Repeatability of metabolic responses to a nutrient deficiency in early and mid lactation and implications for robustness of dairy cows

J. J. Gross and R. M. Bruckmaier¹

Veterinary Physiology, Vetsuisse Faculty University of Bern, Bremgartenstrasse 109a, CH-3012 Bern, Switzerland

ABSTRACT

Nutrient partitioning toward the mammary gland during insufficient energy and nutrient supply is a strategy to ensure survival of the offspring in mammalian species. This homeorhetic priority of the mammary gland is also present in the modern dairy cow, in particular in early lactation. However, despite similar metabolic loads, the adaptive response to a given metabolic load varies considerably among animals. The aim of this study was to investigate if individual cows respond in a consistent manner to a negative energy balance (NEB) in early and mid lactation. Twenty-five dairy cows experienced the usual NEB after parturition and were subjected to a second 3-wk NEB induced by feed restriction in mid lactation. Animals were retrospectively ranked according to their highest plasma nonesterified fatty acid (NEFA) concentration in wk 1 to 4 postpartum. The animals with the 33% highest and 33% lowest values were selected and classified either as the high response (HR) or low response (LR) group. Before parturition, no differences in the studied parameters, dry matter intake, energy balance, concentrations of glucose, NEFA, β -hydroxybutyrate, cholesterol, triglycerides, growth hormone, and insulin-like growth factor-1, were detected between LR and HR. After parturition, milk yield and energy-corrected milk yield was higher for HR compared with LR in wk 2 to 14 and wk 1 to 6, respectively. During feed restriction in wk 15 to 17 postpartum, no differences in energy-corrected milk between LR and HR were found. Energy balance was more negative in HR during the NEB in early lactation, but not different from LR during feed restriction in mid lactation. Although plasma concentrations of glucose, growth hormone, triglycerides, and cholesterol showed group differences in early lactation, but not during feed restriction, the plasma concentrations of NEFA, β -hydroxybutyrate, and insulin-like growth factor-1 in

HR changed repeatedly to a greater extent during the NEB at the 2 stages of lactation compared with LR despite the similar extent of the NEB itself in both groups. The repeatedly greater amplitude of adaptive responses in HR compared with LR at different time points might partly indicate an underlying genetic background to enable a sufficient and rapid supply of mobilization-derived nutrients. The individual characteristics of adaptation to an energy and nutrient shortage might be beneficial when implemented in breeding programs.

Key words: adaptation, robustness, metabolic plasticity, negative energy balance, dairy cow

INTRODUCTION

The inevitable negative energy balance (NEB) at the onset of lactation reflects the most challenging period for dairy cows. To meet energy requirements for maintenance and serving the priority of nutrient partitioning to the needs of lactation, a comprehensive homeorhetic adaptation of metabolic and endocrine systems is required (Bauman and Currie, 1980). Metabolic priority of lactation declines with DIM and mammary gland metabolism becomes more sensitive to the homeostatic control of hormones such as insulin (Bauman and Currie, 1980). The different expression patterns of glucose transporters during the gestation-lactation cycle gives evidence for a more insulin-sensitive glucose uptake into the mammary gland in mid- and late lactation (Mattmiller et al., 2011).

Effects of a NEB in early and mid lactation on performance, metabolic, and endocrine parameters have been reported previously (Gross et al., 2011a,b) with markedly lower extent of metabolic stress during the NEB at the later lactational stage. Codrea et al. (2011) noted that the rate of recovery in milk yield due to a temporary nutritional shortage was unaffected by stage of lactation. Similarly, comparable losses in milk yield during short-term feed restrictions in early, mid-, and late lactation indicated that the metabolic responses due to feed restrictions are dependent on milk yield (Bjerre-Harpøth et al., 2012). However, the deviations

Received December 16, 2014.

Accepted June 17, 2015.

¹Corresponding author: rupert.bruckmaier@vetsuisse.unibe.ch

of plasma metabolites from basal values were shown to be dependent on the stage of lactation (Bjerre-Harpøth et al., 2012). In particular, the early lactation period characterizes dairy cows with diverse robustness to cope with the inflicted metabolic load more or less successfully (i.e., occurrence of metabolic disorders and loss in productive performance when failing to adapt).

Up to now, studies with nutritional challenges in dairy cows at different stages of lactation only focused on the extent of responses in performance and metabolism, but did not consider if cows responding more intensely in early lactation might again react more intensely in mid lactation at an individual level. This unique approach enables the characterization of adaptive responses in dairy cows. Therefore, the objective of this study was to investigate the plasticity in metabolic, endocrine, and performance traits in dairy cows during a NEB at 2 different stages of lactation. The hypothesis tested was that cows responding more intensely to an energy and nutrient shortage in early lactation also react more intensely to a feed-restriction-induced NEB in later lactation.

MATERIALS AND METHODS

Animal Trial

Twenty-five multiparous Holstein dairy cows (3.0 ± 1.1 parities; mean $\pm$ SD) were studied during a period from parturition up to wk 17 postpartum (**p.p.**). Cows experienced a NEB during the first week of lactation, and after getting into a positive energy balance (**EB**), all cows were exposed to a 3-wk feed restriction period (wk 15–17 p.p.) providing 50% of energy and nutrient requirements starting at 98 ± 7 DIM. Feed allowance was adjusted weekly during feed restriction to maintain the degree of NEB. Details on housing and feeding conditions were described earlier (Gross et al., 2011a).

Data Collection

Data on DMI and milk yield were collected daily, whereas BW, BCS, and backfat thickness (**BFT**) were determined weekly. Milk samples for determination of fat and protein content by FTIR were taken twice weekly. Energy balance and energy expenditure for maintenance and lactational requirements were calculated according to the guidelines of the German Society of Nutrition Physiology (GfE, 2001). Blood samples for analysis of plasma concentrations of glucose, NEFA, BHBA, triglycerides, cholesterol, IGF-1, and growth hormone (**GH**) were obtained weekly. Details on feed, milk, and blood analysis as well as calculation of the

RQUICKI index for estimation of insulin sensitivity were reported earlier (Gross et al., 2011a,b).

Statistical Analysis

For analysis of individual responses to an energy deficiency in early and mid lactation, data of the respective parameters of individuals were normalized relative to the mean value and SD of all animals. This method of normalization avoids the bias by different absolute values depending on the lactational stage and reflects the ranking of the individual animal. A regression analysis considering the repeated exposure to a NEB (in wk 3 and 15 p.p.) was performed with cow as the repeated subject. These particular weeks were selected as milk production steeply increased during a period of a NEB (in early lactation), metabolic load was highest (in wk 3 p.p. and wk 15 p.p. after the initiation of feed restriction) and metabolic adaptation still ongoing. In an additional retrospective analysis, cows were ranked according to their highest plasma NEFA concentration in early lactation (observations from wk 1–4 p.p.). The cows with the 33% highest and 33% lowest NEFA concentrations (8 animals each) were selected and classified as either high response (**HR**) or low response (**LR**) groups, respectively. Data were checked for normal distribution by the UNIVARIATE procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC). In cases of not being normally distributed, data were log-transformed. One animal showed a very high plasma BHBA concentration, which was repeatedly confirmed by laboratory analysis. Interestingly, the same animal, after a distinct time-span, again showed elevated plasma BHBA concentration during the nutritional challenge at a later lactational stage. Therefore, this individual animal contributes very much to the finding of a repeated pattern of individual adaptation responses, even in animals that show an extreme reaction. During data evaluation, we had a special focus on this animal, and even repeated our laboratory analyses to exclude any type of mistake in the measurements. We decided to keep this extreme individual in the data set. After log-transformation, all data were normally distributed and the one individual was not identified as an outlier anymore. Weekly data on performance, metabolic, and endocrine parameters of the 2 groups (HR and LR) were compared using the MIXED model in SAS (version 9.4, SAS Institute Inc.) including week, group (HR or LR), parity, and the week $\times$ group interaction as fixed effects and the individual cow as repeated subject. Differences between HR and LR over time were detected by the Bonferroni *t*-test. Significant effects were assumed at a level of $P < 0.05$.

Figure 1. Regression analysis of DMI (A), milk yield (B), energy balance (C), and backfat thickness (D) between the negative energy balance in wk 3 (early lactation) and 15 postpartum (feed restriction). Data are expressed as standard deviation relative to $\mu = 0$.

RESULTS

Performance Parameters

Within all animals, no significant relationship was found between DMI in early and mid lactation during feed restriction (Figure 1A). This can be attributed to the individual limitation and adjustment of feed allowance during feed restriction to maintain a NEB. Before parturition, DMI was not different between LR and HR (Figure 2A; $P > 0.05$). In early lactation, DMI was higher for LR compared with HR in wk 2, 3, and 5 (Figure 2A; $P < 0.05$). During feed restriction in wk 16

and 17 p.p., HR had also a lower DMI compared with LR (Figure 2A; $P < 0.05$).

Throughout the entire experimental period, BW, BCS, and BFT were not different between LR and HR (data not shown; $P > 0.05$). However, within all animals, a correlation was present of $r = 0.66$ of BFT between wk 3 and 15 p.p. (Figure 1D; $P < 0.001$).

Milk yield was higher in HR compared with LR ($P < 0.05$) between wk 3 p.p. and wk 15 p.p. during feed restriction. Within all animals, cows with a higher milk yield in early lactation also had a higher milk yield at wk 15 p.p. (Figure 1B; $P < 0.01$). In wk 16 to 17 p.p., milk yield was not different between HR and LR

Figure 2. Dry matter intake (A), milk yield (B), energy-corrected milk yield (C), and energy balance (D) in cows antepartum, postpartum, and during feed restriction. Data are given as mean values $\pm$ standard error of the mean. Differences between the groups [low response (LR) and high response (HR)] are marked with + ($P < 0.10$) and * ($P < 0.05$).

(Figure 2B; $P > 0.05$). The decline of milk yield during feed restriction was more intense in HR than LR ($P < 0.05$). However, ECM was higher in HR only during the first 6 wk of lactation compared with LR (Figure 2C; $P < 0.05$).

Before parturition, EB was not different between LR and HR (Figure 2D; $P > 0.10$). From wk 1 to 6 p.p., EB was more negative in HR compared with LR (Figure 2D; $P < 0.05$). During feed restriction, no differences in EB were detected between HR and LR. Due to limitation of feed allowance, no obvious relationship was observed between EB in early and mid lactation (Figure 1C).

Metabolic Parameters

Plasma glucose concentration within all animals during a NEB showed a correlation of $r = 0.55$ between wk 3 and 15 p.p. (Figure 3A; $P < 0.01$). Before parturition and until wk 1 p.p., no differences in plasma glucose concentration were detected between HR and LR (Figure 4A; $P > 0.05$). From wk 2 to 4 p.p., glucose concentration was lower in HR compared with LR (Figure 4A; $P < 0.05$). Thereafter and during feed restriction, no differences between groups were found.

Plasma NEFA concentration was higher in HR compared with LR in wk 1 to 6 p.p. and during feed restriction in wk 15 and 16 p.p. (Figure 4B; $P < 0.05$). Plasma NEFA and BHBA concentrations during the NEB in wk 3 and 15 p.p. were closely related [$r = 0.57$ ($P < 0.01$) and $r = 0.88$ ($P < 0.0001$), respectively, Figures 3B and 3C]. The BHBA concentration in plasma was not different between LR and HR before parturition (Figure 4C; $P > 0.05$). However, BHBA values were greater from wk 1 to 6 p.p. (wk 3 and 4: $P < 0.05$) in HR compared with LR. During feed restriction in wk 15 p.p., only a tendency toward greater plasma BHBA concentration in HR was detected (Figure 4C; $P < 0.10$).

Whereas cholesterol concentration in all animals was correlated between the NEB in wk 3 and 15 p.p. (Figure 5A, $P < 0.05$), plasma triglyceride concentration was not repeatedly distributed among animals (Figure 5B, $P = 0.26$). Cholesterol concentration in plasma was not different between LR and HR (Figure 6A; $P > 0.05$). The HR had a higher cholesterol concentration between wk 5 and 8 p.p. (wk 6 and 7 p.p.; $P < 0.05$) compared with LR. During feed restriction, cholesterol concentration did not differ between HR and LR.

Plasma triglyceride concentration did not differ between groups before parturition (Figure 6B; $P > 0.05$). Between wk 1 and 6 p.p., HR showed a higher concentration in plasma triglycerides compared with LR (Figure 6B; $P < 0.05$). Thereafter, no differences for the remaining experimental period were detected.

Endocrine Parameters and Insulin Sensitivity

Throughout the entire experiment, insulin plasma concentration (Figure 7A) and RQUICKI (data not shown) were not different between LR and HR ($P > 0.05$).

Plasma concentration of GH was elevated for HR and LR directly after parturition compared with wk 3 before calving (Figure 7B). In wk 2 p.p., HR had a higher GH concentration compared with LR (Figure 7B; $P < 0.05$). During feed restriction, GH concentration in-

creased slightly in both groups, but was not different between LR and HR (Figure 7B; $P > 0.05$).

Concentration of IGF-1 was not different between groups before parturition (Figure 7C; $P > 0.05$), but was lower in HR compared with LR during the first 3 wk of lactation (Figure 7C; $P < 0.05$). On average, LR had a higher plasma IGF-1 concentration throughout the study. During feed restriction, plasma IGF-1 concentration was lower for HR compared with LR ($P < 0.05$ in wk 16 p.p., $P < 0.10$ in wk 15 and 17 p.p.; Figure 7C). Within all animals, IGF-1 concentrations

Figure 3. Regression analysis of plasma glucose (A), NEFA (B), BHBA (C), and IGF-1 (D) concentrations between the negative energy balance in wk 3 (early lactation) and 15 postpartum (feed restriction). Data are expressed as standard deviation relative to $\mu = 0$.

Figure 4. Plasma glucose (A), NEFA (B), and BHBA (C) concentrations in cows antepartum, postpartum, and during feed restriction. Data are given as mean values $\pm$ standard error of the mean. Differences between the groups [low response (LR) and high response (HR)] are marked with + ($P < 0.10$) and * ($P < 0.05$).

between wk 3 and 15 p.p. were closely related ($r = 0.75$, $P < 0.0001$; Figure 3D).

DISCUSSION

In this study, we investigated the pattern of adaptive performance in cows during a NEB in early and mid lactation. In particular, the hypothesis was tested that cows responding more intensely in early lactation also respond more intensely during an energy deficiency in mid lactation. Feed allowance during the restriction

Figure 5. Regression analysis of plasma cholesterol (A) and triglyceride (B) concentrations between the negative energy balance in wk 3 (early lactation) and 15 postpartum (feed restriction). Data are expressed as standard deviation relative to $\mu = 0$.

Figure 6. Plasma cholesterol (A) and triglyceride (B) concentrations in cows antepartum, postpartum, and during feed restriction. Data are given as mean values $\pm$ standard error of the mean. Differences between the groups [low response (LR) and high response (HR)] are marked with + ($P < 0.10$) and * ($P < 0.05$).

period was adjusted weekly to maintain the degree of NEB in all EB (Gross et al., 2011a). This is an important point in understanding why the EB and DMI themselves are not directly comparable during the NEB in early and mid lactation. Dairy cows of the HR group had a higher milk yield throughout the entire study except for the first 2 wk of lactation and the last 2 wk of the feed restriction period compared with LR. However, ECM was higher in HR compared with LR only during the first 6 wk p.p. Consequently, EB was more negative in HR during the same time. Higher plasma NEFA concentrations were followed by increased BHBA

Figure 7. Plasma insulin (A), growth hormone (B), and IGF-1 (C) concentrations in cows antepartum, postpartum, and during feed restriction. Data are given as mean values $\pm$ standard error of the mean. Differences between the groups [low response (LR) and high response (HR)] are marked with + ($P < 0.10$) and * ($P < 0.05$).

concentrations in HR compared with LR during early lactation. However, and despite a similar BW, EB, and amount of ECM during feed restriction for HR and LR, plasma NEFA concentration increased higher in HR compared with LR. Although not different between LR and HR during feed restriction, plasma BHBA might be additionally responsible for depressing DMI in HR and LR. The inhibitory effect of ketone bodies on DMI of dairy cows was shown recently by Laeger et al. (2010).

Currently, results on nutritional challenges in dairy cows at different stages of lactation only focused on the extent of responses in performance and metabolism (Gross et al., 2011a; Bjerre-Harpøth et al., 2012), but did not consider if cows responding more intensely in early lactation might repeatedly respond more intensely also in mid lactation at an individual level. A comparable energy deficiency in cows of the LR and HR group with a similar milk yield in mid lactation (i.e., based on ECM before the initiation of feed restriction) was accompanied by different concentrations of plasma metabolites. Despite a similar metabolic stress exposure during feed restriction in mid lactation, LR cows had lower concentrations of NEFA and BHBA compared with HR. The adaptive responses to the NEB in early lactation in LR and HR, however, were clearly related to the milk yield and EB in these groups. Interestingly, no studied parameters showed any differences between groups before parturition. Other studies (Janovick et al., 2011; Weber et al., 2013) tried to explain the different performance and metabolic observations after parturition by a distinct differentiation in these factors already before parturition. Though our findings cannot verify the potentially underlying genetic differences, these might play a role in p.p. animal performance of cows of the same breed kept under equal conditions and showing a repeated pattern of metabolic adaptation to a NEB in mid lactation. Furthermore, metabolic programming of the animal during early life conditions additionally might be involved in the development of adaptive responses.

Unfortunately, we could not investigate mammary gland metabolism in the present study. Mattmiller et al. (2011) showed glucose transporters in the mammary gland being expressed in a different pattern with progress of lactation. In mid and late lactation, insulin-sensitive glucose transporters are more expressed compared with early lactation. These findings could be assumed to explain the changing metabolic priority of the lactating mammary gland and the involvement in homeostatic control mechanisms. The extent and relationship between homeostatic and homeorhetic adaptation during lactation was pointed out by Bauman and Currie (1980). This might explain the excessive

amplitudes in our analyzed metabolic and endocrine factors during the NEB in early lactation being part of the homeorhetic adaptation to lactation. In contrast, the repeated similar directed responses with lower amplitudes to feed restriction later in lactation can mainly be attributed to homeostatic control of metabolism.

Although insulin concentration and sensitivity were not different between HR and LR in the present study, endocrine factors involved in the somatotrophic axis clearly showed differences by group and NEB dependent on the stage of lactation. Cows of the HR group showed a higher degree of uncoupling of the somatotrophic axis indicated by a higher concentration of GH at concomitantly lower concentrations of plasma IGF-1 in early lactation compared with LR. Cows of the HR group in the present study had a higher milk yield compared with the LR group. Our findings of plasma IGF-1 concentrations in relation to milk production agree with results of Lucy et al. (2009) and Grala et al. (2011). Recent studies (Lucy et al., 2009; Grala et al., 2011) identified effects of genetic strains having an effect on performance and milk production. Although cows of the present study were of the same breed and experienced the same housing and feeding conditions, a different genetic background may give an obvious explanation for the variable adaptation of metabolism and endocrine factors to energy deficiencies at different stages of lactation. The repeated pattern of a higher response to a NEB in mid lactation induced by feed restriction despite a similar challenge for LR and HR during feed restriction without any needs for a higher response presumably indicated an underlying genetic basis as well as other formative events such as metabolic programming. The potential to provide nutrients from mobilized tissues may be triggered by the needs of and substrates available for the lactating mammary gland. The metabolic priority of this organ in early lactation is reflected by the decline of IGF-1 despite high concentrations of GH (uncoupling of the somatotrophic axis; Lucy et al., 2009; Gross et al., 2011b; Kessler et al., 2013), NEFA, and BHBA to ensure its nutrient supply. Besides dairy cow strain differences in uncoupling of the somatotrophic axis, White et al. (2012) identified differences in expression of phosphoenolpyruvate carboxykinase and pyruvate carboxylase closely linked to milk production and lipolysis. These findings might partly explain our observations related to differences in NEFA and BHBA concentrations between LR and HR. Ha et al. (2015) identified pathways associated with different metabolic loads using a genome-wide approach. They found the variability in lipid metabolism to be based on different SNP at genome level. The more in-depth systems biology approach focusing on adipose

tissue metabolism clearly identified the regulation of contribution of genetic merit besides environmental effects (McNamara and Hillers, 1986; McNamara, 2012; Rocco and McNamara, 2013). As in our study, insulin sensitivity was not different, but only milk production differed between high and low responding animals, the findings of Rocco and McNamara (2013) that lipolysis is more related to milk production might explain our findings regarding the responses of NEFA and BHBA. However, this priority of the mammary gland seems not to be completely diminished in mid lactation as a similar challenge in HR and LR resulted in similarly directed adaptive mechanisms as in early lactation to favor nutrient flow to the mammary gland. Consequently, lactational potential and the degree of priority of the mammary gland may be determined by the genetic potential throughout lactation.

Besides the variability in adaptive performance, it is instructive to consider other possible coping strategies regarding to metabolic challenges at different stages of lactation including for instance hepatic metabolism. The availability of cholesterol plays an important role in exporting excessive FA out of the liver and thereby preventing hepatic steatosis (Kessler et al., 2014). In ruminants, a significant amount of cholesterol is synthesized in small intestinal epithelial cells to support FA absorption (Bauchart, 1993; Iqbal et al., 2010). In the present study, cows of the HR group had a higher cholesterol concentration after 5 wk of lactation that might allow them an earlier and faster handling of NEFA released from adipose tissue compared with cows of the LR group. Though not different between groups, an increased cholesterol concentration during feed restriction might allow a more rapid and effective use of released NEFA instead of their accumulation as triglycerides in the liver. These thoughts are confirmed by the higher concentrations of VLDL in HR that are essential for the hepatic export of FA (Kessler et al., 2014).

CONCLUSIONS

The repeated pattern of adaptive responses to a NEB at 2 different stages of lactation, although not to the same extent, is a notable property that needs further consideration in terms of discussing metabolic robustness in dairy cows. Interestingly, this phenomenon in animals kept under similar conditions could not retrospectively be identified before parturition (i.e., a physiological situation without any noteworthy metabolic load). Most likely, differences in genetic background as well as metabolic programming events are the likely explanation. The prospective implementation in breed-

ing and selection criteria might attract more attention toward metabolic robustness in dairy cows.

ACKNOWLEDGMENTS

This study was in part supported by Swissgenetics (Zollikofen, Switzerland).

REFERENCES

- Bauchart, D. 1993. Lipid absorption and transport in ruminants. *J. Dairy Sci.* 76:3864–3881.
- Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis homeorhesis. *J. Dairy Sci.* 63:1514–1529.
- Bjerre-Harpøth, V., N. C. Friggens, V. M. Thorup, T. Larsen, B. M. Damgaard, K. L. Ingvarsen, and K. M. Moyes. 2012. Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. *J. Dairy Sci.* 95:2362–2380.
- Codrea, M. C., S. Højsgaard, and N. C. Friggens. 2011. Differential smoothing of time-series measurements to identify disturbances in performance and quantify animal response characteristics: An example using milk yield profiles in dairy cows. *J. Anim. Sci.* 89:3089–3098.
- GfE (German Society of Nutrition Physiology). 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchttrinder, Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie. DLG-Verlag, Frankfurt am Main, Germany.
- Grala, T. M., M. C. Lucy, C. V. C. Phyn, A. J. Sheahan, J. M. Lee, and J. R. Roche. 2011. Somatotrophic axis and concentrate supplementation in grazing dairy cows of genetically diverse origin. *J. Dairy Sci.* 94:303–315.
- Gross, J., H. A. van Dorland, R. M. Bruckmaier, and F. J. Schwarz. 2011a. Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. *J. Dairy Sci.* 94:1820–1830.
- Gross, J., H. A. van Dorland, F. J. Schwarz, and R. M. Bruckmaier. 2011b. Endocrine changes and liver mRNA abundance of somatotrophic axis and insulin system constituents during negative energy balance at different stages of lactation in dairy cows. *J. Dairy Sci.* 94:3484–3494.
- Ha, N. T., J. J. Gross, A. van Dorland, J. Tetens, G. Thaller, M. Schlather, R. Bruckmaier, and H. Simianer. 2015. Gene-based mapping and pathway analysis of metabolic traits in dairy cows. *PLoS ONE* 10:e0122325. <http://dx.doi.org/10.1371/journal.pone.0122325>.
- Iqbal, S., Q. Zebeli, A. Mazzolari, S. M. Dunn, and B. N. Ametaj. 2010. Feeding rolled barley grain steeped in lactic acid modulated energy status and innate immunity in dairy cows. *J. Dairy Sci.* 93:5147–5156.
- Janovick, N. A., Y. R. Boisclair, and J. K. Drackley. 2011. Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows. *J. Dairy Sci.* 94:1385–1400.
- Kessler, E. C., J. J. Gross, and R. M. Bruckmaier. 2013. Different adaptation of IGF-I and its IGFBPs in dairy cows during a negative energy balance in early lactation and a negative energy balance induced by feed restriction in mid-lactation. *Vet. Med. (Praha)* 58:459–467.
- Kessler, E. C., J. J. Gross, R. M. Bruckmaier, and C. Albrecht. 2014. Cholesterol metabolism, transport, and hepatic regulation in dairy cows during transition and early lactation. *J. Dairy Sci.* 97:5481–5490.
- Laeger, T., C. C. Metges, and B. Kuhla. 2010. Role of β -hydroxybutyric acid in the central regulation of energy balance. *Appetite* 54:450–455.

- Lucy, M. C., G. A. Verkerk, B. E. Whyte, K. A. Macdonald, L. Burton, R. T. Cursons, J. R. Roche, and C. W. Holmes. 2009. Somatotropic axis components and nutrient partitioning in genetically diverse dairy cows managed under different feed allowances in a pasture system. *J. Dairy Sci.* 92:526–539.
- Mattmiller, S. A., C. M. Corl, J. C. Gandy, J. J. Loor, and L. M. Sordillo. 2011. Glucose transporter and hypoxia-associated gene expression in the mammary gland of transition cattle. *J. Dairy Sci.* 94:2912–2922.
- McNamara, J. P. 2012. Integrating nutritional, genetic and reproductive management in early lactation dairy cattle. *J. Anim. Sci.* 90:1846–1854.
- McNamara, J. P., and J. K. Hillers. 1986. Regulation of bovine adipose tissue metabolism during lactation. 2. Lipolysis response to milk production and energy intake. *J. Dairy Sci.* 69:3042–3050.
- Rocco, S. M., and J. P. McNamara. 2013. Regulation of bovine adipose tissue metabolism during lactation. 7. Metabolism and gene expression as a function of genetic merit and dietary energy intake. *J. Dairy Sci.* 96:3108–3119.
- Weber, C., C. Hametner, A. Tuchscherer, B. Losand, E. Kanitz, W. Otten, S. P. Singh, R. M. Bruckmaier, F. Becker, W. Kanitz, and H. M. Hammon. 2013. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. *J. Dairy Sci.* 96:165–180.
- White, H. M., S. S. Donkin, M. C. Lucy, T. M. Grala, and J. R. Roche. 2012. Short communication: Genetic differences between New Zealand and North American dairy cows alter milk production and gluconeogenic enzyme expression. *J. Dairy Sci.* 95:455–459.