

# Changes in Blood Glucose, Nonesterified Fatty Acids, and Ketones in Response to Weaning and Feed Intake in Young Calves

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## ABSTRACT

Effects of weaning age on blood glucose, ketones, and nonesterified fatty acids were examined using 16 Holstein heifer calves. Animals entered the study at  $6 \pm 3.5$  d of age and were fed 1.8 kg of milk twice daily to 28 (early weaning) or 56 d (late weaning) and a commercial pelleted calf starter from 0 (early) or 28 d (late). Blood was sampled once weekly for 14 wk and analyzed for  $\beta$ -hydroxybutyrate and acetoacetate. Plasma was analyzed for glucose and nonesterified fatty acids. Blood  $\beta$ -hydroxybutyrate increased with increasing grain intake and was greater during wk 0 to 4 and 5 to 8 in calves weaned early than in those weaned late. Blood acetoacetate followed trends similar to  $\beta$ -hydroxybutyrate and averaged 23.8 and 16.1  $\mu\text{M}$  in calves weaned early and late, respectively. Plasma glucose and nonesterified fatty acids declined with age and were lower during 5 to 8 wk in calves weaned early. Data suggest that ketone concentrations resulted from alimentary ketogenesis, which increased rapidly after weaning. (Key words: calves, ruminal development, ketones)

Abbreviation key: ACAC = acetoacetate,  $\beta$ HOB =  $\beta$ -hydroxybutyrate, TDMI = total DM intake.

## INTRODUCTION

Development of mature ruminal function appears to depend upon initiation of dry feed intake. Fermentation of carbohydrates to VFA by microflora in the immature rumen initiates

metabolic development of ruminal function. Changes in size of reticulorumen (27, 30), metabolic activity of ruminal mucosa (16, 25), concentration of ruminal VFA (1, 22), speciation of ruminal microflora (5, 15), and proportion of bacterial N in abomasal N (22) depend on initiation of dry feed intake.

Much of the literature evaluating development of ruminal function has addressed characteristics associated with size or metabolic activity of the organ. However, fewer data are available regarding effects of maturing ruminal function on metabolism by the animal. Murdock and Wallenius (19) suggested that feeding calves rations to promote elevated concentrations of ruminal butyrate would increase rate of ruminal maturation because butyrate influences metabolic activity of ruminal epithelium. Liver and ruminal epithelium metabolize butyrate to ketones in the mature ruminant (3, 11). These ketones are then released to the general circulation and used for energy by peripheral tissues. Increased ruminal butyrate may increase ketone production, thereby increasing concentration of ketones in peripheral circulation. However, it has not been determined whether developing ruminal function affects blood ketone concentrations in young calves or whether age at weaning may influence utilization of circulating ketones.

The objective of this research was to measure concentrations of ketones, glucose, and NEFA in blood of young calves during the periweaning period and to determine if early weaning and dry feed intake alter concentrations and source of these circulating energy metabolites.

## MATERIALS AND METHODS

### Animal Assignments and Feed Management

Sixteen Holstein heifer calves were assigned alternately at birth to a completely randomized

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TABLE 1. Formulation of experimental starter.<sup>1</sup>

Ingredient	(%)
Corn, ground	20.0
Soybean meal, 48%	7.5
Soybean hulls	20.0
Wheat midds	25.0
Cottonseed hulls	7.5
Cottonseed meal	5.8
Alfalfa meal	5.0
Molasses	3.0
Vitamins, minerals, and additives	6.2

<sup>1</sup>Ingredients expressed on an as-fed basis.

experimental design with weaning at 28 (early) or 56 d (late) after initiation of the study. Animals were placed on the 14-wk study at 6 d of age (SD = 3.5 d). Calves were left with the dam to 3 d of age, when they were moved to individual pens bedded with sawdust in an unheated calf barn. Fresh water was available at all times.

Calves were fed 1.8 kg of whole or unsalable milk twice daily (0730 and 1630 h) from nipple pails until weaning. Calves weaned early were offered calf starter for ad libitum consumption to a maximum of 2.7 kg/d once daily (0800 h) from d 0; calves weaned late were offered calf starter once daily from d 28. Refused calf starter was weighed daily and reported weekly. When calves consumed 2.7 kg of calf starter per day (as-fed basis), alfalfa hay was offered for ad libitum consumption. Refused hay was weighed as required.

Calf starter was a commercial formulation (Table 1) containing high fiber by-products to stimulate development of ruminal function (19). Calf starter was sampled once for each shipment and analyzed for DM and CP (2); ADF and NDF (8); Ca, Mg, and K (atomic absorption spectrophotometry), and P (9). Alfalfa hay was sampled weekly and stored at -20°C prior to monthly compositing and analysis.

Body weight was measured at birth, on d 0 (1st d of the study), and every 7 d thereafter.

#### Blood Sampling and Analysis

Once per week (on Wednesday), a sample of approximately 10 ml of blood was taken by

jugular venipuncture into evacuated containers (143 USP units of sodium heparin as anticoagulant) and placed on ice prior to transport to the laboratory. Blood was collected approximately 2 h after a.m. feeding. Plasma was harvested and stored (-20°C) prior to analysis for glucose (Glucose Kit Number 510, Sigma Chemical Co., St. Louis, MO). A second sample of jugular blood (approximately 10 ml) was taken; 2 ml were added to 2 ml 1 M perchloric acid, mixed, and placed on ice prior to transport to the laboratory. Blood was analyzed immediately for  $\beta$ -hydroxybutyrate ( $\beta$ HOB) and acetoacetate (ACAC) as in Heitmann et al. (11). Remaining blood was added to 400  $\mu$ l of 6% EDTA; plasma was harvested and stored at -20°C prior to analysis for NEFA (NEFA C kit, Wako Pure Chemical Industries, Osaka, Japan).

#### Statistical Analysis

Blood variables ( $\beta$ HOB, ACAC, NEFA, glucose), average daily gain, total DM intake (TDMI), and intake of feeds and nutrients were analyzed as a split-plot design using the model:

$$Y_{ijk} = \mu + T_i + C_{(i)j} + P_k + (TP)_{ik} + e_{(ijk)}$$

where:

- $Y_{ijk}$  = dependent variables,
- $\mu$  = overall mean,
- $T_i$  = effect of treatment  $i$ ,
- $C_{(i)j}$  = effect of calf  $j$  nested within treatment  $i$ ,
- $P_k$  = effect of time period  $k$ ,
- $(TP)_{ik}$  = effect of time  $\times$  period interaction,
- $e_{(ijk)}$  = residual.

Term  $C_{(i)j}$  was used as error term to test differences due to treatment. Standard errors of treatment means were calculated as described by Gill (7).

Initial BW was analyzed as a completely randomized design by ANOVA. Final BW and BW at 28 and 56 d were analyzed by analysis of covariance as a completely randomized design using initial BW as covariable. Probability of  $P < .05$  was used throughout unless otherwise noted.

TABLE 2. Chemical composition of feeds.<sup>1</sup>

Item	Calf starter	Alfalfa hay
DM, %	89.1	89.9
	————— (% of DM) —————	
CP	18.9	13.4
ADF	19.8	41.3
NDF	33.5	59.4
Ca	1.26	.83
P	.57	.35
Mg <sup>2</sup>	.34	.29
K <sup>2</sup>	1.54	2.31

<sup>1</sup>Mean of three observations.<sup>2</sup>One observation (calf starter).

### RESULTS AND DISCUSSION

Chemical composition of calf starter and hay is in Table 2. Calf starter averaged 33.5% NDF, consistent with presence of cottonseed and soybean hulls at 8 and 20% of formulation, respectively. Crude protein (18.9%) was slightly higher than that formulated (18%). Alfalfa hay was lower in protein (13.4%) and higher in NDF (59.4%) than typically recommended for calves (6) but reflected limited availability of high quality forage in the Southeast during the 1988 to 1989 drought.

Calves were generally healthy throughout the trial. Twelve incidences of scours, one hernia, two respiratory infections, and one case of bloat were reported. Of scours reported, 11 of 12 cases occurred preweaning; all cases of scours were of minor severity and occurred equally between treatment groups.

Initial and final BW (Table 3) were similar between treatments and averaged 41.1 and 91.2 kg, respectively. Rate of daily gain was unaffected by weaning age (Table 3) averaging .51 and .52 kg/d in calves weaned early and late, respectively. Rate of gain was 17% less than that found by Heinrichs and Hargrove (10) although not different from other studies at the Tennessee Experiment Station (18).

As expected, rate of gain was influenced by week of study. Gain was generally lower prior to weaning and averaged .26, .53, and .66 kg/d during 0 to 4, 5 to 8, and 9 to 14 wk periods, respectively.

Intake of DM was not statistically different between treatments (Table 3) but tended to be greater in calves weaned early. Source of

TABLE 3. Least squares means of BW, gain, and intake in calves weaned early or late.

Item	Early	Late	SE	P
BW, kg				
Initial	41.7	40.5	1.1	NS <sup>1</sup>
28 d	49.5	47.4	1.1	NS
56 d	64.5	62.2	1.7	NS
Final	90.6	91.8	1.0	NS
Gain/day	.51	.52	.03	NS
Intake, kg/d				
DM	1.88	1.77	.05	NS
Milk, <sup>2</sup> wk 1 to 8	.21	.43	.01	.0001
Hay, wk 8 to 14	.52	.47	.06	NS
Grain <sup>2</sup>	1.50	1.28	.24	.007
Hay + grain <sup>2</sup>	1.76	1.52	.05	.004
ADF <sup>2</sup>	.46	.40	.05	.02
NDF <sup>2</sup>	.69	.60	.02	.01
TDN <sup>2,3</sup>	1.51	1.49	.04	NS
CP	.33	.32	.03	NS

<sup>1</sup>NS ( $P > .05$ ).<sup>2</sup>Significant treatment  $\times$  week interaction ( $P < .05$ ).<sup>3</sup>Calculated value of DM of grain (TDN = 80%) + hay (TDN = 59%) + milk (TDN = 129%).

nutrients varied by treatment, however. Calves weaned early consumed less milk and more grain than calves weaned late. Hay intake was similar among treatments. Dry feed intake (hay plus grain) was greater in calves weaned early because of increased grain intake (Table 3). Altered source of nutrients was imposed as part of treatment; therefore, differences in intake of grain and milk were expected.

Intake of ADF and NDF (Table 3) generally reflected intake of grain and milk according to weaning system. However, calculated intake of TDN and CP were similar among treatments indicating that animals are capable of regulating intake of energy from solid and liquid feeds even at an early age.

Concentration of plasma glucose tended to be lower in calves weaned early ( $P < .07$ ) and averaged 85.0 and 90.3 mg/dl in calves weaned early and late, respectively, for the 14-wk study. Concentration of plasma glucose by week of study (Figure 1) declined significantly with advancing age. Concentrations were typical of nonruminants initially (114.5 mg/dl) but reached a nadir at 76 mg/dl by wk 9 to 11 of the study. Weekly means reflected intake of feed, especially from wk 3 to 8. Plasma glucose approached concentrations typical of mature

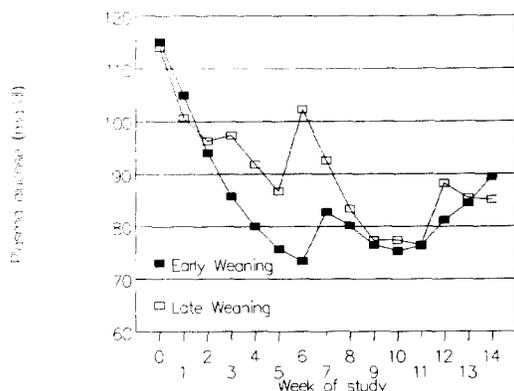


Figure 1. Least squares means of plasma glucose concentration in calves weaned early or late. Effect of week was significant ( $P < .01$ ).

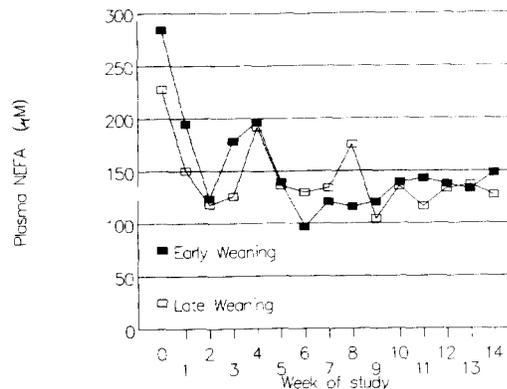


Figure 2. Least squares means of plasma NEFA concentration in calves weaned early or late. Effect of week was significant ( $P < .01$ ).

ruminants (26) postweaning (9 to 14 wk) and did not differ between treatments.

When analyzed by weaning periods (0 to 4, 5 to 8, and 9 to 14 wk), plasma glucose was different ( $P < .01$ ) between treatments during wk 5 to 8 by which time calves weaned early had already been weaned but calves weaned late were fed milk and consumed only small amounts of grain. Mean glucose concentrations during wk 5 to 8 were 78.0 and 91.2 mg/dl in calves weaned early and late, respectively.

Because blood samples were taken 2 h after a.m. feeding, significant treatment differences in glucose concentration during 5 to 8 wk probably was due to elevated glucose in calves fed milk and not a decline in glucose as a result of developing ruminal function per se. As described by Reid (23), changes in blood glucose concentration with advancing age are in response to reduced glucose in erythrocytes and decreased intake of milk, rather than a response to maturing ruminal function. Although erythrocytes were removed prior to glucose analysis in this study, increased plasma glucose as a result of feeding different substrates (milk plus grain versus grain alone) still would impact peripheral concentration. Further, peak glucose concentration may vary with source of glucose entering peripheral circulation; glucose absorbed directly from the small intestine may reach peripheral circulation more rapidly than that entering the circulation via ruminal fermentation of carbohydrate to propionate and hepatic gluconeogenesis. A similar phenome-

non has been reported (1) with ruminal VFA concentration in calves fed high grain diets. Time to peak VFA concentration advanced with advancing age. Thus, as ruminal function develops, both source and timing of glucose entry would be expected to change.

Concentration of plasma NEFA was not significantly affected by treatment within preweaning (0 to 4 wk) and postweaning (9 to 14 wk) periods but was lower ( $P < .01$ ) in calves weaned early during wk 5 to 8. Mean NEFA concentrations during wk 5 to 8 were 116.2 and 140.4  $\mu\text{M}$  in calves weaned early and late, respectively. Concentration of plasma NEFA was affected by week of study (Figure 2) and declined from 255.9  $\mu\text{M}$  at wk 0 to 113.2  $\mu\text{M}$  at wk 6; thereafter, plasma NEFA concentrations did not differ.

Plasma NEFA concentrations consistently were lower than those reported for mature ruminants (13) and lambs (14) but similar to those reported in sheep (28) and lambs (20) sampled within 4 hr of feeding. Time of sampling may affect NEFA concentration, as NEFA are sensitive to plasma glucose and VFA (28). During wk 5 to 8, NEFA in calves fed milk plus grain were higher than in calves already weaned although glucose was significantly higher in these calves. This observation is in contrast with Trenkle and Kuhlemeier (28), who reported an inverse relationship between glucose and NEFA, especially under conditions of fasting. Postweaning increases in plasma propionate and butyrate depressed NEFA from wk 5

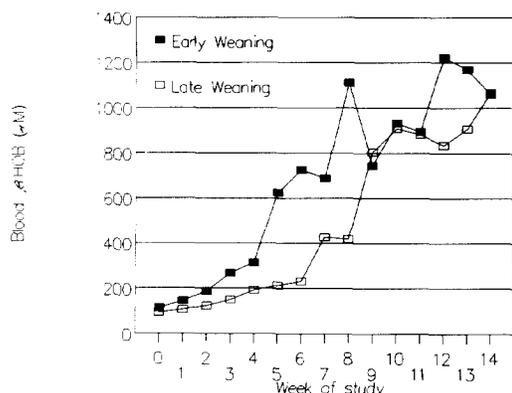


Figure 3. Least squares means of blood  $\beta$ -hydroxybutyrate ( $\beta$ HOB) concentration in calves weaned early or late. Effect of week was significant ( $P < .01$ ).

to 8 in calves weaned early; limited milk feeding and minimal dry feed intake in late-weaned calves relative to energy requirements also may have increased NEFA during this period.

Blood  $\beta$ HOB concentration was greater ( $P < .02$ ) in calves weaned early throughout the 14-wk trial and averaged 680.4 and 491.1  $\mu$ M in calves weaned early and late, respectively. A significant ( $P < .004$ ) week by treatment interaction indicated that magnitude of differences between treatments varied with time. As indicated in Figure 3, calves weaned early maintained higher concentrations of blood  $\beta$ HOB during the first 8 wk of the study. Prior to weaning, blood  $\beta$ HOB increased slowly in calves weaned early and then increased markedly from wk 5 to 8. Calves weaned late showed only a slight increase in blood  $\beta$ HOB concentration after dry feed was offered at 4 wk. Blood  $\beta$ HOB increased markedly at 7 to 9 wk, when significant amounts of dry feed were consumed. At wk 9, increased  $\beta$ HOB in blood of calves weaned late resulted in similar concentration between treatments.

Increases in blood  $\beta$ HOB were closely related to availability of calf starter. Concentration of blood  $\beta$ HOB in early-weaned calves prior to weaning (205.9  $\mu$ M) likely reflected production of acetate and butyrate in the rumen with subsequent metabolism to it by ruminal epithelium. Both of these VFA are ketogenic in young animals (25). Calves weaned late maintained lower levels of  $\beta$ HOB during 0 to 4 wk (131.3  $\mu$ M), probably due to marginal ruminal

fermentation of ingested bedding, milk entering the rumen via backflow, incomplete esophageal groove closure, or lower tract fermentation of digesta. Walker and Simmonds (29) reported that the cecum of young calves is capable of ketone production in the presence of butyrate. During wk 5 to 8, in calves that were weaned early,  $\beta$ HOB concentration increased dramatically to reach 1111.1  $\mu$ M by wk 8. Calves weaned late, though, still received milk during this period although calf starter consumption increased during wk 5 to 8. Consequently,  $\beta$ HOB concentration in blood of calves weaned late increased to 421.0  $\mu$ M by wk 8. After both groups were weaned (9 to 14 wk), however, no differences between groups were observed, and  $\beta$ HOB averaged 952.4  $\mu$ M.

Relationship between blood  $\beta$ HOB and grain intake was not the same for the two treatment groups. For example, calves weaned early consumed .8 kg/d during the 5th wk of the study when blood  $\beta$ HOB concentration averaged 624  $\mu$ M. Calves weaned late consumed .72 kg/d during wk 6, but blood  $\beta$ HOB averaged only 232  $\mu$ M. Magnitude of difference between ketone concentration on relatively similar grain intake suggests that induction of ketogenesis by ruminal mucosa may result from exposure to dry feed intake. This suggestion is in agreement with findings of Walker and Simmonds (29) and Manns and Boda (16), who reported that metabolism of butyrate by ruminal wall of young lambs increased with age, and presumably, dry feed intake. Sutton et al. (25) reported that metabolic activity of ruminal epithelium is induced by presence of ruminal VFA, especially butyrate. Early grain feeding and consequent VFA production possibly caused an increase in metabolic activity of ruminal epithelium, thereby increasing production of a larger amount of  $\beta$ HOB per unit feed intake.

Blood ACAC content tended to be greater in calves weaned early throughout the 14-wk trial and averaged 23.8 and 16.1  $\mu$ M in calves weaned early and late, respectively. Concentration of blood ACAC by week of study (Figure 4) followed by a pattern similar to  $\beta$ HOB, further suggesting that increases in ketone concentration were due to changes in diet associated with weaning. During 0 to 4 wk, concentration of blood ACAC was similar between treatments. During wk 5 to 8, concentrations of

TABLE 4. Coefficients of correlation<sup>1</sup> of blood acetoacetate (ACAC),  $\beta$ -hydroxybutyrate ( $\beta$ HOB), plasma glucose (GLUC), plasma NEFA, average daily gain (ADG), intake of grain (GRNI), dry feed (DRYI), hay (HAYI), and total dry matter (TDMI).

	ACAC	GLUC	NEFA	ADG	GRNI	DRYI	HAYI	TDMI
$\beta$ HOB	.89	-.41	-.17 <sup>b</sup>	.33	.71	.70	.47	.67
ACAC		-.37	-.15 <sup>b</sup>	.23	.56	.52	.35	.52
GLUC			.05 <sup>a</sup>	-.06 <sup>a</sup>	-.27	-.23	-.08 <sup>a</sup>	-.21
NEFA				-.24	-.21	-.15 <sup>b</sup>	-.05 <sup>a</sup>	-.14 <sup>b</sup>
ADG					.41	.37	.16	.37
GRNI						.97	.64	.96
DRYI							.79	.99
HAYI								.81

<sup>1</sup>All coefficients were significant ( $P < .01$ ), except <sup>a</sup> ( $P > .05$ ) and <sup>b</sup> ( $P < .05$ ).

blood ACAC in calves weaned early were greater ( $P < .01$ ) in calves weaned early and averaged 22.6 and 6.4  $\mu$ M in calves weaned early and late, respectively. Marked increase in grain intake in calves weaned early from 5 to 8 wk was probably responsible for increased blood ACAC concentrations. During the 9 to 14 wk period, ACAC concentrations in blood of calves weaned early and late were similar and averaged 39.9 and 31.4  $\mu$ M, respectively.

Production of VFA in the rumen of calves fed high grain diets and weaned at an early age (3 to 6 wk) is well documented (1, 22). Further, high grain diets may increase total VFA concentrations in ruminal fluid relative to diets containing long forage (12). Because ruminal epithelium has the capacity to metabolize both acetate and butyrate from an early age (14, 29), increased concentration of these acids in the rumens of young calves fed high grain diets would result in increased ketone production and peripheral concentrations. Additionally, absorption of VFA is increased with low ruminal pH; although not measured in this study, ruminal pH of calves fed similar diets often are quite low (21), which would further increase rate of ruminal VFA absorption and ketogenesis by ruminal epithelium (24).

Coefficients of correlation among selected variables in the study (Table 4) generally reflect metabolic changes consistent with increasing dry feed intake and ruminal development. Close relationship between  $\beta$ HOB and ACAC ( $r = .89$ ) suggests source of ketones was similar throughout the study. Additionally,  $\beta$ HOB and ACAC correlated .71 and .56, respectively, with grain intake, further indicating that most of the ketones, but particularly  $\beta$ HOB, were of

alimentary origin. Significant correlations of glucose with grain intake ( $r = -.27$ ), dry feed intake ( $r = -.23$ ), and TDMI ( $r = -.21$ ) support the hypothesis that early weaning has a significant impact on plasma glucose concentration insofar as source of glucose is altered.

Relationship between plasma glucose and NEFA ( $r = .05$ ) was not significant, in contrast to data of Trenkle and Kuhlemeir (28). Changes in glucose may have been insufficient to induce concomitant changes in NEFA throughout the study. Low correlation also may have been due in part to changes in NEFA with handling of animals (20), although animals in this study quickly acclimatized to experimental protocol with a minimum of difficulty.

Elevated concentrations of blood  $\beta$ HOB observed in this study were unexpected. From 8

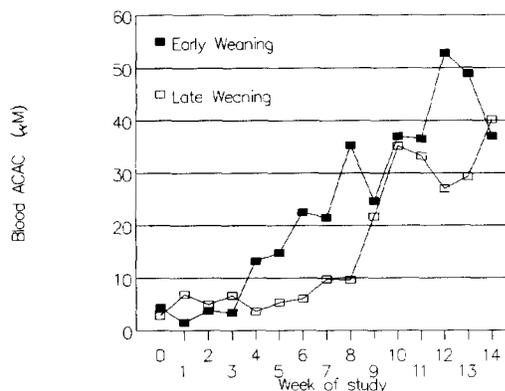


Figure 4. Least squares means of blood acetoacetate (ACAC) concentration in calves weaned early or late. Effect of week was significant ( $P < .01$ ).

to 9 wk, average blood ketone concentration approached or exceeded 1000  $\mu\text{M}$ , a threshold used to indicate incidence of subclinical ketosis (4). Blood ketones of individual animals exceeded 2000  $\mu\text{M}$  on several occasions. Plasma glucose did not fall to concentrations typical of ketosis at any time during the study, however. Hypoglycemia, in conjunction with elevated NEFA and ketones, has been used as an indication of ketosis in cattle (3). Thus, elevated ketone concentration was apparently not consistent with classical ketosis but was a result of excessive alimentary ketogenesis relative to ketone utilization by peripheral tissues. That NEFA were consistently within normal limits also supports this hypothesis. Increased release of NEFA from portal-drained viscera and hindquarters is associated with hepatic ketogenesis (11).

Hyperketonemia may have been exacerbated by time of sampling. Manston et al. (17) reported diurnal variations in  $\beta\text{HOB}$  concentration in lactating but not in nonlactating dairy cattle. Diurnal variation reported actually may have been in response to feeding. In that study, both groups of cattle were fed grain and hay according to nutrient requirements twice daily; peak  $\beta\text{HOB}$  concentrations were reported 3 to 4 h after a.m. feeding, when animals presumably were most hungry (cattle were fed at 0715 and 1615 h) and would have consumed grain most rapidly. It is important to note that calves in the present study were fed grain for ad libitum consumption to a maximum of 2.7 kg/d. This intake was not reached in many calves until the last few weeks of the study. Although calves tended to consume some grain immediately after a.m. feeding, consumption of large amounts of grain immediately after feeding was not observed.

Importance of the observed hyperketonemia is unclear. Although blood ketone concentration indicated potential sub-clinical ketosis, animals remained healthy, gained weight, and consumed feed in increasing amounts throughout the study. Although not measured, elevated ketones may spill over into urine resulting in a loss of energy. Further research is required to determine if hyperketonemia may be a result of over-production of ketones from VFA produced by ruminal fermentation, or an underutilization of ketones by peripheral tissues. It is possible that enzymatic mechanisms, responsible for ke-

tone body metabolism in young calves, cannot utilize increased ketones produced by increasing feed intake.

## CONCLUSIONS

Metabolites in blood of young calves were indicative of events in the rumen and development of ruminal function. Blood ketones increased rapidly with intake of dry feed and increased to concentrations indicative of hyperketonemia. However, calves were healthy throughout the study. Plasma glucose declined with age and was reduced further by early weaning. Plasma NEFA decreased with week of study and during wk 5 to 8 and suggested that increases in blood ketones were a result of alimentary and not hepatic ketogenesis.

Further work is required to evaluate potential losses in metabolic efficiency due to urinary excretion of ketones and potential management strategies to minimize effects, if any, of hyperketonemia.

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