Temporal changes in plasma concentrations of hormones and metabolites in pasture-fed dairy cows during extended lactation

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ABSTRACT

This experiment measured variations in plasma concentrations of metabolic hormones and metabolites in cows undergoing extended lactations of up to 670 d at 2 planes of nutrition. Thirty-seven Holstein-Friesian cows that calved in late winter were selected for varying milk yield and then managed for a lactation of 670 d by delaying breeding until approximately 450 d in milk (DIM). Cows grazed fresh pasture supplemented with pasture silage or hay and crushed wheat or triticale grain. Dietary intake was reduced by approximately 1.8 kg (dry matter) grain/cow per day for 19 of the cows from 300 DIM until the end of lactation to assess the effect of restricted energy intake on the persistency of milk production. Samples of blood were collected monthly from each cow to measure plasma concentrations of selected hormones and metabolites. Dietary restriction beyond 300 DIM reduced yields of milk, protein, and fat, but did not alter the proportion of cows reaching the 670-d lactation target. Dietary restriction had no effect on cow BW or plasma concentrations of any hormones or metabolites. Overall, blood plasma concentrations of insulin-like growth factor-I, leptin, and glucose were elevated from 301 to 600 DIM compared with 0 to 300 DIM, whereas concentrations of growth hormone and nonesterified fatty acids were lower after 300 DIM. Plasma concentrations of insulin and prolactin were unaffected by stage of lactation, but prolactin concentrations increased during summer. These changes were consistent with a decrease in milk yield and an increase in the partitioning of nutrients to body tissue gain, primarily adipose tissue, throughout the later stages of the extended lactation. Cows that continued milking beyond 600 DIM had increased plasma concentrations of growth hormone and decreased concentrations of glucose and leptin compared with cows that milked <600 DIM. These differences, coupled with reduced

body weight gain, indicated an increased priority for nutrient partitioning to milk production at the expense of body tissue gain throughout the extended lactation period in cows with greater lactation persistency.

Key words: extended lactation, hormones and metabolites, lactation persistency, pasture

INTRODUCTION

Research in southeastern Australia (Auldist et al., 2007; Grainger et al., 2009), New Zealand (Kolver et al., 2007), and the United Kingdom (Sorensen et al., 2008) has shown that grazing Holstein-Friesian dairy cows can lactate for much longer than the traditional 300 d. Dairy farm systems that incorporate extended lactations of up to 670 d alleviate the need for cows to conceive during peak production when they are likely to be in negative energy balance. Such systems can also allow farmers to take advantage of higher milk prices in winter and improve monthly cash flow (Borman et al., 2004). Furthermore, the loss of annual production of milk fat and protein compared with that in 300-d lactations can be small (Auldist et al., 2007; Kolver et al., 2007; Grainger et al., 2009). Because of these advantages, economic analyses have shown extended lactation systems can be at least as profitable as systems in which cows calve annually (Malcolm, 2005).

Marked variation exists between cows, however, in their ability to maintain milk production for an extended lactation. Auldist et al. (2007) showed that 83% of cows could lactate for 570 d, but only 42% of cows achieved a 670-d lactation. Kolver et al. (2007) showed subsequently that North American Holstein-Friesians with high production potential were more suitable for extended lactations than New Zealand Holstein-Friesians with lower production potential, and that cow genotype had an important influence on the capacity of cows to sustain long lactations. Other experiments have documented the effects of cow diet on the capacity of grazing cows to sustain extended lactations (Kolver et al., 2007; Sorensen et al., 2008;

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Grainger et al., 2009). These studies have shown that manipulating the amount of supplementary grain and forage within the normal range offered to cows in commercial grazing systems did not influence the capacity of cows to sustain an extended lactation, but that when ME intake is in excess of prevailing milk production and maintenance requirements, some cows commence tissue repletion at the expense of milk production and are unable to lactate for as long as similar cows with more modest ME intake.

Part-lactation studies of changes in homeorhetic hormones and metabolites in blood plasma of cows during extended lactations indicate that those cows that are unable to sustain extended lactations preferentially partition energy toward the deposition of adipose tissue at the expense of milk production, which ultimately leads to cessation of milking (Sorensen and Knight, 2002; Kay et al., 2009; Delany et al., 2010). For example, in those studies, cows less suited to extended lactations generally had higher plasma concentrations of leptin, glucose, and insulin, in addition to lower plasma concentrations of GH. It is necessary to examine these changes for a full 670-d lactation in cows with different lactation persistency because this information has implications for the selection and management of cows well suited for extended lactation systems.

The aim of this experiment was to describe the patterns of change in milk production and blood plasma concentrations of selected hormones and metabolites throughout an extended lactation in cows with differing levels of persistency and under 2 different planes of nutrition. The hypotheses tested were (1) that as the extended lactation progressed, changes in selected hormones and metabolites would reflect an increasing shift in the priority of nutrient partitioning away from milk production and toward body tissue gain; (2) that irrespective of ME intake, observed variation in the capacity of cows to reach the 670-d lactation target would be associated with changes in the plasma concentrations of selected homeorhetic hormones and metabolites that were indicative of differences in the partitioning of energy toward adipose tissue or milk production; and (3) that restricting dietary ME intake (by 1.8 kg DM grain/cow per day) after 300 DIM would not affect the proportion of cows reaching the 670-d target.

MATERIALS AND METHODS

Location

The experiment was conducted at the Department of Primary Industries' research farm located at Ellinbank, near Warragul, Victoria, Australia (38°14'S, 145°56'E). The experiment was conducted following procedures

approved by the Department of Primary Industries, Eastern Animal Ethics Committee.

Cows and Management

At the start of the lactation, 60 Holstein-Friesian cows of mixed age (including 11 primiparous cows) were selected for study from the main research herd. These cows had calved in July and August and were managed for a 670-d lactation by withholding breeding until approximately 450 DIM instead of the usual 90 DIM. They were milked twice daily at approximately 0700 and 1500 h.

When cows reached approximately 90 DIM, the number of cows was reduced to 37. Milk production records from the first 90 d of lactation were used to select cows with a wide range of milk production (the range was 21 to 41 kg/cow per day at 90 DIM). Those cows that had displayed metabolic disorders or mastitis in early lactation were not considered as candidates.

Milking ceased 56 d before the predicted calving date of those cows that reached the target lactation length of 670 d. Milking was also ceased, irrespective of lactation length, for those cows that produced <30 L of milk/wk for 2 consecutive weeks.

Dietary Treatments

Cows were managed as a single herd throughout the experiment. Cows were rotationally grazed on perennial ryegrass-based pasture supplemented with 2.0 to 7.5 kg DM/cow per day of cereal grain (crushed wheat or triticale) at milking times. During some summer and autumn months, when pasture availability and quality were low, pasture was replaced with pasture silage, lucerne hay, or both.

Estimated daily ME intake for the first 300 DIM averaged 204 MJ/cow per day, back-calculated according to the method of Heard et al. (2011). After that, the 37 cows were divided into 2 similar groups balanced for milk production in the first 300 d of lactation, cow age, calving date, BW, and BCS according to the method of Baird (1994). Each group was randomly assigned to 1 of 2 dietary treatments for the remainder of the lactation. Treatments were (1) control ($n = 18$): the same dietary components as for the first 300 d of lactation but with a calculated ME intake of 176 MJ/cow per day; and (2) restricted ($n = 19$): a restricted diet comprising the control diet less approximately 1.8 kg DM grain/cow per day, equivalent to an estimated minimum ME intake of 157 MJ/cow per d.

Nutritive characteristics of the pasture and supplements were not measured in the current study. They would have been similar to those reported by Auldist

et al. (2011) for a companion study conducted on the same farm over the same period.

Measurements and Analyses

Each cow was weighed every month for the duration of the experiment. The BCS of all cows was also assessed at 300 DIM and 650 DIM by trained observers using the 8-point scale of Robins et al. (2003).

Milk yield was measured daily using a DeLaval ALPRO milk metering system, and representative samples of daily milk (a.m. and p.m.) were collected using in-line milk meters every second week (DeLaval International, Tumba, Sweden). These samples were tested for concentrations of fat, protein, lactose, and SCC using a near-infrared milk analyzer (model 2000, Bentley Instruments, Chaska, MN).

A blood sample was taken from the coccygeal vein of every cow on 1 d per month at approximately 0800 h (<1.5 h after milking). Two 10-mL samples were drawn from each cow (1×170 IU of lithium heparin and 1×18 mg of potassium EDTA Vacutainer; BD Vacutainer Systems, Plymouth, UK). Samples were placed immediately on ice before centrifugation at $1,800 \times g$ for 10 min at 4°C. Plasma was harvested and stored at -20°C until analyzed.

Plasma concentrations of glucose were measured using a commercially available reagent (Thermo Infinity Glucose Oxidase Liquid, Thermo Fisher, Noble Park, Victoria, Australia). Plasma NEFA was measured using the commercially available Wako NEFA-C kit (Wako Chemicals USA Richmond, VA) adapted for 96-well microplates (Johnson and Peters, 1993). Plasma insulin was measured using a commercially available RIA kit (Linco Porcine Insulin RIA, Linco Research, St. Charles, MO). The protocol was modified by reducing the volumes of all reagents and samples by 50%. Plasma concentrations of IGF-I were measured by the chloramine-T RIA method described by Gluckman et al. (1983). Interference by binding proteins was minimized by an acid-ethanol cryoprecipitation method validated for ruminants by Breier et al. (1991). Plasma growth hormone (GH) was measured with a double-antibody RIA as described by Downing et al. (1995). Plasma concentrations of prolactin were assayed by double-antibody homologous RIA as described previously by McNeilly and Andrews (1974). Plasma leptin concentrations were measured by a double-antibody RIA (Blache et al., 2000). All samples were conducted in duplicate. Intra- and interassay coefficients of variation were as follows: glucose <3.0% and <5.5%; NEFA <4.0% and <6.0%; insulin <5.0% and <9.0%; GH <5.9% and <6.3%. Leptin, IGF-I, and prolactin concentrations were measured in a single assay with

intraassay variation of <8.5%, <4.3% and <6.2%, respectively.

Statistical Analyses

Statistical analyses were each performed using GenStat for Windows, (GenStat release 11, VSN International, Hemel Hempstead, UK). Total DIM were compared between the dietary treatments using the Wilcoxon (Peto-Prentice) nonparametric survival analysis test described by Grainger et al. (2009). Analyses were performed using the Kaplan-Meier procedure to determine the proportion of cows per treatment surviving in their lactation to 600 and 650 d.

Total yields of milk, protein, fat, and lactose were calculated for each cow for 1 to 300 DIM and for 301 to 670 DIM. Mean protein, fat, and lactose concentrations were calculated by dividing yield totals by milk weights. Data were grouped into the first and second years of the lactation to divide the lactation into data sets that represented the traditional 10-mo lactation (1 to 300 d) and the extended phase of the lactation (301 to 670 d). Each variable was analyzed using a general linear model (REML) in which the fixed effects were specified as year (1 to 300 or 301 to 670 d) $\times$ diet. The random effect was specified as cow. The significance of the main effects of treatment and treatment by year were tested using Wald tests.

To investigate the variation in milk production and plasma hormones and metabolites between cows either better suited to or less suited to extended lactation, groups of cows were divided into those with lactation lengths of greater or less than 600 d (mean 633 ± 5 vs. 543 ± 4 ; $P < 0.001$), and then the data were analyzed using the REML function in GenStat. The small variation in length of lactation reflected the persistency of lactation within the herd during this experiment where cows did not begin to reduce milk output toward the dry-off criteria until approximately 520 DIM. Body condition score measurements taken at 300 and 650 DIM were analyzed at each time point using a one-way ANOVA. Those P -values < 0.05 were considered significant and < 0.1 were considered a trend. Data are presented as means $\pm$ standard error of the difference unless stated.

RESULTS

Proportion of Cows Reaching the Target Lactation Length

Each of the 37 cows in this experiment continued to milk at least until 520 DIM. Only 12/37 cows were still lactating at 670 DIM. Dietary treatment had no effect

Figure 1. Kaplan-Meier survival curves for the proportion of cows still milking during a 670-d lactation for cows offered a control (■) or restricted (□) diet after 300 DIM. Estimated daily ME intake until 300 DIM was 204 MJ/cow per day for all cows, and beyond 300 DIM was 176 MJ/cow per day for the control group and 157 MJ/cow per day for the restricted group.

on the proportion of cows remaining in milk at 600 DIM, with 67% of control cows and 68% of restricted cows still milking, or at 650 DIM, with 22% of control cows and 16% of restricted cows still milking (Figure 1).

Milk Production and Composition

Mean yields of milk and milk protein, fat, and lactose were not different throughout the first 300 d of lactation between dietary treatments (Figure 2). Yields of milk, and milk protein, fat, and lactose were lower beyond 300 DIM for the cows in the restricted group compared with cows in the control group. Concentrations of protein and fat were not affected by diet (Table 1).

Milk protein concentration increased ($P < 0.001$) and fat concentration remained the same for both groups between 301 and 670 d, but lactose concentration decreased ($P < 0.001$) compared with that in the period from 1 to 300 d. Milk yield decreased ($P < 0.001$) after 300 d.

BW and BCS

Dietary intake restriction had no effect on cow BW or BCS (Table 1). However, mean BW increased from 547 kg between 1 to 300 DIM to 598 kg between 301 and 670 DIM (standard error of the difference = 6.01; $P < 0.001$; Figure 2).

Cows that were able to milk >600 DIM (more-persistent cows) had lower mean BW ($P < 0.001$) than cows that milked <600 DIM (less-persistent cows) from 301

to 600 DIM (581 vs. 626 ± 9.4 kg) and lower mean BCS ($P < 0.002$) at 650 DIM (4.45 vs. 4.81 ± 0.10).

Hormone and Metabolite Profiles

Mean plasma concentrations of prolactin and insulin were not affected by stage of lactation (<301 vs. >300 DIM), irrespective of dietary treatment. Plasma GH and NEFA were both lower in the 301-to-600 d phase of the lactation, whereas plasma concentrations of IGF-I, leptin, and glucose increased (Table 2).

Concentrations of hormones and metabolites were not affected by dietary restriction, except that overall mean plasma GH concentration was higher for cows in the control group than for those in the restricted group (Table 2). However, this was evident between 1 to 300 DIM and 301 to 600 DIM, indicating no overall effect of diet on plasma concentration of GH (Figure 3).

Considerable variation was found in the plasma concentration of prolactin according to time of year. Mean plasma prolactin concentrations increased during the

Figure 2. Milk yield and BW for cows offered a control (■) or restricted diet (□) during a 670-d lactation. Arrow represents commencement of feed restriction. Estimated daily ME intake until 300 DIM was 204 MJ/cow per day for all cows, and beyond 300 DIM was 176 MJ/cow per day for the control group and 157 MJ/cow per day for the restricted group. Errors bars represent SEM.

Table 1. Lactation yields of milk and milk protein, fat, and lactose and BW for cows fed the control or restricted diet¹

Item	Control	Restricted	SED ²	P-value
1–300 d				
Milk (L)	7,299	7,104	307.4	0.532
Fat (kg)	305.8	292.2	10.63	0.211
Protein (kg)	221.4	213.1	7.06	0.245
Lactose (kg)	345.6	334.5	14.87	0.460
Fat (%)	4.4	4.4	0.11	0.901
Protein (%)	3.3	3.3	0.04	0.300
BW (kg)	510	512	9.8	0.643
BCS (at 300 DIM)	4.53	4.51	0.046	0.768
301–670 d				
Milk (L)	4,819	3,877	360.9	0.014
Fat (kg)	220.2	174.1	16.84	0.010
Protein (kg)	175.2	140.7	12.55	0.010
Lactose (kg)	230.6	185.7	18.06	0.018
Fat (%)	4.6	4.6	0.12	0.901
Protein (%)	3.7	3.7	0.04	0.300
BW (kg)	567	565	9.8	0.643
BCS (at 650 DIM)	5.10	5.04	0.065	0.033

¹Data are presented for periods of 1 to 300 d and 301 to 670 d of lactation. Dietary restriction occurred after 300 d only. BCS was measured at 300 and 650 DIM only. Estimated daily ME intake until 300 DIM was 204 MJ/cow per day for all cows, and beyond 300 DIM was 176 MJ/cow per day for the control group and 157 MJ/cow per day for the restricted group.

²SED = standard error of the difference.

summer months of November to January (100 to 160 DIM and 450 to 520 DIM) and decreased during the winter months of June to August (300 to 360 DIM, $P < 0.001$; Figure 4).

Those cows that ultimately milked for >600 d (more-persistent cows) had similar plasma concentrations of the assayed hormones and metabolites from 1 to 300 DIM to those of contemporaries milked for <600 d (less-persistent cows; Figures 3 and 4). The more-persistent cows had higher total milk and milk protein and fat yields for the whole lactation than did less-persistent cows. Additionally, the more-persistent cows had greater ($P < 0.05$) yields of milk (14.9 vs. 13.0 ± 0.7 L/d), protein, and fat (2.0 vs. 1.7 ± 0.10 kg/d) and greater ($P < 0.001$) plasma GH concentration compared with less-persistent cows (1.7 vs. 1.5 ± 0.10 ng/mL) between

301 and 600 DIM. In contrast, decreases ($P < 0.001$) in plasma concentrations of leptin (0.7 vs. 1.0 ± 0.04 ng/mL) and glucose (3.7 vs. 3.9 ± 0.04 mM; Figures 3 and 4) were observed in more-persistent cows between 301 and 600 DIM. The more- and less-persistent cows did not differ in their plasma concentrations of IGF-I, insulin, NEFA, or prolactin between 301 and 600 DIM.

DISCUSSION

This experiment has provided the first whole-lactation study of temporal changes in plasma concentrations of metabolic hormones and metabolites in grazing dairy cows undergoing extended lactations of 670 d. Irrespective of the amount of supplementary grain that was offered, changes in metabolic hormones and

Table 2. Plasma hormone and metabolite concentrations for cows fed the control or restricted diet for the periods from 1 to 300 d and from 301 to 600 d of the lactation¹

Item	1–300 d		301–600 d		SED ²	P-value		
	Control	Restricted	Control	Restricted		Diet	Period	Int ³
Growth hormone (ng/mL)	2.3	2.6	1.5	1.7	0.10	0.001	<0.001	0.386
IGF-I (ng/mL)	14.4	14.9	27.0	27.0	0.94	0.790	<0.001	0.668
Leptin (ng/mL)	0.53	0.51	0.83	0.82	0.038	0.585	<0.001	0.762
Prolactin (ng/mL)	8.6	7.1	6.8	7.0	0.75	0.267	0.167	0.098
Glucose (mM)	3.5	3.5	3.7	3.8	0.04	0.369	<0.001	0.437
Insulin (μ U/mL)	6.9	6.6	7.2	7.2	0.36	0.567	0.145	0.590
NEFA (μ M)	184	192	137	145	10.4	0.293	<0.001	0.995

¹Values are the mean of samples taken at monthly intervals. Estimated daily ME intake until 300 DIM was 204 MJ/cow per day for all cows, and beyond 300 DIM was 176 MJ/cow per day for the control group and 157 MJ/cow per day for the restricted group.

²SED = standard error of the difference.

³Int = diet $\times$ period interaction.

Figure 3. Milk yield and plasma growth hormone (GH), leptin, and IGF-I for 1 to 600 DIM for more-persistent cows (lactations >600 d; ●) and less-persistent cows (lactations <600 d; ○). Arrow represents commencement of feed restriction. Errors bars represent SEM.

metabolites in all cows reflected a shift in the priority of nutrient partitioning from milk production to body tissue gain as the extended phase of the lactation progressed (301–670 DIM). The results are in accordance with those from similar investigations into conventional lactations showing that beyond the peak demand for nutrients in early lactation, cow metabolism shifts from a state of catabolism to one of anabolism to conserve energy for a current or subsequent pregnancy and for future lactations (Ronge et al., 1988; Bell, 1995; Bauman, 2000). The current data collected each month during a 670-d lactation expand on studies in which plasma concentrations of hormones and metabolites were measured in extended lactations, but only beyond 300 DIM (Delany et al., 2010), between 1 to 70 and 330 to 440 DIM (Kay et al., 2009), or out to 480 d (Sorensen and Knight, 2002).

The results of the current study supported the first hypothesis. Overall, plasma concentrations of insulin and glucose were greater from 301 to 670 DIM than from 1 to 300 DIM. These observations are consistent with previous observations of a decreased requirement of the mammary gland for glucose during this late stage

of the extended lactation (Bauman, 2000). The observed declines in plasma concentrations of GH and NEFA throughout the extended phase of the lactation were also consistent with a lower physiological importance of milk synthesis as lactation progressed, reduced rates of lipolysis, and increased re-esterification of fatty acids (McNamara and Hillers, 1986; Ronge et al., 1988). The substantial increase in plasma leptin throughout the lactation also indicated that cows were in a state of increasing fat deposition; leptin is secreted almost exclusively by adipocytes and can thus provide an indication of increasing fat mass (Maffei et al., 1995; Considine et al., 1996; Ingvarsen and Boisclair, 2001). Plasma concentrations of IGF-I increased as lactation progressed regardless of decreasing plasma concentrations of GH, which is consistent with the literature (Sorensen and Knight, 2002). Furthermore, more-persistent cows had greater plasma concentration of GH but similar plasma concentrations of IGF-I compared with less-persistent cows. This suggests that a difference in lactation persistency is associated, at least in part, with variation in the functioning of the somatotrophic axis beyond 300 DIM. In the more-persistent cows, the higher concentration

Figure 4. Plasma glucose, insulin, prolactin, and NEFA for 1 to 600 DIM for more-persistent cows (lactations >600 d; ●) and less-persistent cows (lactations <600 d; ○). Arrow represents commencement of feed restriction. Errors bars represent SEM.

of GH, the major galactopoietic hormone in ruminants, would favor increased nutrient supply to the mammary gland (Bauman, 2000). In addition, mammary epithelial cell survival is vital for lactation persistency and is the net result of mammary cell proliferation and apoptosis (Knight and Wilde, 1993; Capuco et al., 2003). This is a highly complex and coordinated process and is influenced by many growth factors and hormones, including GH, IGF-1, and prolactin (Accorsi et al., 2002). Whether differences in the plasma concentration of these hormones contributed to variation in lactation persistency through direct effects on mammary epithelial cell survival was not investigated in this study but cannot be excluded.

In the current study, plasma concentrations of prolactin were influenced more by time of year than by stage of lactation or persistency, and were highest during times of increased daily photoperiod, as has been shown previously (Sorensen and Knight, 2002; Dahl, 2008; Velasco et al., 2008). The strong relationship between photoperiod and plasma concentrations of prolactin leads to difficulty in determining its effect on lactation persistency (Sorensen and Knight, 2002). Because these times coincided with times of increased milk yield, it is possible that prolactin played a role in

the stimulation of milk yield or in mammary epithelial cell survival (Accorsi et al., 2002). However, the absence of variation in plasma prolactin concentration between more- and less-persistent cows indicates that prolactin is unlikely to be a major determinant of persistency.

Every cow increased its BW and BCS during the period from 300 to 670 DIM. This observation, combined with lower yields of milk, milk protein, and milk fat from 301 to 670 DIM compared with 1 to 300 DIM, was similarly consistent with an increased proportion of dietary energy intake being used for tissue gain throughout the latter part of the lactation. Although the observed differences between the 1 to 300 DIM and 301 to 670 DIM periods are presumed to be due to the effects of stage of lactation, seasonal effects such as changes in pasture quality and availability, day length, and climate may also be influencing factors.

It was reported by Kay et al. (2009) that milk solids yield between 1 and 300 DIM was positively correlated with milk solids yield beyond 300 DIM. In the current experiment, cows with high and low milk yields at 90 DIM were enrolled in the study with the aim of ensuring a range in the persistency of cows during an extended lactation. Marked variation was observed in the capacity of cows to reach their 670-d lactation target, with

only 67% of cows able to milk for >600 d irrespective of the amount of supplement offered. When cows were divided into those that milked >600 d (more-persistent cows) and those that milked <600 d (less-persistent cows), several key differences were found between the plasma concentrations of key metabolic hormones and metabolites. These differences support the second hypothesis and are indicative of more-persistent cows partitioning an increased proportion of nutrients from the diet toward milk production compared with less-persistent cows throughout the late stages of the extended lactation.

A lower plasma concentration of leptin in more-persistent cows was also reported by Kay et al. (2009) and Delany et al. (2010). This is probably associated with a lower proportion of body fat in the 301- to 600-d period of the lactation, which may be due in part to the higher milk yield or to increased plasma GH levels promoting lipolysis, gluconeogenesis, and milk synthesis in these cows (Bauman, 2000). Furthermore, decreased plasma concentrations of glucose in more-persistent cows indicated an increased homeorhetic priority of milk synthesis and thus an increased utilization of glucose by the mammary gland (Bauman, 2000). The increased plasma concentrations of GH and lower concentrations of leptin and glucose in more-persistent cows are consistent with similar changes reported for more-persistent North American Holstein-Friesians compared with less-persistent New Zealand Holstein-Friesians (Kay et al., 2009) and more-persistent grazing cows offered moderate levels of cereal grain supplements compared with less-persistent cows consuming TMR (Delany et al., 2010).

We expected that plasma NEFA concentrations would be greater in cows with better persistency, as reported by Kay et al. (2009) but not Delany et al. (2010), because plasma NEFA have previously been used as an indication of increased adipose tissue mobilization through lipolysis (McNamara and Hillers, 1986). This was not evident, possibly because all of the cows would have been in positive energy balance at this time or because changes were very small and could not be detected. Furthermore, evidence exists that throughout late lactation cows are less sensitive to catecholamines and lipolytic hormones such as epinephrine (McNamara and Hillers, 1986). This change in sensitivity may continue throughout the late stages of an extended lactation.

The current study showed that similar proportions of cows in the control and restricted groups were able to milk to 600 DIM (~68% of cows) and 650 DIM (~20% of cows), albeit at a low dry-off threshold. These values are slightly lower than those reported by Auldist et al. (2007) using the same dry-off threshold for cows of a similar genotype. It was concluded that a modest

restriction of intake in cows beyond 300 DIM did not compromise their capacity to reach the 670-d lactation target, supporting the third hypothesis. This result is consistent with the reports of Sorensen et al. (2008) and Grainger et al. (2009) that the amount of supplementary concentrates fed to grazing cows has little effect on the capacity of cows to undergo extended lactations of between 450 and 670 d. Nevertheless, Kolver et al. (2007) showed that the influence of level of supplementation on a cow's capacity for extended lactations depended largely on the genotype of the cow, with New Zealand Holstein-Friesian cows being less likely than North American Holstein-Friesian cows to persist until 670 d when offered 6 kg DM cereal grain/cow per day. Similarly, Grainger et al. (2009) reported a reduced capacity of cows of predominantly Northern Hemisphere genotype to sustain a 670-d lactation when offered a TMR compared with similar cows grazing pasture supplemented with a moderate level of cereal grain. It is clear that important interactions exist between genotype and level of energy intake in relation to the production capacity of the cows, and that these interactions need to be considered when estimating the effects of cow diet on persistency during extended lactation, especially at higher levels of supplementation.

The lack of effect of supplement intake on the capacity of cows to undergo extended lactations was consistent with the fact that few differences were found in concentrations of plasma hormones or metabolites between the control and restricted groups at any stage of lactation. The exception was that plasma concentrations of GH were higher in the restricted cows than in the control cows. This was the case throughout the entire lactation, indicating it was a result of preexisting variation, rather than an effect of the diet. Concentrations of protein and fat were also the same irrespective of diet, but a lower milk volume led to cows on the restricted diet producing significantly less protein and fat in the second year of the lactation compared with the first 300 DIM and compared with the control cows. Additionally, lower intake did not result in any change in BW or BCS in the restricted cows compared with the control cows. Collectively, these results indicate that plane of nutrition between 300 and 670 DIM did not affect the degree to which cows partitioned energy toward adipose tissue, and that cows with reduced energy intake simply diverted less energy toward milk production.

CONCLUSIONS

In conclusion, this experiment has provided the first whole-lactation data about plasma concentrations of metabolic hormones and metabolites during extended

lactations of 670 d. The patterns of change in these hormones and metabolites as the extended lactation progressed reflected an increasing shift in the priority of nutrient partitioning from milk production to body tissue gain. From 300 to 600 DIM, cows that were able to milk >600 DIM (more-persistent cows) had higher yields of milk, protein, and fat, and increased plasma concentrations of GH compared with cows that milked <600 DIM (less-persistent cows). Furthermore, more-persistent cows had lower BW from 301 to 670 DIM, lower BCS at 650 DIM, and lower plasma concentrations of leptin and glucose than less-persistent cows during these late stages of the extended lactation. These data showed that those cows that are better suited to extended lactations display a greater preference for the homeorhetic partitioning of nutrients toward milk synthesis and not body tissue gain. A small reduction in intake of cereal supplements beyond 300 DIM did not affect BW, BCS, blood plasma profiles of the hormones and metabolites measured, or the proportion of cows reaching the 670-d lactation target, but did decrease yields of milk, milk protein, and milk fat. These results have important implications for the selection and management of cows well suited for extended lactation systems.

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