the TMR mixing process. There are 10 factors in the TMR loading and mixing process that can contribute to TMR variation, individually or in combination. Each of these will be discussed in detail. They are:

1. Worn mixer augers, kicker plates, and knives,
2. Mix time after the last added ingredient,
3. Unlevel mixers,
4. Loading position on the mixer box,
5. Load size,
6. Hay quality and processing,
7. Loading sequence,
8. Liquid distribution,
9. Vertical mixer auger speed, and
10. Forage restrictor settings on vertical mixers.

Worn Mixer Augers, Kicker (deflector) Plates, and Knives

TMR particle size consistency, as well as moisture and nutrient consistencies

along the feed bunk (TMR mix quality) can decrease significantly with worn blades, kicker plates, and augers (Oelberg and Stone, 2014). Mixers are factory set with specific agitator clearances of 0.3 to 0.9 cm (Zinn, 2004). As these clearances increase due to wear, mixer efficiency is impaired (Zinn, 2004). The easiest way to evaluate wear on augers is to look for feed under horizontal augers or reels and to look for the feed ring inside vertical mixers. Often, mixing problems become obvious if one simply looks at a full load of feed being mixed. The mixing efficiency on vertical auger mixers depends on the condition of the edge on the auger flighting and on the condition of the kicker plate, shoe, or deflector. The edge of the flighting should not have rounded corners. The degree and speed of wear on the augers, kicker plates, and knives depends on the size of the herd and the amounts of hay, baleage, or straw fed. Routine replacement of blades, kicker plates, and augers are required to keep TMR consistent.

Mix Time After the Last Added Ingredient

Several authors have cited mixing time as a critical element to get consistent mixes (Harner et al., 1995; Groesbeck et al., 2004; Behnke, 2005; Biermann, 2008). Groesbeck et al. (2004) showed that the amount of mix time after the last ingredient was added to a swine diet in a horizontal ribbon mixer was important in reducing the variation in the concentration of salt. One of the most common mistakes in TMR mixing is the lack of mix time after the last added ingredient (usually corn silage or liquid supplement) (Oelberg and Stone, 2014). Oftentimes, the corn silage at the top of the load does not get mixed and is delivered towards the end of the load as pure corn silage. This is even more prevalent as mixer boxes are over-filled. Suggested mix times after the last ingredient with tractors/trucks at nearly full

power (1700 to 2000 rpm engine speed) are 3 to 5 minutes. Inadequate mix times resulted in an inconsistent TMR (Table 1). Increasing mix time from 3.5 to 5 minutes in a 4-auger horizontal mixer reduced the CV for particles retained on each screen and the pan of the Penn State Particle Separator (PSPS).

Unlevel Mixers

Unlevel mixers cause migration of the heaviest and most dense materials in the TMR to the lowest section of the mixer wagon. Figure 1 shows a PSPS analysis of 10 samples taken from a triple–auger vertical mixer that was parked on a ramp that was too short causing the grain-concentrate portion of the TMR to migrate to the back of the mixer box. Notice how the amount in the bottom screen increased from sample 1 (front) to sample 10 (back) and the opposite trend can be observed for the middle screen which would have less dense feedstuffs, such as haylage and corn silage and small particles of hay. This is a very typical pattern in the PSPS analysis for both unlevel mixer boxes and for improper loading position on vertical wagons. A discussion on loading position on mixer boxes will occur in the next section.

Loading Position on the Mixer Box

Loading position on the mixer box refers to the location on the mixer box where the feeder is dumping ingredients. Improper loading position on the mixer box will create a poorly mixed TMR (Oelberg and Stone, 2014). Figure 2 shows the influence of loading liquid in the front versus the middle of a dual-auger vertical mixer on the levels of TMR in the middle and bottom screens of the PSPS. The liquid was a whey product that bound the small feed particles in the pan to the larger particles in the middle screen at the front of the wagon. Then, there was a continued increase in the

amount of material in the pan as you progress to the back of the wagon. The opposite trend was seen for the middle screen. The mixer was moved ahead 4 feet so that the liquid whey could be loaded between both augers or in the center of the mixer box. This resulted in a very consistent TMR shown by the dotted lines. Figure 3 shows the influence of loading a liquid protein supplement in the back of a dual-auger vertical wagon on moisture and protein levels in the TMR. Both moisture and protein increase linearly as you move from front to back of the wagon. This resulted in a very inconsistent TMR along the feed bunk. Because cows are quite territorial within the pen, neither will cows get the same nutrition nor will they get the same effective particle size. This leads to differences in rumen health and digestion, rumination patterns, and manure consistency among cows within the pen fed this ration. Most dual-auger and triple-auger vertical wagons move feed back and forth in the wagon, but it takes time. These results show that feed dumped in either end of these wagons does not get completely mixed, during routine mixing. If mixing time is increased so that the TMR is completely mixed then there is increased risk of decreasing effective particle size in the TMR. The increased mixing time would also increase fuel and labor cost. It's best to load the mixers at the proper position.

Load Size

Over-filling

Over-filling the load capacity can occur on all types of mixer wagons, resulting in poor mix quality of the TMR (Oelberg and Stone, 2014). This is a very common mistake in TMR mixing on many dairy farms and feedlots. Overfilling occurs for several reasons:

- Under sizing the mixer box for the dairy farm,
- Inaccurate pen counts,
- Changes in forage moisture levels or types, i.e. drier silages take up more space, and haylage is bulkier than corn silage, and
- Too large of an increase in bunk calls where the mixer box is already at full capacity.

Reducing the load size in a 4-auger mixer by 5000 lb decreased the CV (Table 2) of the average levels of TMR in all 3 trays of the PSPS and improved TMR mix quality.

Under filling vertical mixers

Under filling of vertical mixers occurs when the TMR does not reach the top of the augers so that all of the ingredients are pushed off the augers and mixed. This happens often on many dairy farms that are mixing for small pens, such as close-up dry and fresh pens (Oelberg and Stone, 2014). Running the vertical augers at a higher RPM can help small loads to mix.

Hay Quality and Processing

Poor hay quality and inadequate processing make TMR very inconsistent and can affect both variation and concentration of milk components in a herd (Figure 4).

Loading Sequence

Several authors have addressed loading sequence as a factor contributing to TMR mix quality (Barmore, 2002; Behnke, 2005; Biermann, 2008; Oelberg and Stone, 2014; Zinn, 2004). The loading sequence will depend on:

- Mixer wagon type (auger-reel versus 4-auger or vertical),
- Ingredient type (density, particle size and

shape, moisture level, and flowability) (Behnke, 2005),

- Inclusion level (Zinn, 2004), and
- Convenience of loading based on where ingredients are stored at the feed center and time available to the feeder (not the most ideal situation on many dairy farms).

Generally, lower density and large particle feeds are loaded first, followed by dry more dense feeds followed by wet feeds, and last with liquid. Of the dry more dense feeds, the lower-inclusion level feeds are added first so that they can be blended properly (Zinn, 2004). Use the ratio of 50:1 to blend lower inclusion dry feeds, such as rumen by-pass fats and vitamin/mineral premixes. Example, if 50 lb of rumen by-pass fat is being added, then the load size should be no more than 2500 lb. The mixer should be running to allow the lower inclusion feed to mix.

TMR mix quality was improved dramatically by increasing mix time after the last added ingredient from 2 to 4 minutes and then changing mix order to further improve the mix quality (Figure 5).

Liquid Distribution

Liquids, such as water, whey, and cane molasses, are routinely added to the TMR to add moisture, sugar, or are used as a carrier for micro-ingredients. Another important reason liquids are added to the TMR is to help reduce sorting by cattle. The liquids, especially cane molasses and liquid whey, are sticky and they help bind the smaller particles to the larger forage particles. As a result, the amount on the pan of the PSPS can shift to the middle and top screens by as much 5 to 7 percentage units depending on type and level of liquid added directly to the TMR.

It is best to add the liquid last to the TMR to prevent any balling or clumping of the drier ingredients (Zinn, 2004; Behnke, 2005; Biermann, 2008). There are 2 challenges of adding liquid directly to the TMR, time and distribution. Depending on the amount of liquid added to the TMR and the sizes of the pumps and pipes to load the liquid, the amount of time it takes to add liquid can range from 2 to 10 minutes per load and sometimes even longer. This can create a bottleneck in getting cattle fed on time for larger operations. Many dairy operations are adding the liquid to the on-farm commodity blend (Oelberg and Stone, 2014). Improper distribution of the liquid can make the TMR very inconsistent along the feed bunk (Oelberg and Stone, 2014). Figure 6 is an example of how liquid should be added to a TMR or to an on-farm commodity blend.

Vertical Mixer Auger Speed

The influence vertical auger speed on TMR mix quality and apparent improvement in dairy cattle performance has been documented in a case study (Oelberg and Stone, 2014). Improved milk and energy-corrected milk (Figure 7) along with improved MUN levels (Figure 8) were associated with improved TMR mix quality after vertical auger speed was increased with proper engine speed and mixer gear box setting.

Forage Restrictor Settings

Most brands of vertical mixers have forage restrictors mounted on the side of the mixer box. The forage restrictors, when properly set, improve hay processing without impeding TMR mix quality. If the forage restrictors are moved too far into the mixer box, mixing can be impeded, resulting in a poorly mixed TMR (Table 3).

Conclusions

An on-farm system to test TMR consistency along the feed bunk and to evaluate mixer performance has been developed. Implementation of this system has improved TMR consistency on many dairy farms across the U.S. The standard for TMR particle size consistency determined on 10 samples is to have a CV of 2.5% or less for particles retained on the middle screen and pan of the PSPS.

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Table 1. Influence of mix time after the last added ingredient on TMR mix quality (CV = coefficient of variation).

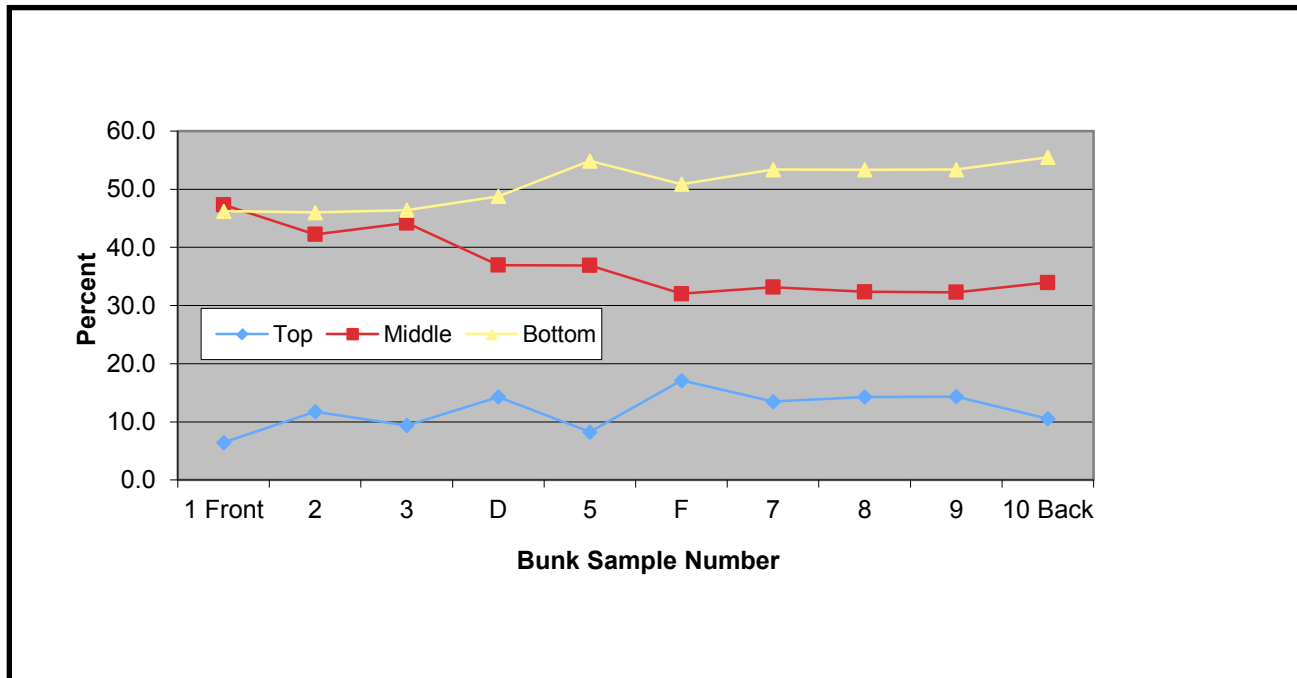
Bunk Sample #	Penn State Shaker Box Results (% of sample)					
	3.5 Minutes			5 Minutes		
	Top	Middle	Bottom	Top	Middle	Bottom
1 Front	10.9	38.2	50.8	14.9	38.8	46.3
2	8.6	38.8	52.6	12.6	41.5	45.9
3	11.6	38.4	50.0	12.5	40.0	47.5
4	15.6	37.8	46.7	14.3	39.3	46.5
5	13.9	39.1	47.0	13.1	39.8	47.1
6	10.8	38.2	51.0	11.7	39.5	48.8
7	9.2	39.1	51.7	12.6	38.8	48.6
8	12.2	41.7	46.0	12.4	38.7	48.9
9	14.1	38.1	47.7	13.0	40.2	46.9
10 Back	11.6	37.3	51.1	11.4	39.3	49.3
Average, %	11.8	38.7	49.5	12.8	39.6	47.6
CV, %	18.52	3.11	4.81	8.15	2.12	2.56

Table 2. Influence of load size in a 4-auger horizontal mixer on TMR mix quality (CV = coefficient of variation).

Bunk Sample #	Penn State Shaker Box Results (% of sample)					
	Over-filled			Normal Filled		
	Top	Middle	Bottom	Top	Middle	Bottom
1 Front	4.9	45.9	49.2	5.6	44.8	49.6
2	2.9	46.3	50.7	6.0	46.0	48.0
3	2.3	44.2	53.5	4.7	46.2	49.1
4	3.8	44.0	52.2	7.4	45.9	46.7
5	4.8	43.8	51.4	5.5	44.5	50.0
6	3.4	47.7	48.9	8.8	42.8	48.5
7	4.3	44.6	51.1	7.0	46.5	46.5
8	3.8	44.2	51.9	8.1	44.1	47.8
9	7.0	37.3	55.7	7.2	43.9	48.9
10 Back	3.6	38.8	57.6	5.9	44.1	50.0
Average, %	4.1	43.7	52.2	6.6	44.9	48.5
CV, %	31.58	7.39	5.22	19.35	2.72	2.58

Table 3. Influence of forage restrictor setting on TMR mix quality (CV = coefficient of variation).

Sample Position	Forage restrictors set all the way in			Forage restrictors set half way in		
	Top	Middle	Bottom	Top	Middle	Bottom
1 Front	4.6	45.4	50.0	6.2	41.6	52.2
2	3.9	45.2	51.0	5.3	41.3	53.4
3	6.0	43.5	50.5	5.4	40.3	54.3
4	4.2	44.2	51.6	6.2	40.5	53.3
5	3.5	46.1	50.3	4.8	41.8	53.4
6	4.8	42.0	53.2	5.1	39.8	55.2
7	2.9	40.0	57.1	5.4	40.1	54.5
8	3.8	41.7	54.5	5.2	41.6	53.2
9	3.8	38.3	57.9	5.1	40.2	54.8
10 Back	1.8	38.3	59.9	5.0	40.4	54.6
Average, %	3.9	42.5	53.6	5.4	40.8	53.9
CV, %	28.12	6.78	6.66	8.86	1.82	1.72

**Figure 1.** Influence of un-level mixer box on TMR particle size distribution on the Penn State Shaker box screens.

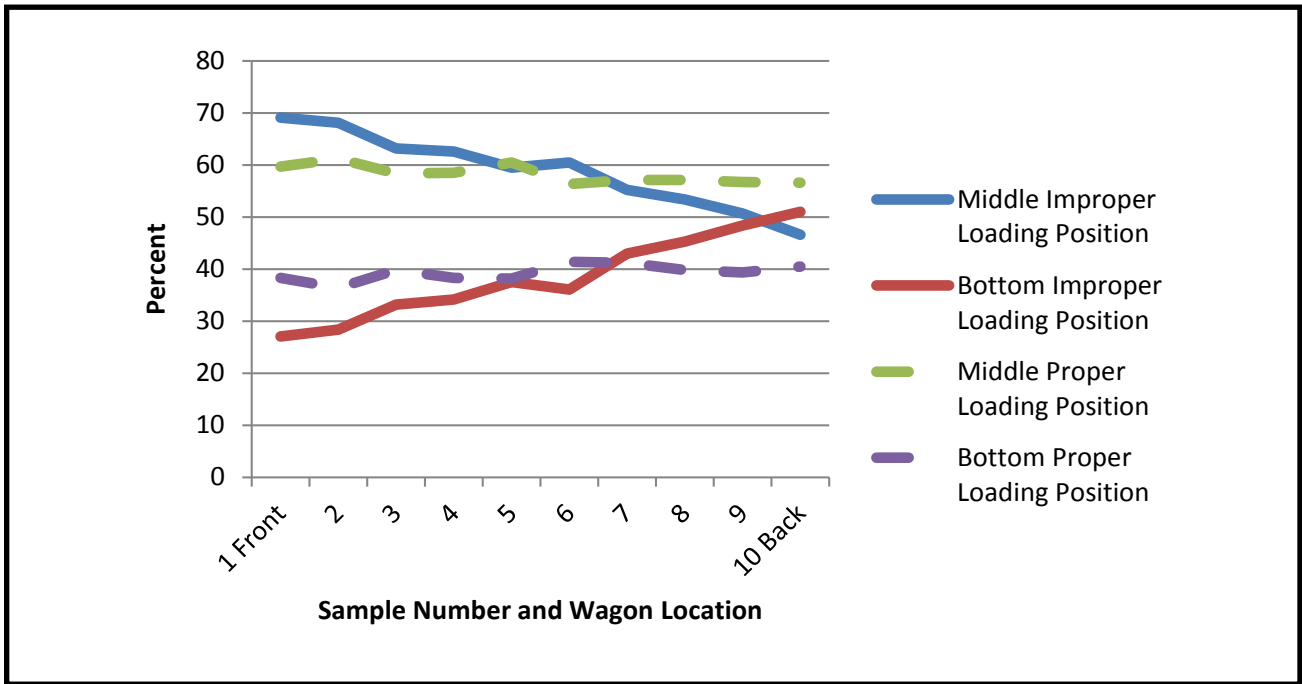


Figure 2. The influence of loading liquid whey in the front vs. center of a dual-auger vertical mixer on levels of TMR in the middle and bottom screens of the Penn State shaker box.

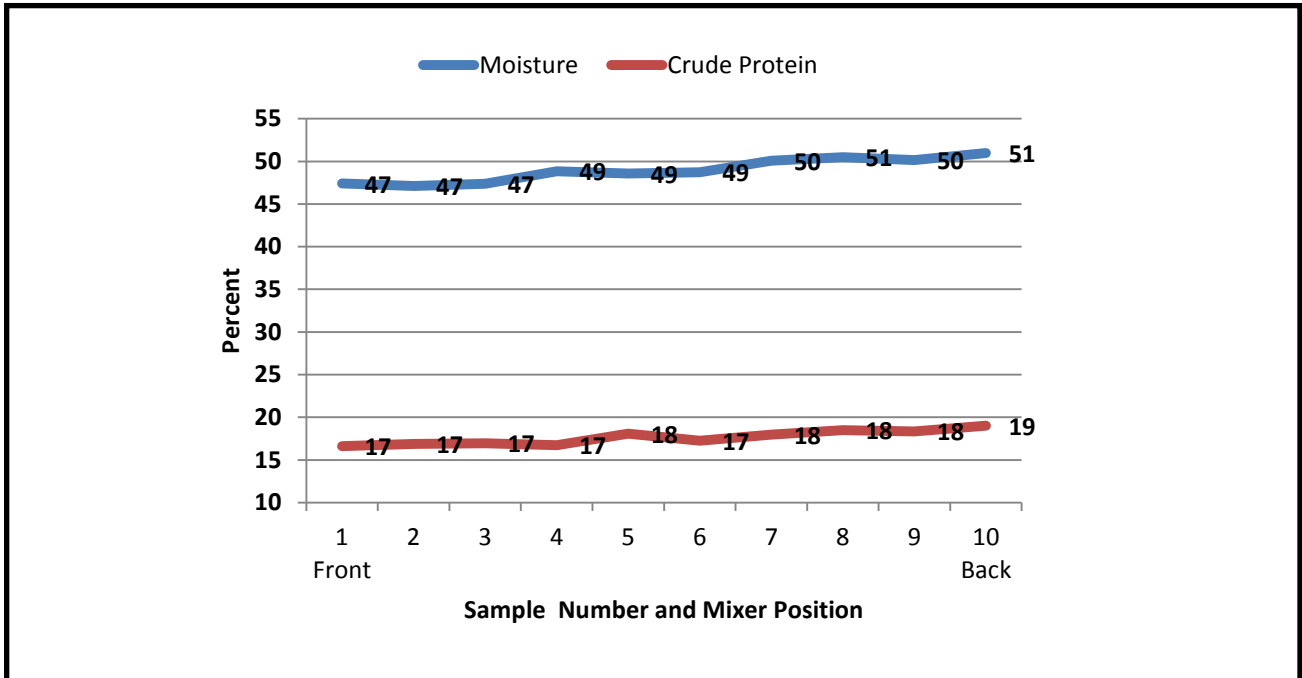


Figure 3. The influence of loading a liquid protein supplement in the back of a dual-auger wagon on moisture and crude protein levels in the TMR.

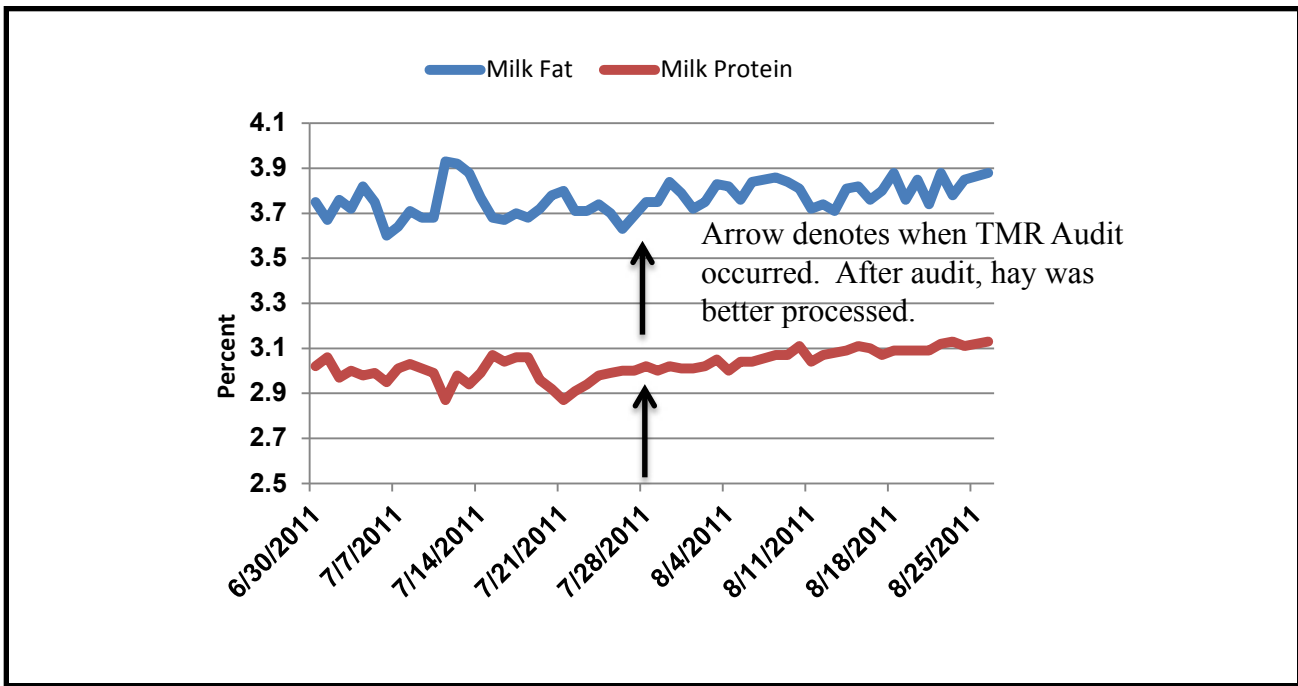


Figure 4. Milk fat and protein concentrations in the bulk tank before and after hay was better processed.

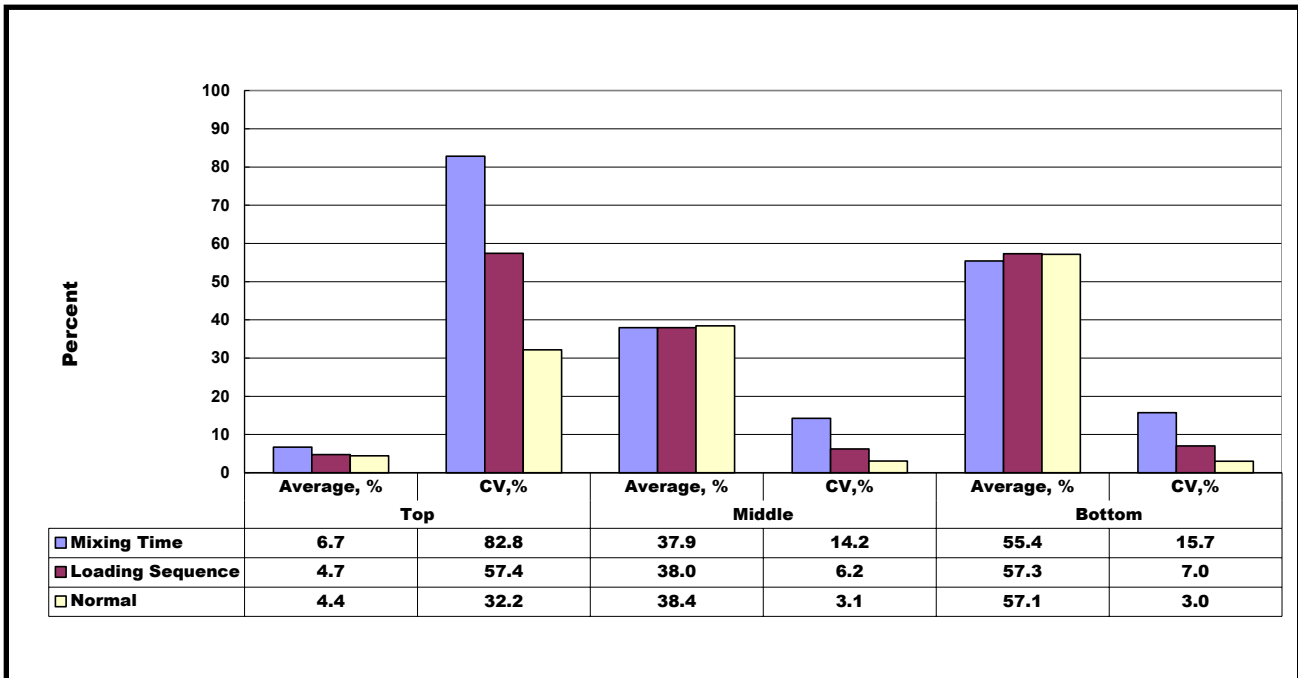


Figure 5. Influencing of mixing time after the last added ingredient and loading sequence on TMR variation (CV = coefficient of variation).



Figure 6. Example of how liquid is added to a TMR.

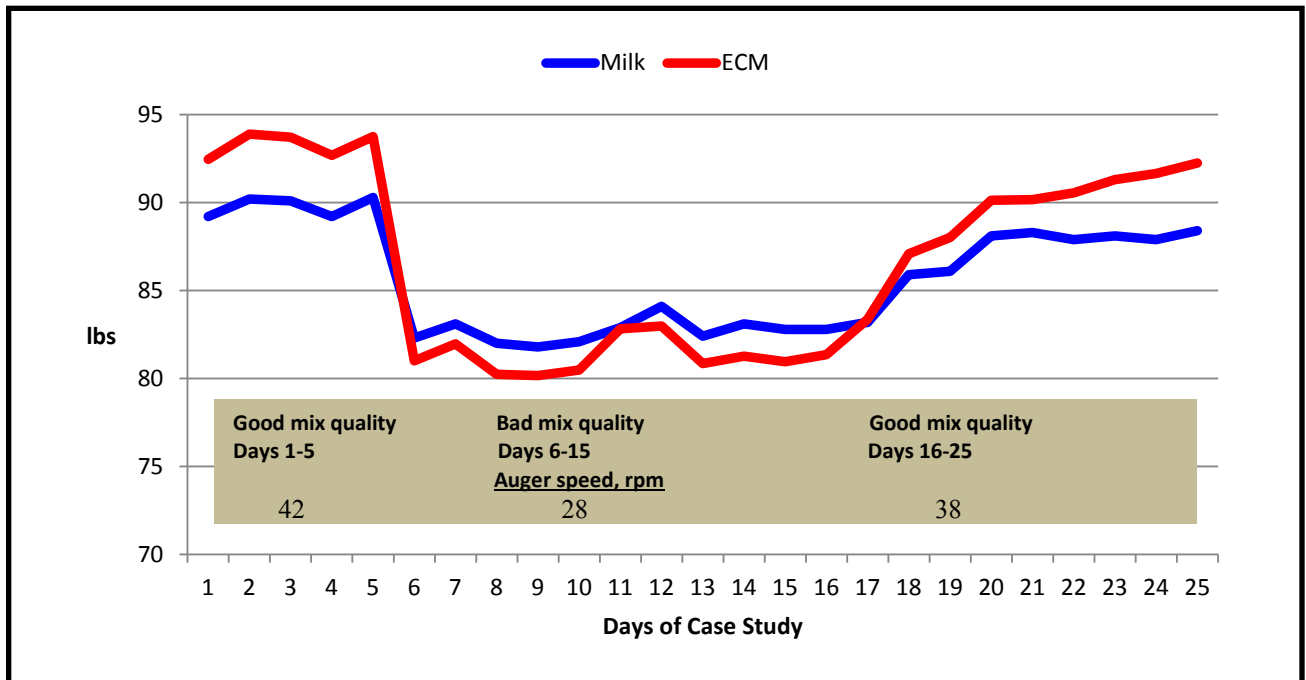


Figure 7. Influence of vertical mixer auger speed on TMR mix quality and milk production.

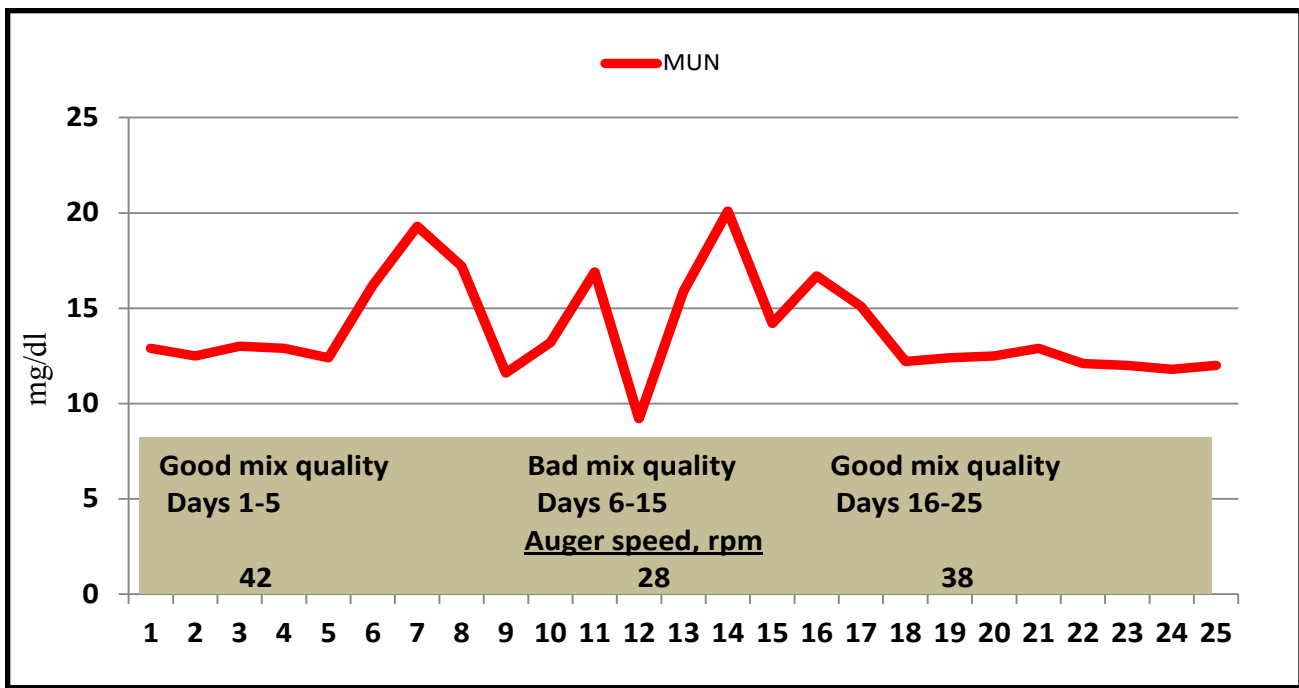


Figure 8. Influence of vertical mixer auger speed on TMR mix quality and milk urea nitrogen (MUN).

Update to the Food Safety Modernization Act

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The President signed the Food Safety Modernization Act (FSMA) into law on January 4, 2011. The 115-page law provides a host of new authorities for the U.S. Food and Drug Administration (FDA) not seen since the creation of the FDA in the federal Food, Drug and Cosmetic Act of 1938.

The centerpiece of the new law is Section 418: Hazard Analysis and Risk-Based Preventive Controls, which requires domestic food and feed facilities that manufacture, process, pack, or hold food or feed products to perform a hazard analysis at each facility for hazards that are “reasonably foreseen,” prepare adequate controls to “significantly minimize or prevent the occurrence” of each, monitor performance, and routinely maintain records. Facilities must maintain such records for at least 2 years.

FDA proposed 2 rounds of regulations for feed, food, produce and imported products to implement this new law. Facilities making products for export to the U.S. are required to comply with the same provision. Facilities that import products must assure that the foreign facilities have developed hazard analyses and written risk-based preventive controls. This can be accomplished by direct inspection of the foreign facilities or the use of FDA-accredited third party entities that can certify compliance with the new law.

FSMA provides FDA with authorities to revoke facility registrations, thereby halting operations at these plants. FSMA also grants FDA mandatory recall and administrative detention authorities under specific conditions or causes.

Implementation of the Safe Food Transportation Act of 1990 is required by FSMA, and FDA must promulgate rules within 18 months enactment of FSMA.

Congress is unlikely to fully fund the requirements of this new law (a budget request of \$300 million), including hiring 4,000 new field staff; 600 or more foreign facility inspections annually and the development of performance standards for affected industry, which are the tolerances/guidance levels for contaminants, among many other requirements.

The cost of preventive control regulations for the feed industry is estimated to exceed \$700 million and may cause delay in FSMA rulemaking. The food and feed industry estimates that training and compliance inspections will take 10 years to fully implement the provisions of FSMA. Focus on the final rules is expected to be on Current Good Management Practices (CGMP), which were not in FSMA.

For more information visit: www.fda.gov/food/foodsafety/FSMA.

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Dairy Sustainability - Using the Real Facts

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Summary

U.S. and global consumers have significant misperceptions about animal agriculture, and in particular, about dairying and dairy products. Two of these misperceptions are that dairy cattle are significant sources of methane and have a large impact on global warming and that cattle compete with humans for food, especially grain. This paper provides quantitative evidence to counter these misperceptions, which can be used to provide factual evidence to consumers that may help them in their life-style choices and their support of government policy and regulations. The evidence also supports the concept that the most sustainable production system is a mixed crop and animal system in terms of minimizing the impact of agriculture on the environment and ensuring an adequate food supply in the future.

Introduction

Sustainability concerns are often viewed as a three-legged stool: environmental, economic, and societal. In animal agriculture, we need to consider animal health and well-being as a fourth leg. While all these concerns are equally important and critical to the future of dairy sustainability, this paper will focus on greenhouse gas (GHG) emissions and the carbon footprint of dairy production and the

role of dairy in the global food supply with the goal of providing solid facts and numbers that can be used to address consumer concerns about U.S. dairy production.

GHG Emissions and C footprint

The GHG include methane (CH₄), carbon dioxide (CO₂), nitrous oxide (N₂O), and halocarbons. In the atmosphere, these GHG enhance the effects of solar and thermal radiation and can increase surface and atmospheric temperatures. In dairy production, the big 3 GHG are CH₄, CO₂, and N₂O. Methane has several natural sources (termites, wetlands, peat bogs, ocean sediments, and wildlife) and man-made or anthropogenic sources (natural gas production, coal mining, wastewater treatment, landfills, and agriculture). In agriculture, methane is derived from enteric fermentation in monogastric animals as well as ruminants, and anaerobic fermentation in manure storage from all species. With farming, CO₂ is counted only if it is derived from fossil fuel use, including electricity generation. CO₂ emitted from cattle is considered part of the natural, biogenic C cycle as the carbon arises from digestion and metabolism of plant material ingested as feed, and plants derive the carbon from fixing CO₂ in photosynthesis. In agriculture, N₂O arises from internal combustion engines, N fertilization, and manure. GHG emissions are often converted to CO₂ equivalents (CO₂e) that take

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into account the different half-life and radiative forcing of the gases and thus their potential for atmospheric warming.

Myth: cattle are major GHG emitters

Fact: Dairy cattle are a minor contributor to total anthropogenic GHG emissions in the U.S.

Globally, dairy animals contribute only 4% to anthropogenic GHG emissions (EPA, 2011a). There are 1.46 billion cattle around the globe, of which 266 million are lactating cows (FAOSTAT, 2015). In developed countries, the contribution of anthropogenic GHG emissions from dairy cattle is even lower due to increased livestock productivity and dilution by emissions from other sectors (Knapp et al., 2014). In the U.S., dairy cattle operations directly contribute 0.55% to anthropogenic GHG emissions (EPA, 2011b; Knapp et al., 2014), and the entire dairy production chain accounts for less than 2.9% (Thoma et al., 2010; Figure 1). The second number may be an over-estimate due to inclusion of GHG emissions associated with the production of co-product feedstuffs, such as soybean meal and dried distillers' grains and other assumptions made in the life-cycle assessment (Thoma et al., 2010). That study resulted in an estimate of 2.05 lb CO₂e/lb milk, whereas a FAO study (2010) gives ~1.10 lb CO₂e/lb milk.

So why are we concerned about CH₄? It's partly political, partly economic. The U.S. EPA has focused on emissions from CH₄ and N₂O in international policy discussions because they are less expensive to mitigate than CO₂ emissions since CO₂ is associated with fossil fuel use and economic development. Frequently at the farm level, CH₄ mitigation approaches can increase profitability, as well as being environmentally beneficial. Secondly, methane from enteric fermentation and manure

comprise more than 40% of the GHG emissions associated with fluid milk production in the U.S. (Thoma et al., 2010). Thus, if we implement strategies to decrease methane per unit of milk produced, we can lower the dairy C footprint. There are good opportunities to further reduce GHG emissions per unit of milk and keep dairy products competitive (Knapp et al., 2011).

With regards to providing an adequate and nutritious food supply, it's more meaningful to look at GHG emissions per unit of product, which is termed methane intensity. In the U.S. and other developed countries, we have the most efficient dairy production systems in terms of GHG emissions per unit of milk (Figure 2).

Fact: Production practices in the U.S. minimize the environmental impact of dairying.

Sustainable Intensification

Globally, it's going to take improvements in production efficiency to produce enough dairy products to feed 9+ billion people in 2050, while minimizing the environmental impact of dairy production. This concept is being called "sustainable intensification" and is typified by dairy production in North America, Europe, Israel and other developed countries. FAO (2011) projects that global demand for dairy products will exceed 1.1 billion tons by 2050 due to increased population and per capita demand, or a 60% increase over 2010 (Cady and Green, 2015). Sustainable intensification has the potential to minimize the impact of increased dairy production on feed, water, and land utilization, as well as reducing GHG emissions per unit of milk. It is possible with existing management strategies and technology to increase milk production while decreasing the number of dairy cows and the feed and water required to support that production. On

a global basis, to achieve this would require increasing milk yields by 100 lb/cow/year, which is significantly greater than historical improvements of 22 lb/cow/year but less than the 285 lb/cow/year in the U.S. and other developed countries (Cady and Green, 2015; FAOSTAT, 2015).

Myth: Confined, intensive animal operations are bad for the environment.

Fact: Production efficiencies achieved in intensively managed dairy operations have the lowest environmental impacts in terms of GHG emissions and resource utilization per unit of product.

Unique Role of Ruminants in Our Food Supply

Ruminant livestock have the unique capability of converting large amounts of inedible plant material to edible foods, e.g. milk and meat. Around the world, grazing land exceeds arable crop land by three-fold. Currently in the U.S. ~400 million acres are cropped, whereas there are over 615 million acres of grazing land. In addition to grazing and harvested forages, ruminants have a higher capacity than monogastric animals to utilize by-product feedstuffs.

Human food production generates a significant amount of by-products as part of growing crops and processing (Figure 3). These by-products include crop residues, milling and oilseed by-products from primary processing, secondary products from the baking industry, etc., spent grains from the brewing, distilling, and ethanol industries, animal proteins from the slaughtering and rendering industries, and recycled food waste. From an economic standpoint, many of these byproducts have significant value and thus are termed

co-products, but from a human food supply perspective they are by-products.

Myth: Livestock and poultry compete for food with humans.

Fact: The only part of U.S. dairy rations that's potentially edible by humans is grain, which comprises less than 20% of the total feed utilized in dairy production.

By-products typically comprise 20 to 25% of livestock and poultry diets in the U.S. (Figure 4). In dairy production, rations also contain significant amounts of forage. The only part of dairy rations that's potentially edible is the grain, most commonly corn, including the grain portion of corn and small grain silages. The grain in silages is a grey area with regards to edible food. In the Midwest, it is very possible for a farmer to make the decision between chopping corn for silage or harvesting it for grain. However, in the Northeast, the growing season is not long enough to produce corn grain and growing corn silage is the best way to maximize crop yield on land that would otherwise be pasture or forest.

Taking into consideration the amount of feed utilized in replacement heifer, dry cow, and lactating cow diets, the grain portion of dairy rations is less than 20% of the total feed. Given that the majority of the grain is corn, which for consumption by U.S. citizens is largely processed, the net amount of edible food used in dairy feeding is less than 10% (Figure 4). By adding 20% grain into lactating cow diets, milk production is increased by 67%, from 45 lb/day for grazing cows to 75 lb/day for cows fed TMR. It's analogous to a fuel additive that gives you more miles per gallon!

How much by-product feedstuffs are produced in food processing? Over the 2009

to 2013 crop years, an estimated 137.5 million tons (as is basis) of by-products were produced from the primary processing of crops, oilseeds, fruits, vegetables, sugar beets, and almonds, and the net production of human food was 136.7 million tons (Knapp, 2015). Where would these by-products go if they weren't fed to livestock and poultry? They can be disposed of by composting, combusting, and fermenting to generate electricity, tilling back into the soil as an amendment, and landfilling. Composting and combusting can eliminate much of the solid mass, but this occurs with a substantial release of CO₂ into the atmosphere (Russomanno et al., 2012). It seems much better to capture this carbon in meat and milk. Annual U.S. landfill capacity is 134 million tons (EPA, 2013). Thus, feeding by-products to livestock and poultry reduces the C footprint of foods consumed by omnivores, vegetarians, and vegans. In essence, the most efficient food production system is a mixed crop and animal system.

Fact: Production and processing of primary crops for human consumption in the U.S. generates as much by-products as it does edible food.

World-wide, by-products from grain and oilseeds generate 410 million tons of feedstuffs each year, with another 1890 tons of crop residues available for feed (Knapp and Cady, 2015). With continued increases in crop yields, it's conservatively estimated that there will be 574 million tons of by-products and 2640 millions tons of crop-residues available in 2050. This amount of feed can go a long way towards feeding livestock without compromising the food supply for humans, and in combination with improvements in animal agriculture, can provide an adequate supply of food for the global population without compromising the environment. The use of by-products reduces the need for grain feeding and results in more

food available for humans. There is a double benefit achieved in utilizing by-products in animal feeding, first, by sparing grain for human consumption, and secondly, by converting inedible feedstuffs to highly nutritious, edible animal products.

Conclusions

In this age of electronic communications, consumers have access to lots of information regarding agriculture and food production. However, not all of it is factual. To be prepared with facts and provide them openly when consumers seek them is in the best interest of all of us who are engaged in animal science and agriculture.

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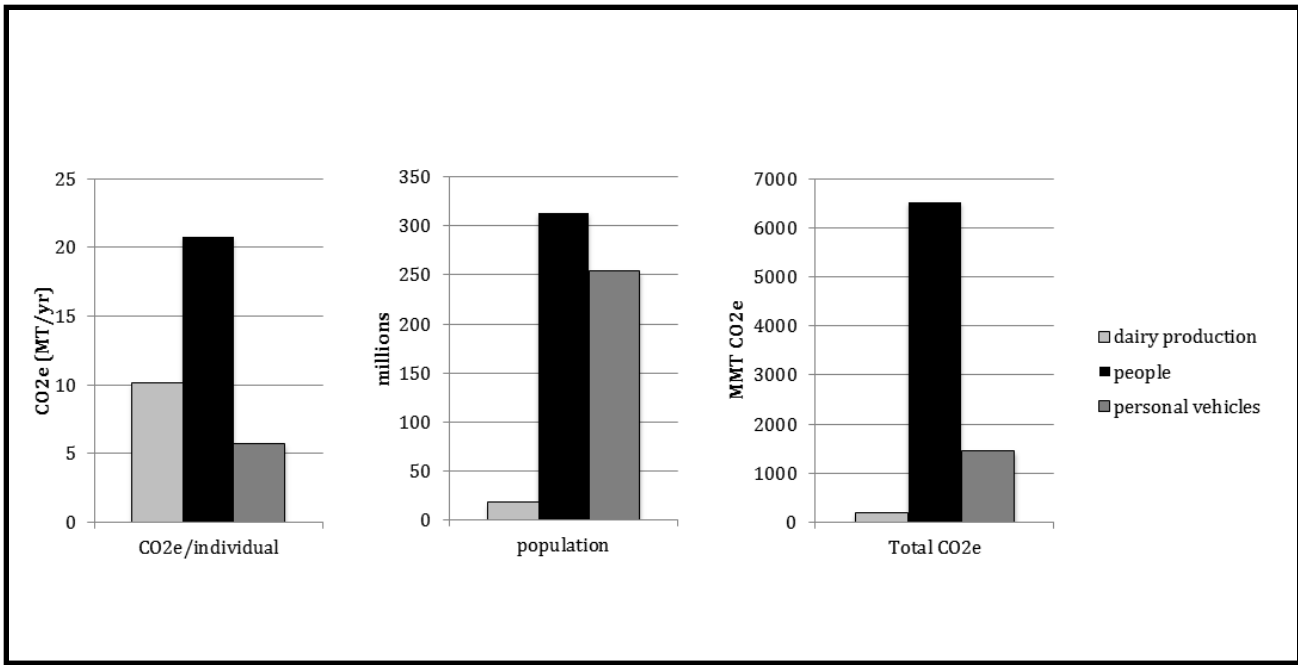


Figure 1. CO₂e from dairy production and personal vehicles compared to 2012 total anthropogenic emissions in the U.S. Dairy production includes the entire chain from farm to consumer and is based on 9.235 million lactating cows + 9.2 million replacement heifers. Data from Thoma et al., (2010), EPA (2013), and USDA-ERS (2015). MT=metric tonne, and MMT=million metric tonnes.

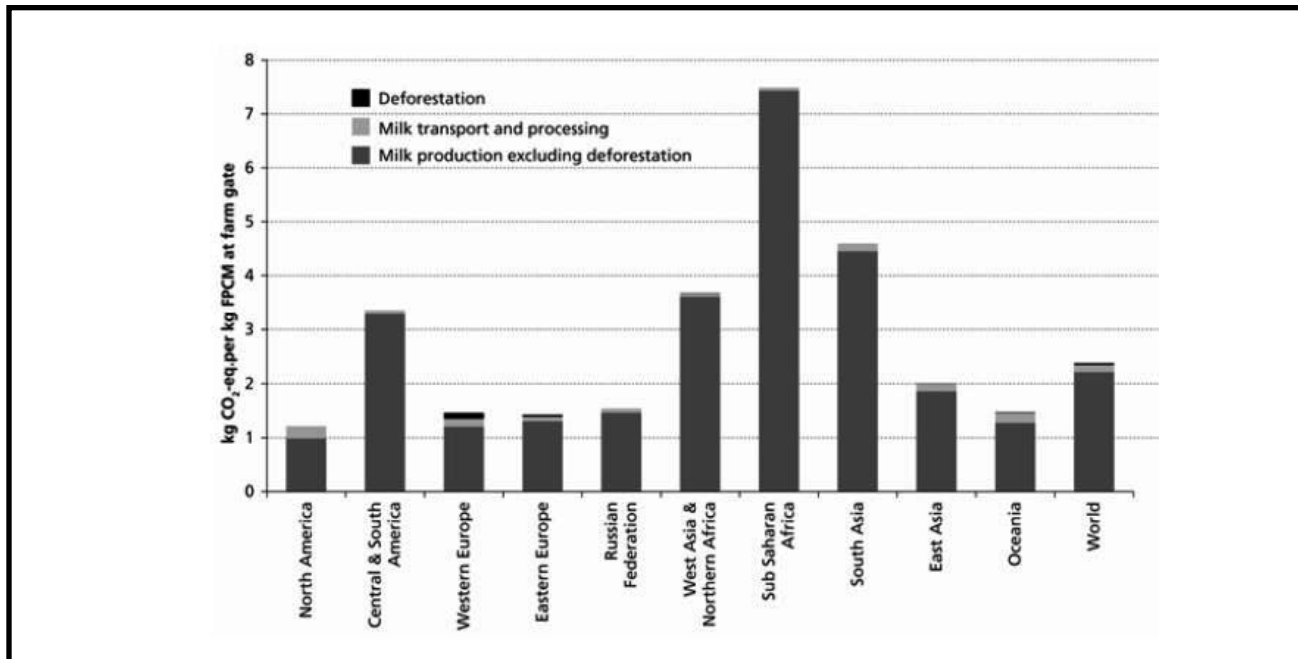


Figure 2. GHG emissions per unit of milk for different regions around the world (FAO, 2010).

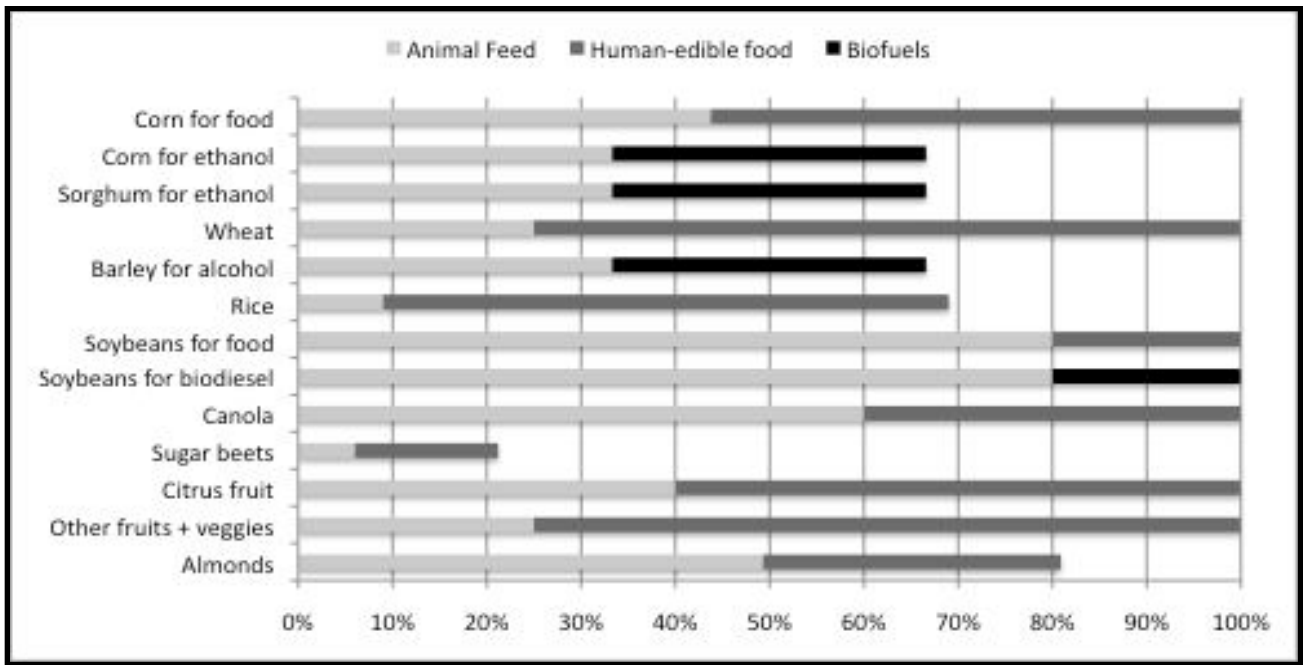


Figure 3. Proportion of by-product animal feeds generated when crops are processed for human food or biofuels. In certain cases, the products of crop processing do not add to 100%. Fermentation to ethanol results in 33% loss of grain mass as CO₂. With rice, sugar beets, and almonds, the discrepancy represents rice hulls, water loss, and almond shells, respectively.

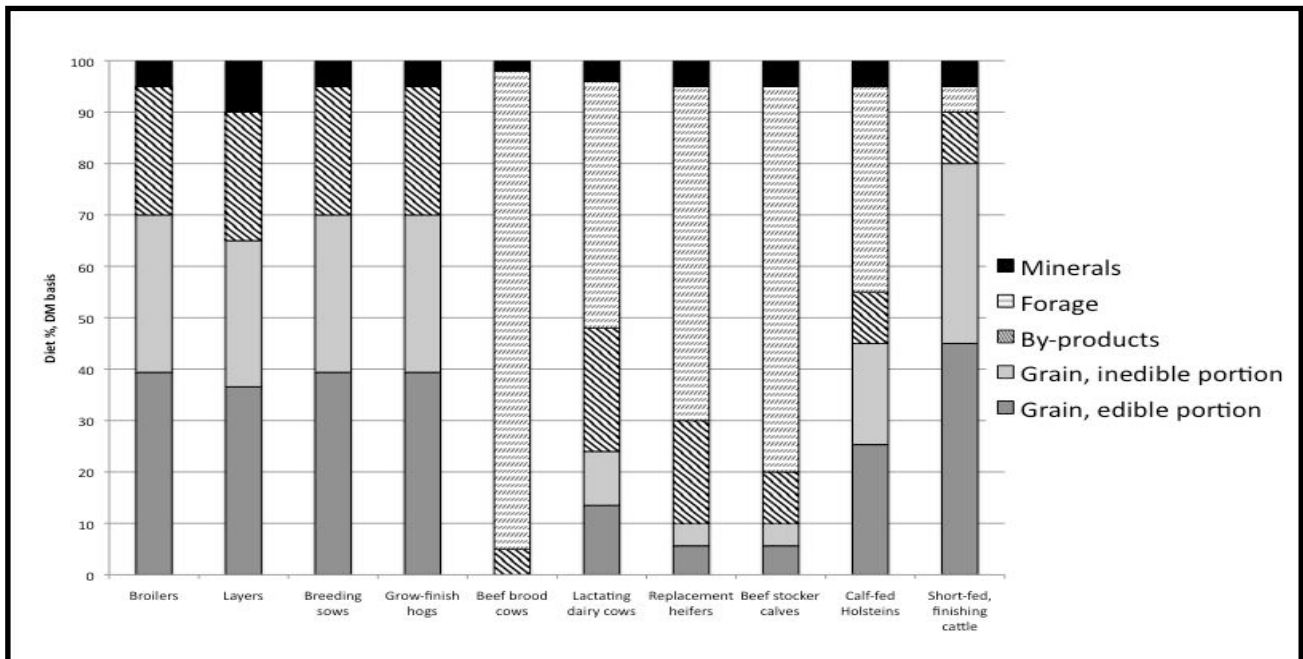


Figure 4. Proportions of grain, by-products, and forage in typical commercial U.S. livestock and poultry diets, dry matter basis.

Why All of the Hype About Feeding Canola Meal?

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Abstract

The U.S. dairy industry is the largest user of canola meal worldwide. A survey conducted in 2011 revealed that respondents believed canola meal provides good value, but they indicated that additional research was needed to better understand how to take advantage of this meal.

Introduction

Canola meal is a relatively new feed ingredient. It was developed in the 1970s to maintain the beneficial properties of rapeseed meal and to remove the anti-nutritional factors that were hampering the use of that meal by the livestock feeding industry. As a result, canola meal and its predecessor, rapeseed meal, are the second most widely traded oilseed meals in the world, as well as being Canada's most valuable crop (Casséus, 2009).

Canola has seen steady growth. In 2014, Canada produced more than 15 million metric tonnes (mmt) (www.canolacouncil.org), and the United States produced over 1.1 mmt (www.uscanola.com) of canola seed. After the oil is removed, approximately 56% of the seed remains as meal. Most of the meal produced by both countries is used by the U.S. dairy industry.

The Canola Council of Canada (CCC) commissioned a survey in 2011 (Evans and Hodgins, 2012) to assess the current perceptions regarding canola meal, as well as industry needs. The results indicated that more data are needed on the feeding value of this product. Oddly enough, a good portion of those taking the survey found that production results obtained when feeding canola meal appeared to be better than predicted by the profile used in nutritional models. This contrasted with models in 2011 that described canola meal as a protein that was highly soluble, provided lower levels of rumen undegraded protein (RUP) than other sources, and was also characterized as being relatively low in energy. There seemed to be a disconnect between calculations by formulators and utilization by cows, and this further underscored the need for additional research to assist the industry. With the rapid growth in canola meal availability and the acceptance of new formulation technologies by the dairy industry, new information was needed to provide accurate feeding values to the industry. As a result, the CCC invested in further research at 5 major North American institutions.

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Review of Recent Findings

So, who is right? Results from recent meta-analyses

A meta-analysis is a statistical procedure for pooling results from several studies and getting a fuller picture of what may be taking place. This approach is also useful for pinpointing where additional research resources need to be directed. How the results are used is determined by the questions asked in the first place.

Huhtanen et al. (2011) wanted to know how increasing ration protein using either soybean meal or canola meal compared for dairy cows. Was one meal superior to the other? Their dataset consisted of 292 treatment results published in 122 studies, carefully restricted to include only studies in which increasing protein in the ration was accomplished by adding canola meal as compared to soybean meal. For each additional pound of protein supplied in the diet, milk production increased by 3.4 lb with canola meal, and 2.4 lb with soybean meal, showing a 1-pound advantage for canola meal. The researchers found these results puzzling; they suggested that the RUP of soybean meal relative to canola meal was overestimated and that canola meal could replace soybean meal.

Martineau et al. (2013) posed a somewhat different question. The researchers looked at the effects of replacing protein in the diet from several vegetable sources of protein by using the same amount of protein from canola meal. There were 49 different peer-reviewed trials included in the dataset that they used. The average amount of canola meal tested was 5.1 lb, with the feeding level from 2.2 to 8.8 lb in the various studies. At the average level of inclusion, canola meal increased milk yield by 3.1 lb when all the protein compared were

considered, but only by 1.5 lb when canola meal was substituted for soybean meal. Milk protein yield followed the same pattern. Once again, canola meal appeared to be superior to other protein sources when included at the same level of protein.

The same group of researchers (Martineau et al., 2014) then conducted an additional meta-analysis study to compare canola with other proteins with respect to concentrations of plasma amino acids. The responses in these studies proved that canola meal increased plasma concentrations of total amino acids, including total essential and all individual essential amino acids, more so than other vegetable protein meals. Furthermore, blood and milk urea nitrogen concentrations were decreased. This meta-analysis strongly suggests that canola meal feeding increased the absorption of essential amino acids, which was responsible for the increased milk protein secretion and the increased protein efficiency.

Something is off. How in the world do you calculate RUP?

Based on most of the models available, canola meal should not be supplying enough RUP to increase the amino acids available to the cow that were revealed by the last meta-analysis (Martineau et al., 2014). If the same amount of protein is supplied by several vegetable protein sources, but plasma amino acids and milk protein yield are higher with canola meal, then the value being ascribed to it must be wrong. Could something be wrong with the methods used to determine RUP? This could have an impact on how diets are formulated.

Of the various models available, the National Research Council (2001) protein evaluation scheme bares similarities to other methods, but it is the least tedious to review.

The A fraction, determined as soluble protein, is instantly degraded in the rumen and is not available to supply amino acids as RUP. The C fraction is unavailable and indigestible, and by definition, not degraded in the rumen at all. The B fraction is calculated as the difference (100 – (A + C)). Some of this fraction is degraded in the rumen and some becomes RUP. How much of that becomes RUP depends on the rate that the fraction is solubilized by rumen microflora (the rate of digestion (Kd)), along with the rate of passage of particles out of the rumen (Kp).

To put this in terms of an equation:

$$\text{RUP} = \text{B fraction} * (\text{Kp}/(\text{Kd} + \text{Kp})) + \text{C fraction}$$

It is important to note that the calculation assumes that the entire portion of the A fraction that becomes soluble in the rumen is degraded there and does not contribute to RUP. Some other models calculate that most of the soluble fraction is degraded in the rumen. These models give the A fraction a very high rate of degradation, from 100 to 500%/hr. With such high rates, very little solubilized material would get past the rumen. Newer research suggests that this is in fact not true.

Table 1 provides a case in point. Swedish scientists Hedqvist and Udén (2006) elegantly demonstrated that proteins could be soluble but may not be degraded. These scientists measured the Kd rates on the soluble fraction of the crude protein and found that these Kd rates are actually quite variable among ingredients. Does this matter? The results clearly show that it does.

Hedqvist and Udén (2006) determined that the portion of the soluble protein that does not break down leaves the rumen with the liquid outflow and contributes to the fraction described by the National Research Council

(2001) as RUP. The effective protein degraded — or the amount that is actually degraded in the rumen — varied from more than 70% of the protein for wheat distillers' grains and soybean meal, to under 50% for canola meal (or rapeseed meal) and flax meal (Table 2). These calculations show that on a meal basis, canola meal actually does contain a high amount of RUP, just as the researchers concluded from the meta-analyses.

Results from newer feeding experiments

Research conducted at the U.S. Dairy Forage Research Center by Broderick et al. (2012) evaluated the variability of canola meal based upon the source. The type of equipment used to extract the oil and the techniques used can have an impact on the value of the protein to dairy cows. These details can be used to optimize meal production parameters. The researchers also looked at how proteins degrade in the rumen and are re-evaluating the use of traditional in sacco methods.

An interesting study conducted by Brito and Broderick (2007) compared lactational performances of cows given 17% diets in which supplemental protein was supplied by urea, soybean meal, cottonseed meal, or canola meal (Table 3). It was expected that the urea diet would supply the least RUP. Unexpected was the fact that the soybean meal diet provided less RUP than either cottonseed meal or canola meal. Cows given the canola meal diet at the same level of protein produced 2.0 lb/day more milk than their counterparts that were given soybean meal.

Continuing in this vein, Faciola and Broderick (2013) compared diets formulated to supply 15 and 17% CP, using either soybean meal or canola meal as the supplemental source (Table 4). Cows receiving the diets with canola

meal again out-produced cows consuming soybean meal by approximately 2.0 lb of milk - unexpectedly, at both levels of protein!

Corn distillers grains are another ingredient that is a good value and widely available, but it is difficult to use in diets that may already be high in corn protein from grain and silage. Two studies have demonstrated that blending distillers with canola meal allows cows to better utilize both ingredients. Mulrooney et al. (2009) learned that milk production and feed efficiency were improved by mixing these 2 vegetable proteins sources (Table 5). Similarly, Swanepoel et al. (2014) evaluated milk production when cows were given either high protein distillers grains or canola meal (Table 6). Both meals have the same amount of protein, and in the treatments, each supplied 20% of the total diet DM with the various combinations of these meals. Once again, the mixtures of the 2 meals were demonstrated to improve milk output, feed efficiency, and gain in body condition score. It would seem that using mixes of canola meal and distillers grains will help dairy producers to get the most from both ingredients.

Canola meal contains more fiber than soybean meal. Because of the fiber content, there was concern that it might not be an appropriate protein for high-forage diets. Schuler et al. (2013) conducted an experiment to compare milk production with diets ranging in forage from 42 to 66% of the total diet DM. All diets utilized canola meal as the supplemental source of protein. As Table 7 shows, there was no loss in energy-corrected milk when cows consumed the high-forage diets.

Canola meal calculator

Canola meal may be ideally suited to dairy rations in a wide range of feeding

situations. However, the real value will depend upon the cost relative to other available protein sources. Comparing costs, however, can be a daunting task. Should ingredients be compared on the basis of CP alone or on RUP? Some protein sources are high in energy and others bring a valuable nutrient, like phosphorus, to the table.

Some years back, Howard and Shaver (2004) put together a spreadsheet, FeedVal4, that allowed ingredients to be compared on the basis of their total CP, RUP, energy, fat, calcium and phosphorus contents. With permission, this system was modified to allow costs of feed proteins to be evaluated. Canola meal does not always win on the basis of cost, but the canola meal calculator will provide fair assessments and has been widely received by the industry. It can be found at canolamazing.com/resources/canola-meal-calculator and is a free resource for all to use to their best advantage.

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Table 1. Rates (Kd) of digestion of the soluble fraction of protein in the rumen for selected ingredients.¹

Vegetable Protein Source	Soluble protein, % of total CP ²	Kd, ³ % degraded/hr
Canola meal (rapeseed meal)	20.4	19
Flax (linseed meal)	58.6	18
Lupins	80.2	34
Peas	77.8	39
Soybean meal	16.9	46
Wheat distillers grains	24.3	62

¹Hedqvist and Udén, 2006.²CP = crude protein.³Kd = rate of digestion.**Table 2.** Calculated effective protein degradation, RUP and RUP contributed by meals.^{1,2}

	Effective protein degradation, %	RUP, % of CP	Protein, % of meal DM	RUP, % of meal DM
Canola meal (rapeseed meal)	44	56	36.9	20.6
Flax (linseed meal)	46	54	26.8	14.5
Lupins	56	44	33.8	14.9
Peas	71	29	25.0	7.25
Soybean meal	73	27	50.6	13.7
Wheat distillers grains	79	21	37.5	7.9

¹Hedqvist and Udén, 2006.²RUP = rumen undegraded protein.

Table 3. Comparison between vegetable proteins and urea.¹

Measurement	Added Protein			
	Urea	Soybean meal	Cottonseed meal	Canola meal
% of ration DM ²	1.9	12.1	14.1	16.5
Microbial protein, g/day	2,340	2,710	2,710	2,780
RUP ³ , g/day	540	990	1,350	1,150
Total protein entering the intestines	2,880	3,700	4,060	3,930
DMI ⁴ , lb/day	48.7	54.3	54.5	54.9
Milk yield, lb/day	72.5	88.2	89.3	90.6
Protein yield, lb/day	2.03	2.71	2.60	2.80
Fat yield, lb/day	2.23	2.69	2.60	2.84

¹Brito and Broderick, 2007²DM = dry matter³RUP = rumen undegraded protein⁴DMI = dry matter intake**Table 4.** Performance of lactating dairy cows fed low- or moderate-protein diets with canola meal or soybean meal.¹

Measurement	15% CP		17% CP	
	Soybean meal	Canola meal	Soybean meal	Canola meal
Dry matter intake, lb/day	54.6	55.6	55.4	56.1
Milk yield, lb/day	86.9	88.4	87.8	90.4
Protein yield, lb/day	2.62	2.66	2.66	2.73
Fat yield, lb/day	3.43	3.50	3.52	3.63

¹Faciola and Broderick, 2013.**Table 5.** Synergistic effects between canola meal (CM) and corn distillers grains (DDGS).¹

Measurement	Diet			
	CM	2/3 CM 1/3 DDGS	1/3 CM 2/3 DDGS	DDGS
DMI, ² lb/day	55.4	55.9	57.0	55.2
Milk, lb/day	77.4	78.8	79.9	75.5
Milk fat, lb/day	2.95	3.19	3.01	2.90
Protein, lb/day	2.37	2.42	3.21	2.26
Energy-corrected milk (ECM), lb/day	80.7	84.5	79.2	78.5
ECM/DMI	1.46	1.53	1.42	1.44

¹Mulrooney et al., 2009.²DMI = dry matter intake.

Table 6. Synergistic effects between canola meal (CM) and corn distillers' grains (DDGS).¹

Measurement	Diet			
	DDGS	2/3 DDGS 1/3 CM	1/3 DDGS 2/3 CM	CM
DMI, ² lb/day	53.0	53.7	54.6	53.6
Milk, lb/day	99.0	104.5	105.5	104.4
Milk fat, lb/day	3.44	3.62	3.58	3.50
Protein, lb/day	2.87	3.05	3.08	3.04
Milk/Feed	1.87	1.95	1.93	1.95
Change in body score/28 days	0.01	0.03	0.08	0.03

¹Swanepoel et al., 2014.²DMI = dry matter intake.**Table 7.** Evaluation of forage levels in diets containing canola meal as the main source of protein¹

Measurement	Forage, % of DM ²			
	42	50	58	66
DMI, ³ lb/day	61.8	59.4	56.8	54.6
Milk, lb/day	88.2	88.9	89.8	86.0
Milk fat, lb/day	2.77	2.81	2.97	3.01
Protein, lb/day	2.61	2.66	2.64	2.51
Energy-corrected milk (ECM), lb/day	83.6	84.5	86.9	85.1
ECM/DMI	1.36	1.44	1.54	1.57

¹Schuler et al., 2013.²DM = dry matter.³DMI = dry matter intake.

Current Knowledge of the Ruminal Fermentation System and What Can We Expect to Learn in the Future

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Introduction

The ruminal fermentation system is simply the fermentation of dietary organic matter (OM) in the rumen, resulting in usable end products (e.g., volatile fatty acids (VFA), microbial protein, and long chain fatty acids (LCFA)) for the dairy cow. This fermentation system has the unique ability to degrade, convert, or alter over 85% of the cow's diet, which means the rumen compared to all of the body's organs exerts the most influence on milk production and composition. The function of the rumen and the microbial community housed within are, therefore, critical to optimizing the performance of the dairy cow. How the rumen is physically structured and fundamentally works is explained well in numerous reviews and books (Van Soest, 1982; Krehbiel, 2014) and will not be the focus of this paper. The focus of this paper will be on our current knowledge of the rumen microbial communities and potential opportunities to manipulate milk synthesis and composition.

Regulation of Milk and Milk Component Synthesis

Regulation of milk and milk component synthesis in the mammary gland is related to 3 areas: milk volume, milk protein yield and composition, and milk fat yield and composition. Milk volume is under osmotic

regulation with lactose as the major osmolyte that regulates the amount of water drawn into the aveoli of the mammary gland (Akers, 2002). As a result, those diets that promote the major precursor for gluconeogenesis, propionate, have the ability to increase lactose synthesis, and in turn, milk volume, especially in early lactation (McCarthy et al., 2013).

Milk protein yield is controlled primarily by the metabolizable energy supply from the diet via insulin, IGF-1, and other energy signaling pathways, and by the substrate supply of essential amino acids (EAA) in the blood (Bionaz, et al., 2012). Changing energy content of diets will change milk protein yield (Reynolds, et al., 1994), while changing energy balance through feed restriction and realimentation has been shown to change milk protein percentage, but not milk fat percentage (Gross, et al., 2011). Increased circulating blood insulin levels have been shown to increase milk protein yield (Winkelman and Overton, 2010).

The EAA profile of rumen-generated microbial protein (RGMP) is highly similar to the EAA profile of milk, and the intestinal digestibility of microbial protein is consistent and generally greater than sources of rumen undegradable protein (RUP) (Block, 2006). Depending on the microbial efficiency, RGMP can theoretically represent 50 to 79% of the total metabolizable protein (MP) needs of

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a high-producing dairy cow (Block, 2006). However, the variability of EAA composition in RGMP is quite high (Clark et al., 1992), which could affect predictions of MP EAA levels in the various software models in the market. The composition of the protein fractions in milk, on the other hand, are minimally influenced by nutritional changes in the cow's diet (Hadrova, et al., 2007).

Milk fat yield and composition are both highly influenced by dietary factors (Bauman, et al., 2008). De novo synthesis of medium and short chain FA in the mammary gland is controlled by substrate supply and production of inhibitory isomers of conjugated linoleic acid (**CLA**). The incorporation of LCFA into milk fat is also affected by the dietary supply as modified by ruminal biohydrogenation. Acidosis and subacute ruminal acidosis (**SARA**) has been intimately linked to incomplete biohydrogenation of LCFA in the rumen and the occurrence of milk fat depression (**MFD**) (Bauman et al., 2008; Khafipour et al., 2009). Therefore, manipulation strategies that change or stabilize the supplies of energy, AA, or LCFA coming from the rumen have an opportunity to change or stabilize milk yield and composition.

The Rumen Microbiome

Microbiome is the term used to describe the totality of all of the microorganisms residing in a particular environment (e.g., rumen). Historically, we have been trained that there are 3 distinct populations of microorganisms that make up the rumen microbiome: bacteria, protozoa, and fungi (Van Soest, 1982). However, with the advent of improved culture techniques and molecular biological techniques, such as 16S rRNA sequencing, we have come to recognize other distinct populations of microorganisms that exert influence on rumen function: archaea (i.e., methanogens) and bacteriophage (i.e., viruses).

Bacteria

The bacteria in the rumen are the most significant population of microorganism with 10¹⁰ CFU per g of rumen contents (Russell, 2002) and representing 60 to 85% of small subunit rRNA (Lin et al., 1997). Our understanding of the diversity of the bacterial species in the rumen has increased from those we can culture, approximately 200 recognized species, to those we cannot, > 3,500 operation taxonomic units (OTU), which is the species level designation when only DNA sequence data are available (Kim et al., 2011). Given this diversity, the bacterial population is normally discussed or grouped based on main substrate fermented: starch-degraders or amylolytic, fiber-degraders or cellulolytic, protein-degraders or proteolytic, fat-utilizers or lipolytic, etc.

Protozoa and fungi

The protozoa are divided into 2 types, flagellated and ciliated, and though low in number (10³ to 10⁶ per g of contents), physically they can amount for 50% of the cellular biomass (Dehority, 2003). The genetic diversity of protozoa is limited to < 50 OTU (Kim et al., 2011). Protozoa, because of their bacterial predation, are major players in N recycling, starch degradation, and fiber breakdown, as well as maintaining a symbiotic relationship with the methanogens (Firkins, 2012).

Fungi in the rumen are considered to be important in fiber degradation due to their extensive mycelial structures that invade plant tissues and their wide array of cellulolytic, hemicellulolytic, glycolytic, and proteolytic enzymes (Liggenstoffer et al., 2010). However, they are present in small numbers (10³ spores per g of contents) with limited genetic diversity (10 to 60 OTU) (Fouts et al., 2012).

Archaea (i.e., methanogens)

The methanogens were for many decades classified as members of the bacterial community, but the work of Woese (1987) redefined the evolutionary status of the methanogens to a new domain, Archaea. The methanogens scavenge H₂ in the rumen, allowing for more complete fermentation of substrates but at the cost of approximately of 5 to 7% of dietary gross energy (GE) through methane emissions, which makes cows targets for greenhouse gas (GHG) reduction strategies worldwide (Hristov et al., 2013). The Archaea are present at 10⁷ cells per g of contents.

Bacteriophage (i.e., viruses)

Bacteriophage, often referred to as phage, are bacterial viruses that are involved in lysis of bacterial cells, as well as horizontal transfer of genetic material (Kleive et al., 1996). They occur at 10⁹ to 10¹¹ particles per mL of rumen fluid and are distinctly associated with the predominant bacterial populations in the rumen (Berg Miller et al., 2012).

Manipulation of the Rumen Microbiome

Historically, we have assumed that we have changed the microbial population dynamics in the rumen with a variety of feed additives. These shifts in populations were ascribed by the circumstantial evidence of changes in pH, VFA concentrations, microbial protein flow, and digestibility of different diet fractions (e.g., fiber, starch, protein, etc.). Assessment of actual changes in microbial populations was limited to those few organisms that were culturable (Krause et al., 2013). Over the last 30 years, the development of molecular biological techniques have given rise to the field of metagenomics, which allow for culture-independent analysis of the changes

in the rumen microbial ecosystem (Krause et al., 2013). For a very good review of these techniques, please refer to Chaucheyras-Durand and Ossa (2014) and McCann et al. (2014). In the future, these techniques will continue to increase our understanding of the dietary and environmental impacts on the rumen microbiome, and how those impacts can be replicated to provide consistent and measurable performance responses in dairy cattle.

The idea of consistency leads to a discussion of responders and non-responders. Why do some cows respond to a feed additive or diet change while others do not? Genetic finger printing studies demonstrate how animal-to-animal variation controls the rumen microbial ecosystem. Work at the USDA Forage Center in Wisconsin by Weimer and colleagues illustrated clearly the impact of the cow when they performed near-total exchange (>95%) of rumen contents between 2 cows with very different ruminal pH, VFA concentrations, and bacterial community compositions (BCC) and followed the changes in BCC for the next 60+ days (Weimer et al., 2010b). Ruminal pH and VFA for both cows returned to pre-exchange levels within 24 hr. However, the BCC of both cows returned to original pre-exchange profiles in 14 and 61 days, respectively. These results show that the cow has tremendous control over ruminal pH and VFA content, even though the BCC is the source of VFA production.

If a cow can recover its original BCC from a perturbation, such as a one time, near total exchange of rumen contents, then how do cows and their rumen microbiome respond to perturbations from daily dietary changes or feed additives? In work examining BCC under MFD conditions, Weimer et al. (2010a) screened 18 cows to find clusters of the cows that demonstrated MFD with either rapidly

fermented starch (**RFS**) or monensin (**M**) addition, or the combination of both (**RFS/M**). The researchers then compared the BCC of the liquid and particle associated bacterial populations within each cluster of cows to determine how the BCC shifted with each dietary treatment. Several interesting results were observed: 1) Cows within a cluster (i.e., having the same response in milkfat to a specific treatment) had different BCC, 2) The liquid and particle associated BCC were similar within individual cows, 3) Cows sensitive to RFS or M demonstrated large changes in BCC while cows sensitive to both RFS/M and non-responding cows showed small changes in BCC, and 4) For cows that demonstrated a MFD response to diet, the BCC did not return to its original structure with removal of monensin from the diet. Together, these results suggest that while the cow dictates its individual BCC, the responses to MFD inducing diets are directly associated with changes in BCC.

If the cow exerts such remarkable control over the rumen through passage rate (i.e., intake), buffering through saliva production, and rate and extent of absorption of VFA, then how similar is the rumen microbiome between cows consuming the same diet? Jami and Mizrahi (2012) compared the rumen bacterial populations across 16 cows fed the same diet consisting of 70% concentrate:30% forage. The researchers found that of 250 OTU identified, 32% were present in 90% of the cows and only 19% of the OTU were present in all of the cows. In terms of abundance (i.e., amount) of each OTU in individual samples, there was < 60% similarity across samples. These results point to low similarity in the rumen bacterial populations both in presence and level of the OTU, which may have an impact on how effective dietary changes are across a group of animals. On the other hand, there may be a core bacterial population that if properly defined

and targeted could allow for more consistent responses to dietary changes.

Opportunities to Manipulate the Rumen Microbiome and Possibly Cow Response

Shifting dairy cattle diets to generate more propionate (i.e., propiogenic) versus acetate is often accomplished by increasing dietary starch content or starch fermentability, or by addition of monensin (McCarthy et al., 2013). However, the effect of these treatments on the rumen microbiome has been shown to be variable. Belanche et al. (2012) fed 11.7 vs. 30% starch diets to lactating Holstein cows, and found decreases in protozoa (-38%), fungi (-59%), and methanogens (-27%), while total bacteria did not change with high starch diets. However, known cellulolytic bacterial populations were unaffected by increasing dietary starch content. Working with lactating Holstein cows, Lettat et al. (2013) fed diets with 0, 50, or 100% of the dietary forage as corn silage (**CS**), which linearly increased dietary starch from 17.0 to 30.0%, and found a 4-fold decrease in protozoa, a 2-fold increase in total bacteria, and a 1.5-fold increase in methanogens. And, while ruminal pH declined linearly with increasing CS in the diet, the known cellulolytic bacterial populations did not significantly change. Thoetkiattikul et al. (2013) fed crossbred dairy cows diets containing either 2, 10, or 21% starch and found a linear decrease in the genera of cellulolytic bacteria with increasing starch level. So, while increasing dietary starch can increase propionate available for gluconeogenesis, the rumen microbiome response across cows has not been defined such that a consistent response can be expected.

The most common benefit afforded to monensin is the inhibition of Gram-positive (**G+**) bacteria, which shifts the ruminal fermentation to greater propionate

concentration at the expense of acetate concentration (McGuffey et al., 2001). However, numerous studies demonstrate no effect on the acetate:propionate ratio (Oelker et al., 2009; Mathew et al., 2011; Reveneau et al., 2012), which has been attributed to rapid adaptation of G+ bacteria (Weimer et al., 2008). As previously described, ruminal bacterial populations exhibit variable changes to monensin supplementation (Weimer et al., 2010a). Monensin supplementation does not change total protozoal levels in the rumen but does cause small variations in the population composition (Arakaki et al., 2000; Reveneau et al., 2012). Archaeal populations in the rumen show little change to monensin supplementation (Hook et al., 2009). Therefore, the effect of monensin on the rumen microbiome may be related to impacts on individual bacterial species rather than whole populations.

The variability of the nutrient composition of microbial flow from the rumen is well documented for both EAA profile of the RGMP (Clark et al., 1992; Martin et al., 1996) and the fatty acid profile (Or-Rashid et al., 2007). That variability may be related to the proportions of protozoa and bacteria flowing from the rumen, as well as proportion of liquid vs. solid-associated bacteria (Belanche et al., 2011). Firkins and colleagues at The Ohio State University have done extensive research on the recycling or selective retention of protozoal populations in the rumen, and how that will affect RGMP flow (Firkins et al., 2007). The presence of protozoa has been shown to increase the ratio of 2 major bacterial phyla, Firmicutes:Bacteroidetes, and increase ammonia-N levels in rumen contents (Ozutsumi et al., 2005). Treatments to alter or reduce EAA profile variation in RGMP should consider effects in both the liquid and solids fractions, since the liquid and solids-associated populations of both protozoa and bacteria have

different EAA profiles and different flow rates from the rumen (Martin et al., 1996; Hook et al., 2012).

The manipulation of milk fat content and composition through alterations in the rumen microbiome is heavily challenged by the relationship between acidosis/SARA and biohydrogenation of LCFA. The definition of acute acidosis is the sudden and uncompensated drop in rumen pH to < 5.0 (Krause and Oetzel, 2006), while the definition of SARA is prolonged periods of moderately depressed ruminal pH (Krause and Oetzel, 2006; Plaizier et al., 2009). There is disagreement in the literature as to the pH threshold for SARA onset varying from 5.5 to 6.0 (Krause and Oetzel, 2006; Plaizier et al., 2009). While the ruminal fermentation conditions of SARA are similar across studies, the changes in the rumen microbiome vary widely, depending on the causative agent (Khafipour et al., 2009). Khafipour et al. (2009) demonstrated that grain-induced SARA causes significant increases in *S. bovis*, an amylolytic bacterium producing lactic acid, and concomitantly, *M. elsdenii*, a lactate-utilizing bacterium. When Khafipour et al. (2009) induced SARA with pelleted alfalfa, there were no changes in *S. bovis* or *M. elsdenii*, but *Prevotella* spp. increased significantly in relation to other known bacterial species. These differences in bacterial composition could drive how we work to prevent or treat SARA on a farm level. Grain-induced SARA is directly related to diet formulation; whereas, alfalfa pellet-induced SARA is related to both diet formulation and feeding management as particle size of dietary components is a key factor in the latter.

Based on the biohydrogenation theory of MFD (Bauman et al., 2008), dietary and feeding management factors that alter ruminal fermentation (e.g., elevated starch or grain, oil,

presence of monensin, or reduction in particle size) will result in altered ruminal FA metabolism due to changes in the rumen microbiome, increasing the flow of polyunsaturated FA (PUFA) through an alternative pathway of biohydrogenation. The SARA induction models described by Khafipour et al. (2009) fit with the biohydrogenation theory as *M. elsdenii*, elevated during grain-induced SARA, and *Prevotella* spp., elevated during alfalfa pellet-induced SARA, have been implicated in MFD (Palmonari et al., 2010; Jami et al., 2014).

More recently, Jami et al. (2014) was able to demonstrate a relationship between the ratio of 2 major bacterial phyla, Firmicutes:Bacteroidetes, in the rumen and daily milk fat yield. Across 15 cows fed the same diet, an increasing Firmicutes:Bacteroidetes ratio was positively correlated with milk fat yield ($R^2 = 0.51$). In humans and mice, an increased Firmicutes:Bacteroidetes ratio in the gut microbiome is associated with increased energy harvest and body fat tending towards obesity (Ley et al., 2006; Turnbaugh et al., 2006). Additionally in the work of Jami et al. (2014), among the 42 common core genera (i.e., those genera found in >50% of the cows sampled), *Prevotella*, found in the Bacteroidetes phylum, were strongly negatively correlated with milk fat yield (Pearson $R = -0.69$, $P = 5 \times 10^{-3}$). On the other hand, *Bifidobacterium* and *Lactobacillus*, both common probiotic genera, were positively correlated with milk fat yield. Weimer et al. (2010b) also found specific species of bacteria were associated with the responsiveness of individual cows to MFD inducing dietary treatments. Therefore, even with a limited core of bacterial species across cows, defining and targeting those bacterial species involved with specific performance responses may represent an opportunity to modulate milk composition in the future.

Aside from diet formulation and feeding management, the opportunities for controlling SARA and MFD by changing the rumen microbiome may rest with probiotics. Probiotics are, by definition, viable microorganisms or endproducts of their fermentation that when consumed in adequate amounts confer a health benefit on the host (FAO, 2001). In the dairy cattle industry, there are 2 general groups of probiotics, bacterial-based and fungal-based, which are termed, direct-fed microbials (DFM).

Bacterial-based DFM normally contain a variety of species with wide ranging metabolic activities. There are limited demonstrations of changes in the rumen microbiome with bacterial-based DFM. Chiquette (2009) using culture-dependent techniques, demonstrated that *E. faecium* and *S. cerevisiae* (ES) fed under SARA conditions did not affect *R. flavefaciens*, *F. succinogenes*, *R. albus*, or *M. elsdenii* levels in lactating dairy cows. More recently, Chiquette et al. (2012) using a combination of ES and *P. bryantii* (PB) were able to demonstrate an increase in *R. flavefaciens*, but no effect on *F. succinogenes*, *R. albus*, or *M. elsdenii* levels in lactating dairy cows fed to induce SARA. The supplementation of PB alone had no effect on any measured bacterial populations.

Fungal-based DFM are either a live yeast or yeast culture. Live yeast are defined as active dry yeast products that must contain >15 billion live yeast cells/g (AAFCO, 2011). Yeast cultures (YC) are products from yeast fermentation that contain live yeast and fermentation by-products and are not dependent on live yeast for their physiological effects (AAFCO, 2011). There are numerous benefits to rumen function attributed to fungal-based DFM: 1) stimulate growth of beneficial microorganisms, 2) improved fiber digestion, 3) reduced lactate concentrations, 4) reduced O_2 concentrations, 5) improved starch utilization, and 6) moderation of ruminal pH.

The suggested mode of actions that elicit those benefits include: production of stimulatory AA, peptides, vitamins, and organic acids; out competing lactate-producing bacteria for available carbohydrates; and scavenging of O₂ by live yeast cells. There are numerous thorough reviews of yeast-based DFM available in the literature (Chaucheyras-Durand et al., 2012).

There are numerous studies that demonstrate changes in the rumen microbiome with yeast supplementation. Harrison et al. (1988) fed YC to ruminally fistulated Holstein cows, and using culture-dependent techniques, they found yeast supplementation to increase total anaerobic bacteria and cellulolytic bacteria compared to controls. Mathieu et al. (1996), using culture dependent techniques, also found an increase in total bacteria but a decrease in cellulolytic bacteria with supplementation of YC. Arakaki et al. (2000) examined the impact of YC on protozoa counts in ruminally fistulated steers and found that while the total protozoal counts did not change, the protozoal species composition changed with *Dasytricha* increasing and *Entodinium* decreasing. Using culture independent techniques, Mosoni et al. (2007) examined the effect of YC supplementation on 3 known species of cellulolytic bacteria, *F. succinogenes*, *R. flavefaciens*, and *R. albus*, and found that YC caused 2 to 4-fold increases in *R. flavefaciens* and *R. albus* but had no effect on *F. succinogenes* populations. More recently, Pinloche et al. (2013) examined the effect of YC on the rumen microbiome in early lactation Holstein cows fed to induce SARA. The supplementation of YC produced a 2-fold increase in *Megasphaera spp.* and *Selenomonas spp.*, both lactate utilizing bacterial populations, and a 2-fold increase in *Fibrobacter spp.* and *Ruminococcus spp.*, both cellulolytic bacterial populations. Conversely, there was a 25% decrease in *Prevotella spp.* and 7-fold decrease in *Mitsuokella spp.*, both starch-

degrading bacterial populations. AlZahal et al. (2014) also demonstrated several fold increases in *F. succinogenes*, *Anaerovibrio lipolytica*, *R. albus*, and anaerobic fungi when active dry *Saccharomyces cerevisiae* was supplemented to lactating Holstein cows fed to induce SARA. All of these results support the mode of action of yeast on rumen function, and in turn, the performance responses of supplemented animals.

Summary

The advent of molecular biological techniques that allow for whole genome analysis of the ruminal microbiome have allowed researchers to examine the effects of dietary changes or additives on whole populations of microorganisms and individual species in relation to the performance responses observed in the host animals. These studies illustrate how the cow has tremendous control over both rumen function and the microbial populations within the rumen and that there is a limited common core bacterial population across groups of cows consuming the same diet. The individual cow control and limited core explain some of the animal to animal variation observed in performance with dietary changes. Future research should focus on understanding how to manipulate this common core microbial population in order to generate consistent responses across a wide group of animals.

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Saturated and Unsaturated Fatty Acid Pretreatment Regulates [1-¹⁴C] C16:0 Metabolism in Madin-Darby Bovine Kidney Cells

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Metabolic fates of fatty acids (FA) may be influenced by circulating FA concentration. The capacity for FA oxidation, glucose production, and energy metabolism in transition cows is linked to activity of pyruvate carboxylase (PC) enzyme. PC enzyme activity is related to PC mRNA abundance, which is increased during feed restriction and transition to lactation, metabolic states when blood non-esterified FA are elevated. Previous work in our lab demonstrated an ability of C18:3n-3 *cis* to ameliorate PC gene expression after depression by either C16:0 or C18:0 in Madin-Darby bovine kidney (MDBK) cells, a model of metabolic control in ruminants. Our objective was to determine effects of copresence of saturated and unsaturated FA pretreatments on cellular partitioning of [1-¹⁴C] C16:0 metabolism to CO₂ or acid-soluble products (ASP) in MDBK cells. Cells at 80% confluence were exposed for 21 h to either individual FA bound to bovine serum albumin (C16:0, C18:0, C18:1n-9 *cis*, or C18:3n-3 *cis*) or FA cocktails in 10:90, 25:75, 50:50, 75:25 or 90:10 ratios for combinations of C16:0: C18:3n-3 *cis* or C18:0: C18:3n-3 *cis* or C18:1n-9 *cis*: C18:3n-3 *cis*. Total pretreatment FA concentration was 1.0 mM. Following pretreatment, cells were incubated with 1.0 mM [1-¹⁴C] C16:0 for 3 h. Pretreatments with either C16:0 or C18:0 alone significantly ($P < 0.01$) depressed subsequent oxidation of [1-¹⁴C] C16:0 to ASP by 62.7 and 41.2%, respectively, compared to C18:3n-3 *cis* pretreatments. Pretreatments with C18:1n-9 *cis* either alone or in any combination with C18:3n-3 *cis* did not significantly ($P > 0.10$) depress subsequent [1-¹⁴C] C16:0 oxidation to ASP. Similar patterns were seen with [1-¹⁴C] C16:0 oxidation to CO₂. ASP production from [1-¹⁴C] C16:0 was positively correlated ($r = 0.68$, $P < 0.01$) with PC gene expression, while CO₂ production from [1-¹⁴C] C16:0 did not show a correlation ($r = 0.30$, $P > 0.10$) with PC expression. Results show regulation of ketone production by MDBK cells in response to FA pretreatments. Activation of PC gene expression by unsaturated FA may play a critical role in determining metabolic fates of FA. Modifying diets of close-up dairy cows to contain limited quantities of unsaturated FA, particularly C18:3n-3 *cis*, may be a feeding strategy to improve transition cow outcomes.

Calcium Hydroxide Treated Corn Stover as an Alternative Forage Source for Lactating Holstein Cows: Effects on Milk Production and Milk Composition

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Crop residues can potentially replace traditional forage feedstuffs in diets for dairy cattle. However, crop residues typically have low nutritional value, limiting their inclusion in diets for lactating cows. The objectives of this study were to determine the effect of prestorage hydration and treatment with 6.6% Ca(OH)₂ on feeding value of corn stalks as an alternative feed for lactating cows and the impact on milk production, milk composition, and feed intake. Mid-lactation multiparous Holstein cows (n = 30) were stratified by parity and milk production and randomly assigned to one of three diets. Corn stalks were chopped, hydrated, and treated with 6.6% Ca(OH)₂ (DM basis) and stored in Ag-bag silos. Treated corn stover was fed in a TMR at 0, 15, and 30% of the diet DM. Treated corn stover replaced either alfalfa haylage (15% stover) or replaced alfalfa haylage and an additional portion of corn silage (30% stover). Cows were individually fed in tie stalls for 10 wk. Milk production was not altered by treatment ($P = 0.80$). Compared with 0% stover diet, DMI was reduced when the 15% stover diet was fed (57.0 vs. 49.9 ± 1.9 lb/day, $P < 0.05$) and tended to be reduced (57.0 vs. 50.8 ± 1.9 lb/day, $P = 0.08$) when cows were fed 30% stover diet. Milk production per unit DMI (lb/lb) tended to increase for cows fed 15% stover diet compared with 0% stover diet (1.41 vs. 1.62 ± 0.07 , $P = 0.08$) but was not different between cows fed 0% and 30% stover diets (1.41 vs. 1.50 ± 0.07 , $P = 0.62$). Milk composition, energy corrected milk (ECM) production, and ECM produced per unit of DMI (lb/lb) was not different ($P > 0.05$) among treatments for the 10-wk feeding period. Cows fed 15 and 30% diets had stable DMI and daily milk production over the 10-wk treatment period, but DMI for cows fed 0% stover increased slightly (time x treatment effect, $P < 0.05$). Results indicate that Ca(OH)₂-treated stover can replace up to 30% of the diet DM by replacing either alfalfa haylage or alfalfa haylage and an additional portion of corn silage. These data indicate that corn stover processed through prestorage hydration with Ca(OH)₂ results in an alternative feedstuff for mid-lactation dairy cows and provides improvements in efficiency of converting feed to milk without altering milk production or milk composition.

Effects of Pre- and Post-Weaning Nutrition on Growth, Efficiency, and Rumen Fermentation Characteristics of Holstein Calves

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Feeding pre-weaned dairy calves high planes of nutrition has been shown to increase weight and frame size at weaning; however, little information exists on the effects of pre-weaning nutrition on weaned calf performance up to 28 wk of age. The objective of this study was to evaluate the interaction of pre-weaning and post-weaning nutrition on animal performance, blood metabolites, and rumen fermentation parameters. Holstein calves (95.9 ± 11.2 lb BW at birth; 39 heifers and 18 bulls) were assigned at 1 d of age to 1 of 4 treatments in a randomized complete block design with a 2×2 factorial arrangement of treatments. Pre-weaning milk replacer (MR) treatments were a 22% CP, 20% fat (as-fed basis) MR (C) or 28% CP, 20% fat MR (H), with weaning based on starter intake. Post-weaning treatments were low NFC (27% NFC on DM basis; LNFC) or high NFC (42% NFC; HNFC) grower diets fed individually for ad libitum intake from 12 to 28 wk of age. BW, skeletal measurements, and blood samples were taken every 2 wk during the pre-weaning period. Post-weaning, BW were taken every 2 wk and skeletal measurements, blood, and rumen fluid samples were collected monthly. Pre- and post-weaning periods were analyzed separately and overall from birth to 28 wk of age. Calves fed H were 15 d older, 39.7 lb heavier, and consumed 58% more DM through weaning compared to C ($P < 0.01$); however, feed efficiency (FE) was similar between H and C from birth to weaning ($P = 0.24$). From weaning to 11 wk, DMI was 53% greater for C ($P < 0.01$); however, ADG from weaning to 11 wk was similar, resulting in greater ADG from birth to 11 wk for H ($P < 0.01$). Hip height, hip width, and heart girth increased 2.7, 3.6, and 3.7%, respectively, for H over C at 8 wk of age ($P < 0.01$). Post-weaning, ADG was improved for HNFC ($P = 0.01$), resulting in a 19.2 lb advantage in BW at 28 wk ($P = 0.04$). Total DMI was similar between post-weaning treatments, and FE was significantly improved for HNFC from 12 to 28 wk ($P < 0.01$). Rumen fermentation and blood profiles were altered in favor of decreased acetate ($P = 0.09$), increased butyrate ($P = 0.01$), and reduced rumen NH₃ and plasma urea N ($P < 0.01$) for HNFC. Overall, calves fed H+HNFC were 27.3 lb heavier at 28 wk compared to calves fed H+LNFC, but similar in BW to calves fed C+HNFC. These results suggest that calves fed a high plane of nutrition early in life should continue to receive high planes of nutrition post-weaning to maintain pre-weaning growth advantages.

Genes Expressed in Milk Fat May Reflect Trace Mineral Status in Dairy Cows

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Determining trace mineral status in dairy cattle is difficult. To evaluate trace mineral status, some biological measure must accurately reflect the animal's current status for the trace nutrient (e.g. glutathione peroxidase activity and Se). For some minerals, the ability to determine status is possible but not practical on a large scale or recurring basis (e.g. liver Cu and Cu status). Proteomic analysis has shown that the milk fat globular membrane (MFGM) contains large concentrations of proteins associated with membrane/protein trafficking. We wanted to determine if mRNA expression of proteins associated with metal transport in the MFGM could be used to evaluate mineral status. The specific objectives of this study were to determine whether intake and source of Cu, Zn, and Mn affected erythrocyte Cu/Zn superoxide dismutase (SOD) activity and expression of genes in milk fat that are related to Cu, Zn, and Mn transport. Thirty multiparous (n = 18) and primiparous (n = 12) lactating Holstein cows were fed a diet void of supplemental Cu, Zn, and Mn (9, 41, and 41 mg/kg, respectively) for 30 d and then fed 1 of 3 diets for 30 d. One diet (UNSUP) contained no supplemental Cu, Zn, and Mn (9, 41, and 41 mg/kg); one diet (SUL) contained Cu, Zn, and Mn from sulfates (total concentrations = 17, 59, and 54 mg/kg, respectively); and one diet (GLY) contained Cu, Zn, and Mn in the glycinate form (B-TRAXIM[®] 2C, Pancosma; total concentrations = 20, 66, and 58 mg/kg). Using the NRC (2001) model and absorption coefficients (AC), UNSUP provided about 82% of requirements for Cu and Zn for primiparous cows and 95% for multiparous cows. Assuming an AC of 0.05 and 0.20 for Cu and Zn from supplements, supplemented diets provided 1.3 to 2.3 times more absorbed Cu and Zn than NRC requirements. Expression of several metal transport genes were analyzed using qPCR. Expression of Copper Chaperone for SOD (CCS), a protein that transports Cu to SOD, and SOD was negatively correlated ($P < 0.06$). Expression of most genes was not affected by treatment. Zip8 expression tended ($P = 0.10$) to be greater in cows fed supplemental Cu, Zn, and Mn, regardless of source. When supplemental Cu, Zn, and Mn were fed, cow requirements were likely exceeded and no differences were observed between mineral sources. Milk fat is easily obtained from lactating cows and our results (i.e., Zip8) demonstrate the potential of using expression of metal transport genes extracted from milk fat as indicators of trace mineral status.