Effects of Monensin and Stage of Lactation on Variation of Blood Metabolites Within Twenty-Four Hours in Dairy Cows

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ABSTRACT

Effects of prepartum administration of a monensin controlled release capsule (CRC) and stage of lactation on variation of blood metabolites within 24 h were determined in 16 dairy cows. Cows were fed a total mixed ration ad libitum twice daily at 0700 and 1300 h. At calving, cows were switched from a close-up dry cow diet to a lactating cow diet. Cows were blood sampled every 3 h for 24 h at 3 stages of lactation, including 1 wk before calving (wk -1), 1 wk after calving (wk 1), and 6 wk after calving (wk 6). Serum concentrations of glucose, β -hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), and urea exhibited significant variation within 24 h. Glucose and NEFA were, respectively, 0.09 and 0.08 mM lower between 1030 and 2230 h than between 2230 and 1030 h. β -Hydroxybutyrate and urea were, respectively, 95.1 and 0.49 mM higher between 1030 and 2230 h than between 2230 and 1030 h. Monensin did not significantly affect glucose, NEFA, and urea in this study. Monensin reduced BHBA at wk 1, but not at wk -1 or wk 6. Glucose was lower and BHBA and NEFA were higher at wk 1 compared with wk -1 and wk 6. Urea was higher at wk 6 compared with wk -1. The variation within 24 h of glucose, BHBA, and NEFA were not affected by monensin and stage of lactation. Diurnal variation of urea was affected by stage of lactation, but not by monensin.

(Key words: variation within 24 h, blood metabolites, lactation stage, monensin)

Abbreviation key: CRC = controlled release capsule.

INTRODUCTION

Sutton et al. (1988) and Eicher et al. (1999) observed diurnal variation in the concentrations of glucose,

BHBA, blood urea nitrogen, and NEFA in peripheral blood of cows that were fed twice daily. Nielsen et al. (2003) found variation within 24 h of the blood plasma concentrations of BHBA and NEFA, but not that of glucose, in cows fed TMR. The blood concentrations of BHBA and insulin increased and glucose decreased after meals in cows fed twice daily (Sutton et al. 1988). The study of Sutton et al. (1988) also found that NEFA diminished after the first meal of the day. In cows fed 6 times daily, concentrations of blood metabolites and metabolic hormones did not show significant variation within 24 h (Sutton et al., 1988). Blood concentrations of urea have also been shown to increase during the first few hours after a meal with a peak occurring 1.5 to 4 h after the rumen ammonia peak (Gustafsson and Palmquist, 1993). Nonesterified fatty acids are thought to increase during the night due to a reduction in insulin (Fröhli and Blum, 1988; Sutton et al., 1988; Blum et al., 2000).

In competitive and noncompetitive environments, feeding activity increases after the delivery of fresh feed (Philips and Rind, 2001; Beauchemin et al., 2002; DeVries et al., 2003). Pushing the feed toward the animal results in an increase in feeding activity (DeVries et al., 2003). Dairy cows fed a TMR are normally provided with fresh feed only once or twice daily. The increase in feeding behavior after feed delivery will, therefore, result in variation in feeding behavior throughout the day (Beauchemin et al., 2002; DeVries et al., 2003). This variation in feeding behavior contributes to variation in pH, VFA, and ammonia in the rumen within 24 h (Keunen et al., 2002; Kononoff and Heinrichs, 2003). These variations in rumen conditions, in turn, contribute to variation within 24 h in the concentrations of blood metabolites in peripheral blood.

A monensin controlled release capsule (CRC) has been approved in Canada as an aid in the prevention of subclinical ketosis in lactating dairy cows. Monensin alters rumen fermentation by decreasing the production of acetate and methane and increasing the production of propionate (Bergen and Bates, 1984; Russell

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and Strobel, 1989). Prepartum administration of the monensin CRC has been shown to lower blood BHBA and reduce incidence of subclinical ketosis (Duffield et al., 1998a,b), elevate blood glucose (Green et al., 1999), increase protein digestibility, and improve nitrogen balance in early-lactation dairy cows (Plaizier et al., 2000). Sodium monensin premix has been found to reduce microbial degradation of dietary protein and ammonia production in the rumen (Hanson and Klopfenstein, 1979). It has been suggested that monensin may increase meal frequency in lactating cows (Lunn et al., 2004). This frequency can affect variation in rumen fermentation within 24 h, which contributes to similar variation in blood metabolites.

The objectives of this study were to determine the variation within 24 h of the concentration of glucose, urea, BHBA, and NEFA in cows at 1 wk before calving, 1 wk after calving, and 6 wk after calving and determine the effect of prepartum administration of a monensin CRC on these variations.

MATERIALS AND METHODS

Experimental Procedures

Sixteen second- and third-lactation Holstein cows were blocked in pairs based on their expected calving date. Cows within each block were randomly assigned to 1 of 2 treatments, monensin and control. Monensin treatment consisted of administration of a monensin CRC (Rumensin CRC Provel, Division Eli Lilly Canada Inc., Guelph, ON, Canada). The control treatment consisted of administration of a placebo CRC (Provel). The monensin CRC contained 32 g of monensin sodium blended into a hexaglycerol distearate matrix core. The monensin CRC delivered (mean $\pm$ SD) 335 ± 33 mg/d of monensin for approximately 95 d. The placebo CRC was identical to the monensin CRC, but contained no monensin sodium in the core. The CRC was administered approximately 3 wk before the expected calving date.

Cows entered the experiment between February 1998 and January 1999. Calving dates were evenly distributed throughout the experimental period. Approximately 4 wk before the expected calving date, animals were moved to the physiology wing of the Elora Dairy Research Station where they were housed in individual tie stalls. Animals were fed a TMR ad libitum twice daily at 0700 and 1300 h. At calving, animals were switched from a close-up dry cow diet to a lactating cow diet. For the first 3 wk after calving, cows also received 1.8 kg of alfalfa hay once daily. Detailed descriptions of these diets are given in Plaizier et al. (2000). Cows had unlimited access to fresh water. Two cows were excluded from the precalving measurements due to ear-

lier than expected calving dates, resulting in insufficient length of the collection period. One cow was excluded from the postpartum determinations due to a displaced abomasum. The pairs of these cows were not excluded from the experiment. Blood samples were obtained using a jugular vein catheter every 3 h for 24 h at approximately 7 d before the expected calving date (wk -1) and at 7 (wk 1) and 42 d (wk 6) after calving. Average DM intakes were 10.4 ± 2.4 , 15.5 ± 1.89 , and 20.5 ± 1.85 kg/d (mean $\pm$ SD) at wk -1, wk 1, and wk 6, respectively. Average milk yields were 28.8 ± 5.36 and 39.1 ± 5.67 kg/d at wk 1 and wk 6, respectively. More details about the cows and experimental procedures are given in Plaizier et al. (2000).

Blood Sampling and Analyses

Blood for the harvesting of serum was collected into 10-mL red top (plain) Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Blood was left to clot at room temperature, which ranged from 17 to 24°C, for 1 h and subsequently centrifuged at $900 \times g$ for 20 min. Serum was stored at -20°C.

Serum was analyzed at the Animal Health Laboratory of the University of Guelph, using a BM/Hitachi 911 analyzer (Boehringer Mannheim, Mannheim, Germany) using a BHBA reagent (procedure no. 310-UV, Sigma Diagnostics, St. Louis, MO; McMurray et al., 1984), a glucose kit (Cat. no. 1 448 668, Boehringer Mannheim, Mannheim, Germany; Trinder, 1969), a blood urea nitrogen kit (Cat. no. 1 489 321 Boehringer Mannheim; Talke and Schubert, 1965), and a NEFA kit (Randox Laboratories, Ardmore, UK; Matsubara et al., 1983) for these respective analyses.

Statistical Analyses

The ANOVA was conducted using the SAS Mixed Procedure (SAS Institute, 1996) as recommended by Wang and Goonewardene (2004) for the analysis of animal experiments with repeated measures. The effects of treatment (monensin or control), lactation stage (wk -1, wk 1, or wk 6), hour, and their interactions were considered fixed. Block and interactions of other factors with block were considered random. Covariance structures relative to hourly measurements that were tested included simple, compound symmetry, first-order autoregressive, first-order antedependence, and unstructured (Wang and Goonewardene, 2004). Final mixed models were accepted only if the converge criteria was met, the estimated G matrix was a positive definite, and the degrees of freedom for hour were the same as those obtained by running the same model using the GLM procedure (SAS Institute, 1996). The covariance

Table 1. Concentrations of blood metabolites of cows at different stages of lactation receiving a monensin controlled release capsule (M) or a placebo (C) precalving.

	Lactation stage ¹						SE	Effect, <i>P</i> <		
	Wk -1		Wk 1		Wk 6			M	LS	M × LS
	M	C	M	C	M	C				
Glucose, mM	3.64	3.53	3.10	2.64	3.56	3.55	0.28	0.23	<0.0001	0.12
BHBA, μM	568	525	1122 ^b	1459 ^a	639	612	119	0.18	0.0003	0.07
NEFA, mEq/L	0.32	0.24	0.68	0.81	0.19	0.19	0.07	0.91	<0.0001	0.40
Urea, mM	5.28	4.93	5.81	5.27	6.08	5.80	0.48	0.84	0.08	0.93

^{a,b}Treatment means within lactation stage with different letters differ (*P* < 0.05).

¹Wk -1 = 1 wk before calving; wk 1 = 1 wk after calving; and wk 6 = 6 wk after calving.

structure that resulted in the lowest value for the fit statistic was chosen (Wang and Goonewardene, 2004). The full model used for the SAS Mixed Procedure (SAS Institute Inc., 1996) was:

```
PROC MIXED;
Class TR Block LS ST;
model Y = T LS LS*TR
Hour Hour*Diet ST*LS ST*LS*TR/DDFM = satterth;
Random Block Block*TR LS*TR*Block;
Repeated ST/ Type=arh(1) Subject=LS*TR*Block;
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where Y = dependent variable (glucose, BHBA, urea or NEFA); TR = treatment (monensin or control); LS = stage of lactation; and ST = sampling time.

Significance of differences in blood metabolites among periods within 24 h was determined using orthogonal contrasts.

RESULTS AND DISCUSSION

Glucose

Monensin did not affect glucose at any stage of lactation although glucose was numerically higher in monensin-treated compared with placebo-treated cows during wk 1 (Table 1). Averaged across monensin and control, serum glucose was 3.57 mM at wk -1, 2.84 mM at wk 1, and 3.59 mM at wk 6. This average was lower at wk 1 compared with wk -1 and wk 6 (*P* < 0.0001).

Table 2. Effects (*P*-values) of sampling time (ST) and the interactions of prepartum administration of a monensin controlled release capsule (M) and lactation stage (LS) with sampling time (ST) on concentrations of blood metabolites.

Item	ST	M × ST	LS × ST	M × LS × ST
Glucose, mM	0.0003	0.47	0.42	0.34
BHBA, μ M	<0.0001	0.65	0.82	0.42
NEFA, mEq/L	<0.0001	0.78	0.89	0.54
Urea, mM	<0.0001	0.93	0.037	0.61

Serum glucose exhibited significant (*P* < 0.001) variation within 24 h, with a higher concentration during the night compared with during the day (Table 2, Figure 1). Glucose was on average 0.09 mM lower (*P* < 0.05) between 1030 and 2230 h than between 2230 and 1030 h. The variation of glucose within 24 h in our study was not influenced by monensin or stage of lactation (Table 2, Figure 1).

Monensin can affect the metabolism of glucose by increasing the availability of glucose by shifting rumen fermentation toward propionate, reducing methane production, and reducing rumen digestion of dietary protein (Bergen and Bates, 1984). Monensin might also increase glucose availability by increasing the proportion of dietary starch that is digested postruminally (Haïmoud et al., 1995). Prepartum administration of a monensin CRC increased the glucose concentration in peripheral blood of postpartum cows in the studies from Duffield et al. (1998a) and Green et al. (1999), but only a numerical trend was found in the study from Duffield et al. (2003). The latter was explained by the lack of power and low number of cows compared with the study of Duffield et al. (1998a). The lack of effect of monensin on blood glucose in the current study might also be explained by the relatively small number of experimental animals and insufficient statistical power of the experiment. Given the variation in glucose among cows at wk 1, a significance level of 0.05, and a power of 0.8, only differences between monensin and control greater than 20% would have been significant. Reduced blood glucose in postpartum cows compared with prepartum cows and subsequent increases in blood glucose as lactation progresses were also observed by Stephenson et al. (1997), Duffield et al. (1998a), Green et al. (1999), and Duffield et al. (2003) and correspond to changes in the energy status of the cows.

Variation within 24 h of the concentration of glucose in peripheral blood of cows was also observed by Borrebaek et al. (1990), Eicher et al. (1999), and Blum et al. (2000). In contrast, Nielsen et al. (2003) did not observe

Figure 1. Diurnal variation in blood serum glucose in cows receiving a placebo (Control) or a monensin controlled release capsule (Monensin) precalving or at 1 wk before calving (■), 1 wk after calving (▲), and 6 wk after calving (▼).

such a variation in cows that were provided with TMR 4 times daily. Serum glucose was higher overnight compared with during the day when the intensity of feeding activity, the extent of rumen fermentation, and the availability of substrates for gluconeogenesis were expected to be higher. In the cows of the current study, rumen pH was indeed higher during the day compared with night, which suggests higher rumen VFA during the day than at night (Duffield et al., 2004). Sutton et

al. (1988) and Borrebaek et al. (1990) observed that glucose decreased and that insulin and ketone bodies increased after meals in cows fed twice daily and that glucose and ketone bodies were negatively correlated during the day. Hence, the decrease in blood glucose after meals could be due to the increase in insulin, which might be related to higher blood levels of ketone bodies, or a direct action of ketone bodies on blood glucose (Borrebaek et al., 1990).

BHBA

Monensin reduced BHBA at wk 1, but not at wk -1 or wk 6 (Table 1). Averaged across monensin and control, serum BHBA was 558.6 μM at wk -1, 1271.1 μM at wk 1, and 609.9 μM at wk 6. Lactation stage affected BHBA significantly, as BHBA was higher ($P = 0.0003$) at wk 1 compared with wk -1 and wk 6. Significant ($P < 0.001$) variation within 24 h was exhibited by BHBA (Table 2, Figure 2). Levels of BHBA were, on average, 95.1 μM higher ($P < 0.001$) between 1030 and 2230 h than between 2230 and 1030 h. Variation within 24 h of BHBA was affected by stage of lactation, but not by monensin (Table 2, Figure 2).

Results of the current study are consistent with Duffield et al. (1998a), Green et al., (1999), and Duffield et al. (2003), who found that prepartum administration of the monensin CRC reduced BHBA at wk 1, 2, and 3 postpartum, during 6 wk postpartum, and within 7 d postpartum, respectively. These effects were explained by improvement of the energy status of postpartum cows by shifting rumen fermentation toward propionate, thereby reducing the production of ketone bodies from oxidation of fatty acids (Bergen and Bates, 1984; Duffield et al., 1998a; Duffield et al., 2003). In the cows of the current study, monensin indeed reduced the acetate to propionate ratio in the rumen (Fairfield, 2003). Monensin could also reduce BHBA by reducing butyrate in the rumen, which is converted to BHBA in the rumen epithelium (Duffield et al., 1998a; Green et al., 1999). Similar to the current study, Duffield et al. (1998a) found that by wk 6 postpartum monensin no longer reduced BHBA and that blood BHBA levels had reduced substantially from the first week after calving. Moreover, Green et al. (1999) observed that monensin had only a limited effect on BHBA precalving.

Reduced blood BHBA in prepartum cows compared with postpartum cows and subsequent increases in blood BHBA as lactation progresses were also observed by Stephenson et al. (1997), Duffield et al. (1998a), Green et al. (1999), and Duffield et al. (2003) and correspond to changes in the energy status and fat mobilization of the cows. Variation within 24 h of blood BHBA was also demonstrated by Borrebaek et al. (1990),

Figure 2. Diurnal variation in serum BHBA in cows receiving a placebo (Control) or a monensin controlled release capsule (Monensin) precalving or at 1 wk before calving (■), 1 wk after calving (▲), and 6 wk after calving (▼).

Eicher et al. (1999), and Blum et al. (2000). A rise in BHBA occurred after meals, which is believed to result from an increase in conversion of butyrate into BHBA in the epithelium (Borrebaek et al., 1990). As cows in the current study were fed at 0700 and 1300 h, most meals must have occurred during the day (Beauchemin et al., 2002; DeVries et al., 2003), which explains higher blood BHBA during the day compared with during the night. Stage of lactation might have affected the variation of BHBA within 24 h, due to the higher blood BHBA

in wk 1 compared with wk -1 and wk 6. In addition, variation within 24 h of blood BHBA at wk 1 can be attributed to variation within 24 h of butyrate metabolism in the rumen epithelium as well as variation within 24 h of ketone body production in the liver. At wk -1 and wk 6, the energy status of the cows was better than at wk 1, which would lead to a reduction of ketone body production and less contribution of variation within 24 h in hepatic ketone production to variation within 24 h of blood BHBA.

NEFA

Monensin did not affect NEFA at any stage of lactation (Table 1). Averaged across monensin and control, serum NEFA was 0.28 mEq/L at wk -1, 0.74 mEq/L at wk 1, and 0.18 mEq/L at wk 6. Lactation stage affected NEFA significantly, as NEFA was significantly ($P < 0.001$) higher at wk 1 compared with wk -1 and wk 6. Serum NEFA exhibited significant ($P < 0.001$) variation within 24 h (Table 2, Figure 3) and was on average 0.08 mM lower ($P < 0.001$) between 1030 and 2230 h than between 2230 and 1030 h. Variation of NEFA within 24 h was not affected by stage of lactation and monensin (Table 2, Figure 3).

Prepartum administration of monensin can reduce NEFA prepartum indicating improved energy status and reduced fat mobilization (Stephenson et al., 1997; Duffield et al., 2003). Despite changes in BHBA that indicated improved energy status, Stephenson et al. (1997) and Duffield et al. (2003) did not observe that monensin significantly reduced NEFA postpartum. This lack of significance was explained by lack of statistical power. As the current experiment included fewer cows than the studies of Duffield et al. (2003) and Stephenson et al. (1997), an absence of the effect of monensin on NEFA postpartum could be expected. Decreased blood NEFA in prepartum cows compared with postpartum cows were also observed by Stephenson et al. (1997) and Duffield et al. (2003), and correspond to a shift in rumen fermentation, reduction in energy status, and increased fat mobilization.

A nightly rise in NEFA was also observed by Fröhli and Blum (1988) and Blum et al. (2000). Sutton et al. (1988) only observed this increase in cows fed a high concentrate twice daily, but not in cows fed low or high concentrate diets fed 6 times daily, or in cows fed a low concentrate diet twice daily. Nielsen et al. (2003) also observed a peak before the first feeding of the day and a large effect of the timing of feed delivery on the daily pattern of NEFA. This effect of diet and time of feed delivery can be explained by dietary variation in insulin. Infrequent feed delivery will result in a large

Figure 3. Diurnal variation in serum NEFA in cows receiving a placebo (Control) or a monensin controlled release capsule (Monensin) precalving or at 1 wk before calving (■), 1 wk after calving (▲), and 6 wk after calving (▼).

variation of feeding behavior throughout the day (Beauchemin et al., 2002; DeVries et al., 2003), resulting in a large variation in insulin (Sutton et al., 1988; Borrebaek et al., 1990) and, therefore, fat mobilization (Blum et al., 2000). Hence, when feed is delivered during the day, most meals occur during the day, which would result in lower NEFA during the day compared with during the night.

Figure 4. Diurnal variation in serum urea in cows receiving a placebo (Control) or a monensin controlled release capsule (Monensin) precalving or at 1 wk before calving (■), 1 wk after calving (▲), and 6 wk after calving (▼).

Urea

Monensin did not alter serum urea at any stage of lactation (Table 1). Averaged across monensin and control, serum glucose was 5.13 mM at wk -1, 5.53 mM at wk 1, and 6.14 mM at wk 6. Lactation stage did not affect serum urea significantly. Serum urea exhibited significant ($P < 0.001$) variation within 24 h (Table 2, Figure 1) and was on average 0.49 mM higher ($P <$

0.001) between 1030 and 2230 h than between 2230 and 1030 h. Variation of urea within 24 h was affected by stage of lactation, but not by monensin (Table 2, Figure 4).

Green et al. (1999), Duffield et al. (1998a), and Duffield et al. (2003) observed that monensin increased serum urea in postpartum cows. This effect might be explained by the protein-sparing effect of monensin in the rumen (Hanson and Klopfenstein, 1979; Bergen and Bates, 1984), which increases the supply of protein to the small intestine. That the current study failed to show this effect might be due to the small size of our study and differences in the dietary rumen-degradable and rumen-undegradable protein contents among studies.

The substantial variation within 24 h in blood urea might be explained by the variation in feeding behavior throughout the day. As cows were supplied with TMR at 0700 and 1300 h, most meals would have occurred immediately after these times (Beauchemin et al., 2002; DeVries et al., 2003). This contributes to variation within 24 h in the concentration of urea in peripheral blood, as this concentration peaks and subsequently drops in the first few hours after a meal (Gustafsson and Palmquist, 1993).

CONCLUSIONS

The concentrations of glucose, BHBA, NEFA, and urea in peripheral blood of cows provided with TMR twice daily showed substantial variation within 24 h. The variations of blood metabolites within 24 h were not affected by monensin. With exception of urea, these variations were not affected by stage of lactation. Monensin reduced BHBA at 1 wk after calving, but not at 1 wk before calving or 6 wk after calving. Monensin did not significantly affect glucose and NEFA in this study, but that was most likely due to a small number of experimental animals. Differences in glucose, BHBA, and NEFA among the different stages of lactation reflected differences in the energy status of the cows between 1 wk before calving and 1 and 6 wk after calving.

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