Postprandial Changes of Selected Blood and Ruminal Metabolites in Ruminating Calves Fed Diets with or Without Hay

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ABSTRACT

Eight ruminally cannulated Holstein bull calves were used in a switchback design to evaluate effects of hay on postprandial changes in ruminal pH, ammonia and VFA, blood ketones, and plasma glucose, NEFA, VFA, and urea N. Calves were fed 4.5 kg/d of calf starter with or without hay for ad libitum consumption. Blood and ruminal fluid were sampled every 2 h for 12 h on d 21 (periods 1 and 2) or d 13 (period 3). Ruminal pH declined from 6.6 at 0 h to 5.3 by 2 h. Changes in ruminal and plasma VFA were consistent with low pH and rapid fermentation of soluble carbohydrate. Ruminal ammonia, molar percentage butyrate, and blood ketones, plasma urea N, and plasma molar percentage butyrate were lower when hay was fed. Postprandial increases in blood β-hydroxybutyrate and acetoacetate were reduced when hay was fed, and correlated .65 and .50, respectively, to ruminal butyrate. Concentration of β-hydroxybutyrate averaged 1.24 and 1.87 mmol/L at 4 h postfeeding. These data suggest that rapid consumption of limited amounts of grain increased in ruminal VFA and blood ketones; increase in ketones was smaller when hay was included in the diet.

(Key words: calves, calf starter, ketones, volatile fatty acids)

Abbreviation key: ACAC = acetoacetate, βHBA = β-hydroxybutyrate, H+ = hay offered for ad libitum consumption, H– = no hay offered.

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INTRODUCTION

Efficient growth of weaned calves may be accomplished by feeding high grain diets with minimal forage. Rapid ruminal fermentation of soluble carbohydrate in high grain diets causes reduced ruminal pH, which can alter proportions of VFA produced in the rumen and induce a shift in types and proportions of ruminal microflora. Increased circulating blood ketones may occur in response to metabolism of butyrate by ruminal epithelium and liver (23). Although diurnal variations in concentrations of blood ketones have been reported in mature cattle (14, 20), it is not clear how blood ketones respond to high grain diets or whether inclusion of forage can affect blood ketone concentrations. Recently, Quigley et al. (17) reported increased blood ketones with increasing dry feed intake in young calves. Most circulating ketones were from alimentary ketogenesis, which was influenced by ruminal VFA. Calves were sampled 2 h after feeding, which may have influenced blood ketone concentration.

The objective of this study was to determine changes in concentrations of blood and ruminal metabolites with time after feeding in ruminating calves fed high grain diets with or without hay.

MATERIALS AND METHODS

Eight Holstein bull calves averaging 16 wk of age (SE = .5) and previously equipped with ruminal cannulas were housed individually in 1.2- × 4.8-m pens bedded with shavings in an unheated barn. Calves were weaned at 8 wk of age to a diet of calf starter, which was fed for ad libitum consumption until initiation of the study. Animals were assigned randomly to treatment in a simple switchback design. Treatments were calf starter (4.5 kg/d per calf) with (H+) or without (H–) alfalfa hay offered for ad libitum consumption. Periods 1 and 2 each included 20 d of acclimation and 1 d of samp-

TABLE 1. Chemical composition of feeds used in experiment.

Item	Starter	Hay
DM, %	85.4	86.0
	(% of DM)	
CP	20.0	16.2
ADF	20.4	42.6
NDF	37.1	61.6
Ca	1.03	1.21
P	.66	.32
Mg	.35	.35
K	1.13	1.44

ling. Period 3 was 13 d of acclimation and 1 d of sampling. Feed was offered once daily (1000 h), and water was available at all times. Indwelling jugular catheters were installed on the day prior to sampling to expedite blood collection and to minimize animal stress. Blood and ruminal fluid were collected at 0, 2, 4, 6, 8, 10, and 12 h postfeeding. During period 1, a 12-h sample was not collected.

Approximately 10 ml of blood were collected from the jugular catheter, and a 2-ml subsample was deproteinated immediately by addition to 2 ml of 1 *M* perchloric acid. Remaining blood was added to 400 μl of 6% EDTA and placed on ice before samples were transported to the laboratory. Plasma was harvested by centrifugation and stored (–20°C) prior to analysis for glucose and NEFA (17), plasma urea N (Sigma kit 640, Sigma Chemical Co., St. Louis, MO), and VFA (18). Deproteinated blood was analyzed for β-hydroxybutyrate (βHBA) and acetoacetate (ACAC) as in Williamson and Mellanby (27).

Approximately 40 ml of ruminal fluid were collected through the ruminal cannula at sites throughout the rumen. Ruminal pH was measured immediately, and the sample was frozen quickly in liquid N. Sample was placed on ice, transported to the laboratory, and stored (–20°C) until analyzed for VFA (18) and NH_3 N (Sigma 640 kit).

Samples of calf starter and hay were collected daily and composited by period for analysis of DM and CP (1); ADF and NDF (8); Ca, Mg, and K (atomic absorbance spectrophotometry); and P (10).

Data were analyzed by ANOVA using a generalized linear mixed model algorithm (2). Terms of the model included animal, treatment, period, time after feeding, and selected interactions. Error terms were selected by the algorithm assuming that random effects (time after feeding and interactions containing time after feeding) were distributed as multivariate normal. Also, data at 0 h postfeeding were analyzed by ANOVA using a simple switchback design. Level of significance at $P < .05$ was used unless otherwise noted.

RESULTS AND DISCUSSION

One animal was removed from the study because of chronic digestive upset and excessive feed refusal; data were excluded from analyses. All other calves were healthy throughout the experiment.

Chemical composition of starter (Table 1) was consistent with inclusion of high fiber byproducts (cottonseed and soybean hulls), and

TABLE 2. Intake of ingredients and nutrients by calves fed 4.5 kg/d of grain with (H+) or without (H–) hay for ad libitum consumption.

Item	H–	H+	SE[1]	P[2]
DM, kg/d	3.7	4.5	.1	**
Grain, kg/d	3.7	3.7	.1	NS
Hay, kg/d	0	.8	.2	**
CP, kg/d	.74	.86	.01	**
ADF, kg/d	.76	1.08	.04	**
NDF, kg/d	1.38	1.84	.05	**
Ca, g/d	38.1	47.8	1.0	**
P, g/d	24.7	27.0	.3	**

[1]Pooled SE of the mean.

[2]Probability of a significant treatment effect.

**$P < .01$.

TABLE 3. Least squares means[1] of ruminal pH and selected ruminal and blood metabolite concentrations in calves fed 4.5 kg of grain/d with (H+) or without (H–) hay for ad libitum consumption.

Item	H–	H+	SE	P^2
Rumen				
pH	5.4	5.5	.2	NS
NH_3 N, mg/dl	18.4	12.0	1.6	**
VFA, mmol/L	127.1	118.9	11.0	NS
Acetate, mol/100 mol	53.3	55.3	1.2	NS
Propionate, mol/100 mol	28.6	30.8	.8	†
Butyrate, mol/100 mol	13.5	9.8	.6	**
Isobutyrate, mol/100 mol	.5	.3	.1	NS
Valerate, mol/100 mol	3.4	3.3	.1	NS
Isovalerate, mol/100 mol	.7	.6	.1	NS
Blood plasma				
Glucose, mmol/L	4.98	5.03	.08	NS
NEFA, μmol/L	109.4	118.2	18.7	NS
βHBA,[3] mmol/L	1.37	.88	.15	**
ACAC,[3] mmol/L	.034	.021	.006	†
PUN,[3] mg/dl	10.9	10.2	.5	†
VFA, mmol/L	1.23	1.22	.16	NS
Acetate, mol/100 mol	94.6	95.0	.5	NS
Propionate, mol/100 mol	4.1	4.0	.3	NS
Butyrate, mol/100 mol	1.3	1.0	.1	†

[1]Means are across all sampling times.

[2]Probability of a significant treatment effect.

[3]βHBA = β-Hydroxybutyrate, ACAC = acetoacetate, PUN = plasma urea N.

†$P < .10$.

**$P < .01$.

hay was of medium quality. Intake of DM, hay, and nutrients (Table 2) were increased with hay feeding; calves consumed .8 kg/d of hay DM when fed H+, which resulted in a forage:concentrate ratio of 18:82 (DM basis).

Ruminal pH and concentrations of selected ruminal and blood metabolites averaged across all sampling times are in Table 3. In the rumen, NH_3 N and molar percentage of butyrate were reduced ($P < .01$), and propionate tended ($P < .10$) to be greater, when calves were fed H+. Blood βHBA was reduced ($P < .01$), and ACAC, plasma urea N, and plasma molar percentage of butyrate tended to be reduced ($P < .10$), when calves were fed H+. Other parameters were unaffected by treatment and reflected intake of high grain diets by calves (17, 18).

Low ruminal pH (5.5) of calves on both treatments suggests that offering hay for ad libitum consumption had little effect on maintenance of ruminal pH. Although this finding contrasts with reports of others (9, 28), it may be explained by our observation that calves rapidly consumed grain in large amounts as soon as it was offered. This was particularly true in periods 2 and 3, when calves often consumed all grain within 4 h. Generally, calves ate hay only after most of the grain was consumed.

Change in ruminal pH with time after feeding (Figure 1) indicates a rapid decline from 6.6 to 5.3 by 2 h after feeding, consistent with observed intake of grain. Ruminal pH reached a nadir of 5.1 at 4 h after feeding. Clearly, hay had no effect on pH of the rumen to 12 h postfeeding; the mediating effect of hay likely was overwhelmed by the rapid fermentation of grain in the rumen after it was fed.

Ruminal pH less than 6 has been associated with reduced cellulolysis, inhibition of growth of certain ruminal bacteria (12) and ruminal protozoa (15), and a shift in ruminal fermentation away from acetate and toward propionate and butyrate. Further, low pH can cause an increase in growth of lactogenic bacteria, a subsequent increase in lactate production, and, in extreme cases (pH less than 5), development of ruminal parakeratosis (4). Unfortunately, ruminal lactate concentrations were not analyzed in this study.

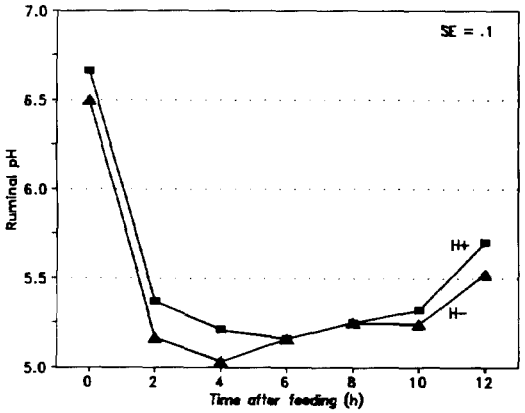

Figure 1. Ruminal pH with time after feeding in calves fed starter (4.5 kg/d) with (H+) or without (H–) alfalfa hay for ad libitum consumption.

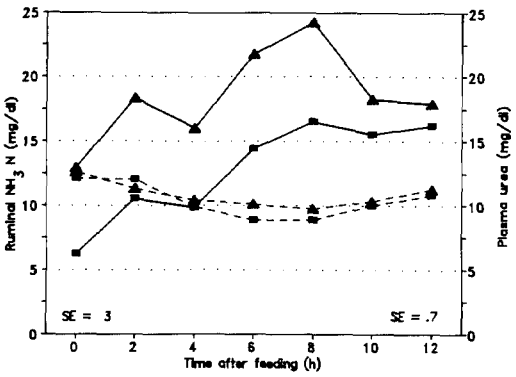

Figure 2. Concentrations of ruminal NH_3 N and plasma urea N with time after feeding in calves fed starter (4.5 kg/d) with (■) or without (▲) alfalfa hay for ad libitum consumption. Primary (left) Y-axis (—) and secondary (right) Y-axis (- - -).

Feed processing and source of fermentable carbohydrate can have a marked impact on rate of fermentation and resultant pH change. Declines in ruminal pH associated with whey-containing diets (3) or those containing ground corn compared with whole, shelled corn (9) have been reported. Wheat, included in our rations as wheat middlings (25% of ration), is degraded rapidly and extensively in the rumen (5) and may have contributed to the large, rapid decline in pH.

Concentration of ruminal NH_3 N tended to be lower in calves fed H+ ($P < .08$) at 0 h; concentration increased with time after feeding (Figure 2) to 16.5 and 23.0 mg/dl by 8 h in calves fed H+ and H–, respectively. By 10 to 12 h, the difference between treatments declined. Lower ruminal NH_3 N in calves fed H+ may have been a result of increased ruminal dilution and outflow or a result of altered ruminal fermentation. Similar slopes of the lines in Figure 2 suggest that NH_3 N concentrations in both groups of calves responded similarly to grain intake during the first few hours after feeding. Concentration of plasma urea N (Figure 2) tended to be lower when calves were fed H+, associated with lower ruminal NH_3 N. Postprandial changes in plasma urea N were variable for both treatments and correlated poorly ($r = .12$; $P > .10$) with changes in ruminal NH_3 N.

Concentration of total VFA in the rumen, similar at 0 h, increased by 2 h after feeding in both treatments (Figure 3) as a result of rapid fermentation of ingested feed by ruminal bacteria. The increase in VFA did not differ by treatment and correlated ($r = .81$) with decline in ruminal pH. Similar relationship between pH and VFA has been reported (3). Total VFA concentration approached 150 mmol/L by 2 to 6 h after feeding, which is similar to other reports in young calves (16) and indicates rapid fermentation of carbohydrate (3). After 6 h, concentration of VFA began to decline, although prefeeding concentrations were not achieved by 12 h.

Molar proportion of VFA in the rumen changed with time after feeding (Figure 3). At 0 h, acetate was greater ($P < .02$), and other VFA tended to be less when calves were fed H+, indicating altered basal fermentation when hay was fed. Average prefeeding molar percentages of acetate, propionate, and butyrate were 60.1, 27.2, and 7.6 and 55.6, 28.3, and 11.0, respectively, in calves fed H+ and H–. By 12 h, molar percentages were 51.5, 31.8, and 11.3 and 51.3, 27.3, and 15.2, respectively. Most notable was the molar proportion of ruminal butyrate, which increased throughout the 12-h period and was lower in calves fed H+ ($P < .01$). Molar percentage of propionate increased throughout the period (H+) or to 6 h (H–; Figure 3). Increases in percentages of butyrate and propionate were at the expense of percentage of acetate and minor VFA. Al-

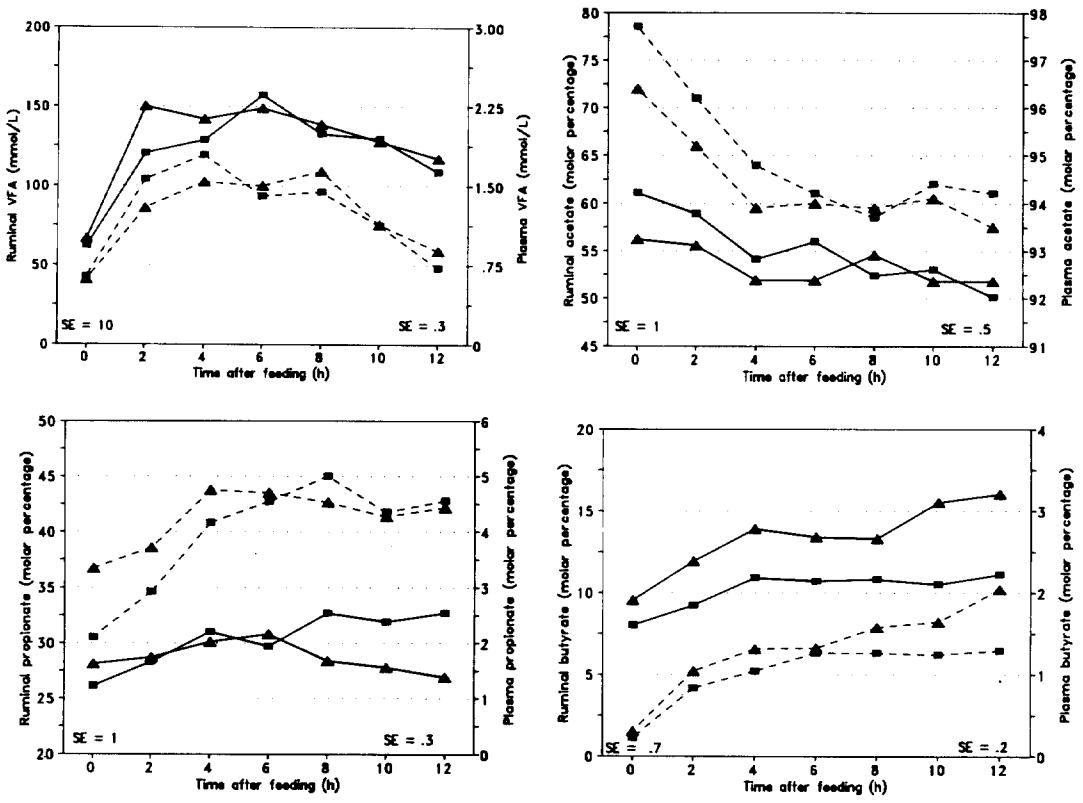

Figure 3. Concentrations of ruminal and plasma VFA and molar percentage of acetate, propionate, and butyrate with time after feeding in calves fed starter (4.5 kg/d) with (■) or without (▲) alfalfa hay for ad libitum consumption. Primary (left) Y-axis (—) and secondary (right) Y-axis (- - -).

though not measured in this study, increased butyrate and NH_3 N have been associated with increased numbers of ruminal protozoa in cattle fed high grain diets (6). However, protozoa are especially sensitive to low pH [less than 5.5 (13)] and may have been inhibited by low pH observed in our study.

Associated with feeding and changes in ruminal metabolite concentrations were changes in concentrations of jugular blood metabolites. Plasma VFA (Figure 3) increased from .6 mmol/L at 0 h to 1.6 to 1.8 mmol/L by 4 h (H+) and 8 h (H–) without effect of treatment. Change in total plasma VFA correlated (r = .34) with ruminal VFA, although peak plasma VFA concentration (Figure 3) followed peak ruminal VFA concentration (Figure 2) by 2 h, which may be associated with the lag time for absorption from the reticulorumen. Results were similar to other reports of blood VFA concentrations in ruminating calves (18) and lambs (19).

As expected, VFA in blood was mostly acetate with small amounts of propionate and butyrate. No other acids were observed in measurable quantities. Prefeeding molar percentage of acetate was greater ($P < .04$) and of propionate lower ($P < .03$) when calves were fed H+. With feeding, molar percentages of propionate and butyrate increased at the expense of acetate (Figure 3), in agreement with data of Evans et al. (7). Molar percentage of butyrate was greater ($P < .01$) when calves were fed H–, particularly from 8 to 12 h postfeeding.

Plasma glucose and NEFA were unaffected by consumption of hay. Glucose declined to 4 h (Figure 4) and then increased to 5.3 mmol/L by 10 h after feeding. Plasma NEFA declined throughout the 12-h sampling period (Figure 4). We observed marked increases in βHBA

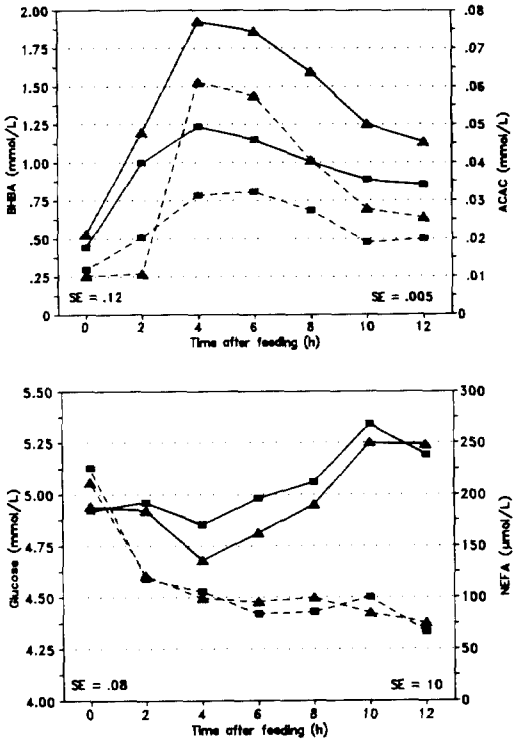

Figure 4. Concentrations of plasma β-hydroxybutyrate (βHBA), acetoacetate (ACAC), NEFA, and glucose with time after feeding in calves fed starter (4.5 kg/d) with (■) or without (▲) alfalfa hay for ad libitum consumption. Primary (left) Y-axis (—) and secondary (right) Y-axis (- - -).

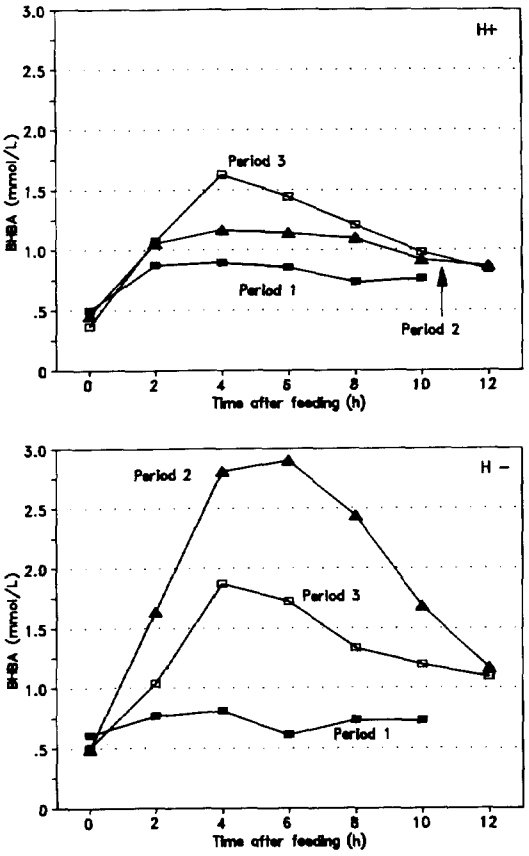

Figure 5. Concentrations of plasma β-hydroxybutyrate (βHBA), by period with time after feeding in calves fed starter (4.5 kg/d) with (H+) or without (H–) alfalfa hay for ad libitum consumption.

and ACAC in jugular blood with feeding (Figure 4), but particularly when calves were fed H– ($P < .01$). Concentration of βHBA peaked at 4 h and averaged 1.24 and 1.87 mmol/L when calves were fed H+ and H–, respectively. Blood ACAC peaked at .031 and .059 mmol/L when calves were fed H+ and H–, respectively.

The rapid increase in blood ketones with feeding appeared to be a result of increased ruminal butyrate (Figure 3); subsequent metabolism to ketones was by ruminal epithelium. Blood βHBA and ACAC were correlated (r = .65 and .50; $P < .01$), respectively, with ruminal butyrate (millimoles per liter). Ruminal epithelium develops the ability to metabolize butyrate to ketones from an early age (11); thus, metabolism usually is a function of butyrate absorption. Decreased ruminal pH may have exacerbated postprandial increases in blood ketones because VFA (particularly butyrate) are absorbed more rapidly from the rumen when pH is low (22). Similar ruminal pH between treatments during the feeding period suggests that increased ruminal butyrate and blood ketones when calves were fed H– were not a result of low pH per se but that consumption of hay reduced concentration of these metabolites independent of ruminal acidity. Changes in substrate available for fermentation and rumen microbial populations may influence the production of butyrate in the rumen (3).

Figure 5 shows effect of period on blood βHBA when calves were fed H+ and H–. When calves were fed H+, peak concentration of βHBA increased from .89 mmol/L in period

1 to 1.63 mmol/L in period 3. This observation is consistent with increased rate of grain intake from periods 1 to 3. As calves aged, they exhibited greater appetite for the fixed amount of grain fed throughout the study, and they increased their rate of grain intake as the study progressed. When calves were fed H–, blood βHBA increased dramatically during periods 2 and 3. During period 2, βHBA in blood of two of the four animals exceeded 3.8 mmol/L at 4 and 6 h postprandial. Calves with elevated ketones in periods 2 and 3 were fed H+ in the preceding period; the transition from a diet containing forage to one containing all concentrate may have contributed to elevated blood ketones by altered ruminal VFA or by altered ruminal butyrate metabolism or absorption.

Increased blood ketones with time after feeding in sheep fed pelleted rations twice daily (26) and lactating cattle fed limited concentrate and hay twice daily (21) have been reported. Blood ketones were lower in both studies than those we observed, although the increase from prefeeding to peak concentrations was similar (approximately 350%). Fermentation of available carbohydrate in the rumen, low ruminal pH, and rapid metabolism of butyrate may have contributed to elevated concentration of ketones observed in this study.

Physiological effects of elevated ketones in blood of young calves are not clear. Data of Targowski et al. (24, 25) indicated that ketones may reduce the mitogenic response of bovine lymphocytes in vitro and in vivo. Effects of alimentary ketogenesis on immune function of cattle fed production rations have not been determined.

CONCLUSIONS

Blood metabolites were influenced markedly by time after feeding in young calves fed high grain diets once daily. Changes in metabolites appear to be associated with rapid ruminal fermentation of soluble carbohydrates, resulting in depressed pH and increased VFA and NH_3 N. Increase in circulating ketones was associated with VFA production and appeared to be especially sensitive to ruminal butyrate concentration. Feeding hay for ad libitum consumption reduced blood ketones, probably by reducing production of ruminal butyrate.

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