Associations among hormones and metabolites during the transition period and early lactation milk production

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ABSTRACT

The transition period is loosely defined as the period from three weeks pre-calving to three weeks post-calving, and is regarded as a very important determinant of a cow's lactation performance. Data were collated from three experiments in which blood was sampled repeatedly pre- and post-calving and milk production was recorded post-calving. Data were pooled within treatment and associations between blood hormones and metabolites, and milk production determined using regression analysis. Milk and milksolids (MS) yield were positively associated ($P < 0.05$) with pre-calving plasma leptin and glucose concentrations and milk yield was negatively associated ($P < 0.05$) with pre-calving non-esterified fatty acid (NEFA) and β-hydroxy butyrate concentrations. Milk yield was negatively associated ($P < 0.05$) with plasma growth hormone, aspartate aminotransferase, and glutamate dehydrogenase concentrations in the first days of lactation, and with insulin-like growth factor and glucose concentrations during the first five weeks post-calving. In comparison, plasma NEFA and albumin (ALB) concentrations post-calving and the ratio of NEFA to ALB were all positively associated ($P < 0.05$) with milk and MS yield during the first five weeks of lactation. Data are consistent with a positive effect of calving body condition on milk production and an increased milk production with increasing condition score loss post-calving.

Keywords: transition cow; blood hormones; blood metabolites; milk production.

INTRODUCTION

The transition period is regarded as a very important determinant of a cow's lactation performance. The general definition of the transition period encompasses the interval from approximately three weeks either side of calving and is, arguably, the greatest time of homeorhetic adjustment in the lactation cycle of the cow.

Homeorhesis refers to coordinated changes in metabolism, wherein many metabolic processes are altered to support a physiological state (Bauman & Currie, 1980). During the transition period, there is an increased nutrient requirement for fetal growth and lactogenesis pre-calving, and to sustain milk production during lactation. This is facilitated by a range of physiological changes, including intestinal hypertrophy, increased gluconeogenesis and insulin resistance, and the loss of body condition score (BCS) (Bauman & Currie, 1980).

Although there has been considerable research effort into cow nutrient requirements during this period, results and recommendations are inconsistent (see review by Overton & Waldron, 2004). If management during the transition period affects milk production, there should be an association amongst plasma metabolites and hormones and milk production variables. The objective of the current study was to determine if such a relationship exists and, if so, what implications the associations have for milk production.

MATERIALS AND METHODS

Experimental design and treatments

Full details of the three experiments used in this analysis are presented by Roche *et al*. (2004; 2005; 2006). Briefly, the three experiments were run at DairyNZ's Scott and Lye Farms in Hamilton between 2003 and 2005. During the three experiments, 188 multiparous Holstein-Friesian or Jersey x Holstein-Friesian cows were randomly allocated to one of eight pre-calving dietary treatments. Pre-calving treatments included: energy intakes of 60, 90, 120 and 150 MJ metabolisable energy (ME)/cow/d (Roche *et al.*, 2004), dry matter intakes (DMI) of 5 and 12 kg dry matter (DM)/cow/d (Roche *et al.*, 2005) and iso-energetic intakes (114 MJ ME/cow/d) of pasture/pasture silage with or without concentrate supplementation (Roche *et al*., 2006).

At calving, cows were either grazed together on *ad libitum* fresh pasture (Roche *et al.*, 2004), or randomly allocated to one of two post-calving dietary treatments in a 2 x 2 factorial arrangement. Post-calving dietary treatments included DMI of 10 and 15 kg DM/cow/d (Roche *et al*., 2005) or iso-energetic intakes (179 MJ ME/cow/d) of pasture/pasture silage with or without concentrate supplementation (Roche *et al*., 2006).

Grazing management

Cows had access to a fresh allocation of pasture daily. The experimental groups were grazed within

the same paddock and separated by double strands of electric fence to control pasture allowances both pre-calving and post-calving. Back-grazing behind the day's allocation was prevented by electric fences and the cows had access to water in their respective treatment areas. To achieve different pasture allowances, and hence intakes, areas of different sizes were allocated daily to each treatment group.

Measurements

Individual milk yield was recorded twice daily (Westfalia Surge, Oelde, Germany) for the first 35 days of lactation. Fat, protein and lactose concentrations were determined by Milkoscan (Foss Electric, Hillerød, Denmark) on individual evening and morning milk samples collected on two days each week.

Blood was collected from each cow by coccygeal venipuncture into heparinised (10 mL) evacuated tubes before treatment allocation (covariate) and on Day -21, -14, -7, 0, 1, 2, 3, 4, 7, 14, 21, 28 and 35 relative to calving. Plasma was harvested after centrifuging at 1,120 *g* for 10 minutes at 4°C, and analysed for non-esterified fatty acids (NEFA), β-hydroxy butyrate (BOH), glucose, aspartate aminotransferase (AST), glutamate dehydrogenase (GDH), growth hormone (GH), insulin-like growth factor-1 (IGF-1), albumin (ALB), insulin and leptin. The NEFA (colorimetric method), BOH (BOH dehydrogenase assay), glucose (hexakinase method) and AST (IFCC UV test) analyses were all performed on a Hitachi 717 analyser (Roche, Basal, Switzerland) at 30°C by Alpha Scientific Ltd., Hamilton. The inter- and intra-assay coefficient of variation (CV) was <2% for all assays. Growth hormone (Downing *et al.*, 1995), IGF-1 (Gluckman *et al.*, 1983), insulin (Hales and Randle, 1963) and leptin (Blache *et al.*, 2000) were measured in duplicate by double-antibody radioimmunoassay with an inter- and intra-assay with a CV of <6%.

Statistical analysis

Data from all three experiments were pooled within treatment and associations among blood hormones and metabolites, and milk production variables determined using regression analysis. Due to the large number of analyses undertaken only metabolites with significant associations have been included for discussion. Milk data from week one of lactation were not included in the analysis because of missing data for some cows due to timing of calving relative to milk sampling.

TABLE 1: Associations among pre-calving plasma metabolites and weekly milk and milksolids yield. SE = Standard error; BOH = β-hydroxy butyrate; NEFA = Non-esterified fatty acids; MS = Milksolids; ALB = Albumin; IGF-1 = Insulin-like growth factor-1.

Y variable	X variable	Days pre-calving	Slope ± SE	r^2	P value
Milk yield (kg/wk)	BOH (mmol/L)	-21	-18.5 ± 10.4	0.02	0.08
		-14	-9.9 ± 5.6	0.02	0.08
		-7	-24.2 ± 9.9	0.04	<0.05
	Glucose (mmol/L)	-21	24.4 ± 8.4	0.05	<0.01
		-14	17.0 ± 7.8	0.03	<0.05
		-7	14.8 ± 8.1	0.03	0.07
	Leptin (ng/mL)	-21	6.2 ± 6.1	0.01	0.31
		-14	19.7 ± 9.8	0.05	<0.05
		-7	26.5 ± 9.6	0.10	<0.01
	NEFA (mmol/L)	-21	-22.2 ± 8.8	0.03	<0.05
		-14	-12.4 ± 5.7	0.03	<0.05
		-7	-12.8 ± 6.8	0.03	0.06
MS yield (kg/wk)	ALB (g/L)	-21	0.06 ± 0.08	0.01	0.45
		-14	0.14 ± 0.07	0.03	<0.05
		-7	0.12 ± 0.07	0.02	0.09
	Glucose (mmol/L)	-21	2.51 ± 0.61	0.09	<0.001
		-14	1.65 ± 0.59	0.05	<0.01
		-7	1.48 ± 0.62	0.04	<0.05
	IGF-1 (ng/mL)	-21	0.03 ± 0.01	0.03	<0.05
		-14	0.05 ± 0.02	0.05	<0.001
		-7	0.02 ± 0.02	0.01	0.40
	Leptin (ng/mL)	-21	1.17 ± 0.45	0.06	<0.01
		-14	2.31 ± 0.76	0.11	<0.01
		-7	2.62 ± 0.77	0.15	<0.001
	NEFA (mmol/L)	-21	-1.40 ± 0.66	0.03	<0.05
		-14	-0.79 ± 0.44	0.02	0.07
		-7	-0.84 ± 0.53	0.02	0.11

RESULTS AND DISCUSSION

The dataset presented is a collation of periodic measurements of metabolic status in pasture-based dairy cows during the periparturient period of significant physiological change. These data provide insight into periparturient metabolic disturbances, their implications for aspects of animal health, and subsequent milk production.

Although associative analyses are an important component of inductive reasoning, caution is required in interpreting such outputs and, in particular, in the inference of cause and effect. For example, plasma calcium concentration during the colostrum period was negatively

associated (P <0.05) with milk (Slope = -23.09) and milksolids (MS) yield (Slope = -1.72) in the current dataset. However, it is unlikely that increasing blood calcium resulted in reduced milk production. It is more plausible to induce the hypothesis that higher producing cows have lower blood calcium because of the greater requirement for milk calcium.

Caution is also urged when interpreting the importance of the associations under investigation. Although a statistically significant (P <0.05) association may be evident, the change may not result in a biologically meaningful difference in milk production. Furthermore, the size of the coefficient of determination (r^2) reflects the relative importance of the metabolite in question in explaining the recorded variation in the dependent variable and must, therefore, be given due consideration.

Pre-calving

Data indicate a positive association (P <0.05) among milk and MS yield and pre-calving leptin and glucose concentrations and a negative association with plasma NEFA concentrations (Table 1). Furthermore, pre-calving plasma BOH was negatively associated with milk yield and both IGF-1 and ALB concentrations were positively associated with MS yield. Greater circulating concentrations of leptin, glucose, IGF-1 and ALB coinciding with lower circulating NEFA and BOH are indicative of a more positive energy balance. The results are, therefore, consistent with a positive association between pre-calving energy balance and milk production. This conclusion is supported by the positive effect of pre-calving BCS gain and calving BCS on milk production reported by Roche *et al.* (2009). If body reserves are being mobilised pre-calving and as a result, cows are thinner at calving, milk and MS yield will be reduced.

However, despite this consistent association between these energy balance indicators and milk production, each metabolite explained less than 10% of the variation in recorded milk and MS yield. This indicates either that

TABLE 2: Associations among plasma metabolites and hormones during the colostrum period (Days 1 to 4 post-calving), weekly milk yield and average fat and protein % between Weeks 2 and 5 post-calving. SE = Standard error; ALB = Albumin; AST = Aspartate aminotransferase; GDH = Glutamate dehydrogenase; GH = Growth hormone; IGF-1 = Insulin-like growth factor-1; NEFA = Non-esterified fatty acids; BOH = β-hydroxy butyrate.

Y variable	X variable	Days post-calving	Slope ± SE	r^2	P value
Milk yield (kg/wk)	ALB (g/L)	1	2.1 ± 0.8	0.05	<0.01
		2	2.1 ± 0.7	0.06	<0.01
		3	2.2 ± 0.8	0.07	<0.01
		4	2.2 ± 0.7	0.07	<0.01
	AST (mmol/L)	1	-0.40 ± 0.17	0.09	<0.05
		2	-0.07 ± 0.10	0.01	0.49
		3	-0.03 ± 0.06	0.00	0.62
		4	-0.02 ± 0.07	0.00	0.83
	GDH (mmol/L)	1	-0.33 ± 0.16	0.06	<0.05
		2	-0.27 ± 0.10	0.09	<0.05
		3	-0.08 ± 0.08	0.02	0.32
		4	-0.12 ± 0.14	0.01	0.37
	GH (ng/mL)	1	-0.35 ± 0.30	0.01	0.24
		2	-1.00 ± 0.31	0.05	<0.001
		3	-0.77 ± 0.36	0.02	<0.05
		4	-0.49 ± 0.42	0.01	0.24
	Glucose (mmol/L)	1	-0.2 ± 3.9	0.00	0.97
		2	-5.4 ± 4.5	0.01	0.23
		3	-10.4 ± 5.0	0.02	<0.05
		4	-12.4 ± 5.2	0.03	<0.05
	IGF-1 (ng/mL)	1	-0.91 ± 0.39	0.03	<0.05
		2	-0.77 ± 0.43	0.02	0.08
		3	-1.13 ± 0.45	0.03	<0.05
		4	-1.61 ± 0.49	0.06	<0.001
	NEFA (mmol/L)	1	10.4 ± 4.7	0.03	<0.05
		2	18.5 ± 4.6	0.08	<0.001
		3	19.2 ± 4.3	0.09	<0.001
		4	17.0 ± 4.5	0.07	<0.001
MS yield (kg/wk)	ALB (g/L)	1	0.20 ± 0.06	0.08	<0.001
		2	0.20 ± 0.06	0.09	<0.001
		3	0.21 ± 0.06	0.10	<0.001
		4	0.21 ± 0.06	0.11	<0.001
	GH (ng/mL)	1	-0.05 ± 0.02	0.03	<0.05
		2	-0.10 ± 0.02	0.10	<0.001
		3	-0.09 ± 0.03	0.06	<0.001
		4	-0.10 ± 0.03	0.06	<0.001
	IGF-1(ng/mL)	1	-0.05 ± 0.03	0.02	0.08
		2	-0.02 ± 0.03	0.00	0.58
		3	-0.06 ± 0.03	0.02	0.08
		4	-0.11 ± 0.04	0.05	<0.01

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Table 2 continued:

Y variable	X variable	Days post-calving	Slope ± SE	r^2	P value
	NEFA (mmol/L)	1	0.47 ± 0.35	0.01	0.19
		2	1.26 ± 0.35	0.06	<0.001
		3	1.28 ± 0.33	0.08	<0.001
		4	1.25 ± 0.33	0.07	<0.001
Protein (%)	BOH (mmol/L)				
		1	-0.07 ± 0.08	0.00	0.39
		2	-0.14 ± 0.08	0.02	0.07
		3	-0.19 ± 0.08	0.03	<0.05
		4	-0.15 ± 0.06	0.03	<0.05
	GH (ng/mL)	1	-0.004 ± 0.002	0.02	0.08
		2	-0.003 ± 0.003	0.01	0.30
		3	-0.005 ± 0.003	0.02	0.06
		4	-0.010 ± 0.003	0.04	<0.01
	Glucose (mmol/L)	1	0.01 ± 0.03	0.00	0.70
		2	0.12 ± 0.04	0.06	<0.001
		3	0.14 ± 0.04	0.06	<0.001
		4	0.14 ± 0.04	0.06	<0.001
	IGF-1 (ng/mL)	1	0.003 ± 0.003	0.01	0.28
		2	0.008 ± 0.003	0.03	<0.05
		3	0.009 ± 0.004	0.03	<0.05
		4	0.012 ± 0.004	0.04	<0.01
	Insulin (µIU/mL)	1	0.007 ± 0.003	0.03	<0.05
		2	0.009 ± 0.004	0.03	<0.05
		3	0.008 ± 0.004	0.02	0.06
		4	0.016 ± 0.006	0.04	<0.01
	Leptin (ng/mL)	1	0.11 ± 0.06	0.03	0.07
		2	0.20 ± 0.06	0.08	<0.001
		3	0.27 ± 0.07	0.10	<0.001
		4	0.25 ± 0.08	0.07	<0.01
	NEFA (mmol/L)	1	-0.07 ± 0.04	0.02	<0.05
		2	-0.08 ± 0.04	0.02	<0.05
		3	-0.08 ± 0.04	0.03	<0.05
		4	-0.07 ± 0.04	0.02	0.06
	NEFA:ALB	1	-2.8 ± 1.5	0.03	0.07
		2	-2.9 ± 1.5	0.03	<0.05
		3	-3.5 ± 1.4	0.05	<0.05
		4	-3.9 ± 1.4	0.06	<0.01

the majority of the measured variation in milk production is not a function of prepartum energy balance or that the metabolites and hormones measured do not adequately represent the cow's energy balance status. Considering the number of metabolites and hormones included and the published evidence for their association with energy balance, it appears likely that pre-calving energy balance has little impact on subsequent lactational performance. This is consistent with the reported effects of pre-partum DM intake and milk production (Roche *et al.,* 2004; 2005).

Colostrum period

The colostrum period is defined as the first eight milkings after calving. Plasma GH, IGF-1, glucose, AST and GDH concentrations during the four days immediately post-calving were negatively associated (P <0.05) with milk yield (Table 2), and GH (P <0.001) and IGF-1 (P <0.05) were negatively associated with MS yield. In comparison, plasma ALB and NEFA concentrations were positively associated (P <0.01) with milk and MS yield. The greater circulating concentration of NEFA and ALB reflect a greater availability of fatty acids and amino acids for milk synthesis and the evident positive association is consistent with greater milk production with increasing BCS loss post-partum (Roche *et al.*, 2009).

However, BCS loss can also reduce milk production under certain circumstances, with excessive mobilisation reported to be associated with reduced 4% fat corrected milk and milk protein % (quadratic response; Roche *et al.*, 2007). The negative association among GH, GDH, and AST during the colostrum period and subsequent milk yield is consistent with such a quadratic effect. Glutamate dehydrogenase and AST are liver enzymes used to indicate the degree of liver fat infiltration; the greater the concentration of the enzyme circulating in blood, the greater the concentration of triglyceride in liver tissue. Non-esterified fatty acids that do not undergo β-oxidation in the liver are re-esterified to triglycerides and released into circulation as very low density lipoproteins. Although the capacity of the bovine to re-esterify fatty acids is high during periods of negative energy balance, its ability to export very low density lipoproteins is low. Elevated concentrations of these liver enzymes in early lactation may, therefore, reflect hepatocyte triglyceride accumulation, a metabolic condition commonly referred to as "fatty liver disease", and the negative association with milk production is consistent with this premise.

Growth hormone promotes lipolysis (Roche *et al.*, 2009) and can be exogenously administered to cows as recombinant bovine somatotropin (rBST) to increase galactopoiesis (Richard *et al.*, 1985). However, this positive effect of rBST on milk

TABLE 3: Associations among post-calving plasma metabolites and hormones, weekly milk and milksolids yield and average fat % between Weeks 2 and 5 post-calving. SE = Standard error; ALB = Albumin; IGF-1 = Insulin-like growth factor-1; NEFA = Non-esterified fatty acids; MS = Milksolids.

Y variable	X variable	Days post-calving	Slope ± SE	r^2	P value
Milk yield (kg/wk)	ALB (g/L)	7 - 35	2.10 ± 0.78	0.06	<0.01
	Glucose (mmol/L)	7 - 35	-14.9 ± 6.3	0.03	<0.05
	IGF-1 (ng/mL)	7 - 35	-2.36 ± 0.55	0.09	<0.001
	NEFA (mmol/L)	7 - 35	29.8 ± 6.6	0.10	<0.001
	NEFA:ALB	7 - 35	1153 ± 258	0.14	<0.001
MS yield (kg/wk)	ALB (g/L)	7 - 35	0.21 ± 0.06	0.09	<0.001
	IGF-1 (ng/mL)	7 - 35	-0.16 ± 0.04	0.08	<0.001
	NEFA (mmol/L)	7 - 35	2.79 ± 0.48	0.15	<0.001
	NEFA:ALB	7 - 35	96 ± 20	0.16	<0.001
Fat (%)	NEFA (mmol/L)	28	0.25 ± 0.09	0.04	<0.01
	NEFA (mmol/L)	35	0.24 ± 0.12	0.02	<0.05

production is only evident after approximately 60 days in milk (Richard *et al.*, 1985). In the current dataset, circulating GH during the colostrum period may, instead, be indicative of a negative energy balance pre-calving and a consequential lower BCS. Roche (2007) reported that cows subjected to a restricted feed allowance pre-partum had a greater peripartum increase in circulating GH concentrations than a cow receiving adequate energy for her physiological state. This would explain the negative association with milk yield in the current dataset.

The negative association between milk and MS yield and IGF-1 may reflect an effect of genetic merit for milk production. Hart *et al.* (1978) and Lucy *et al.* (2009) compared aspects of endocrinology in cows differing in their genetic merit for milk production and higher yielding cows tend to have lower concentrations of circulating IGF-1, possibly a consequence of a more suppressed somatotropic axis in early lactation (Lucy *et al.*, 2009).

Milk protein % was negatively associated (P <0.05) with plasma GH, NEFA and BOH concentrations, and the NEFA to ALB ratio during the first week post-partum (Table 2). In comparison, it was positively associated (P <0.05) with plasma IGF-1, insulin, leptin, and glucose concentrations (Table 2). It is possible that the greater negative energy balance, indicated by this combination of hormone and metabolite profiles, resulted in greater milk production from mobilised body tissue, reducing the concentration of milk protein through dilution.

Early lactation

There was a negative association (P <0.05) between milk yield and glucose concentration, and milk and MS yield and IGF-1 during the first five weeks post–calving (Table 3). In contrast, there was a positive association (P <0.05) between milk and MS yield and NEFA and ALB concentration and NEFA to ALB ratio (Table 3). These data are consistent with an increasing milk production with increasing negative energy balance, that is cows losing more BCS post-calving. Such a result was previously published by Roche *et al.* (2007), where greater BCS loss to nadir was associated with a greater peak milk and 4% fat-corrected milk yield.

Milk fat % was also positively associated (P <0.05) with NEFA concentrations during weeks four and five of lactation (Table 3), consistent with the increased availability of pre-formed fatty acids for mammary uptake.

CONCLUSIONS

In conclusion, the metabolite data are largely indicative of an association between pre- and post-partum energy balance and milk yield. Cows gaining either BCS pre-calving or, at least, calving in better BCS produce more milk than cows with a metabolite profile indicative of less BCS gain or BCS loss pre-partum. In comparison, BCS loss post-partum was associated with greater milk yields, but lower milk protein %. Elevated concentrations of liver enzymes that have been previously postulated to indicate hepatic triglyceride accumulation were associated with reduced milk yields.

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