Effects of Nutrient Source and Time of Feeding on Changes in Blood Metabolites in Young Calves[1]

J. D. Quigley III[2] and J. K. Bernard[3]

Department of Animal Science, University of Tennessee, Knoxville 37901

ABSTRACT: Sixteen Holstein calves were used in a completely randomized design to evaluate effects of dry feed intake and time after feeding on concentration of selected peripheral blood metabolites. Calves entered the study at 13 (SD = 2.9) d of age and were fed milk replacer (10% of BW) twice daily to weaning at 28 (Grain) or 84 (Milk) d and calf starter from 1 (Grain) or 56 (Milk) d. Blood was sampled every 14 d at 0 and 2 h after the morning feeding and analyzed for β-hydroxybutyrate (βHBA), glucose, nonesterified fatty acids, urea N, L(+) lactate, and VFA. Blood βHBA and VFA increased with increasing dry feed intake, but particularly at 2 h postfeeding. Molar proportion of VFA as acetate declined and propionate and butyrate increased with increasing feed intake. Glucose at 2 h postfeeding declined after weaning to levels lower than 0-h values. Prefeeding glucose concentrations increased with increasing grain intake. Lactate declined throughout the study without effect of treatment or time after feeding. Data indicate that marked changes occur with increasing grain intake and time after feeding. Increased blood βHBA seems to be a response to alimentary ketogenesis.

Key Words: Calves, Weaning, Blood Chemistry, Ruminal Development

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Introduction

Development of ruminal function, a consequence of the initiation of dry feed intake, stimulates fundamental changes in nutrients available to the young ruminant. Before weaning, sources of energy and protein are derived mainly from intestinal absorption of milk. After weaning, however, energy is derived primarily from ruminal fermentation and subsequent absorption of VFA.

Recently, we reported rapid increases in the concentration of peripheral blood ketones that were correlated to dry feed intake ($r = .7$; $P < .01$) in young calves (Quigley et al., 1991a). Peak concentration of β-hydroxybutyrate (**βHBA**) reached 1.2 m*M*, a level indicative of subclinical ketosis in mature ruminants (Bergman, 1984). However, concentrations of other energy metabolites indicative of ketosis, glucose and nonesterified fatty acids (**NEFA**), suggested that blood ketone concentrations were a consequence of alimentary ketone production. Because calves were sampled at 2 h postfeeding, it is possible that ketone concentrations were a consequence of feeding; samples taken before feeding may not exhibit similar characteristics.

Our objective was to evaluate effects of nutrient source (grain vs milk feeding) on changes in blood metabolites in young dairy calves when sampled at 0 and 2 h postfeeding.

Materials and Methods

Animal Assignments and Feed Management. Sixteen Holstein heifer calves (13 [SD = 2.9] d of age) were assigned randomly to a completely randomized experimental design with source of nutrients as treatments. Calves were fed calf starter from d 1 of the study and weaned at 28 d (**Grain**), or starter beginning at 56 d and weaning at 84 d (**Milk**). Calves were left with the dam to 3 d of age, when they were moved to individual hutches (2.18 m diameter) and housed there throughout the experiment.

[1]The authors acknowledge the technical assistance of S. A. Nagel.

[2]To whom correspondence should be addressed.

[3]Martin Exp. Sta., Martin, TN.

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Table 1. Chemical composition of feeds used[a]

Item	Calf starter		Milk replacer	
	Mean	SE	Mean	SE
DM, %	89.2	.3	90.5	1.6
	% of DM			
CP	20.2	.4	23.7	.2
Ash	7.9	.2	2.7	.2
NDF	34.7	.4	0	—
	mg/g of DM			
Ca	12.4	.6	5.7	.2
P	7.8	.2	6.5	.1
Mg	3.7	.1	1.5	.1
K	12.2	.1	18.3	.1

[a]n = 7.

Milk replacer (Maxi-Lac, Tennessee Farmers Cooperative, La Vergne, TN) was fed twice daily (approximately 0730 and 1700) from nipple bottles at 10% of BW per day, based on BW of the previous week. Water was available at all times. Calf starter was a commercially pelleted formulation offered at the morning feeding for ad libitum consumption. Starter and milk replacer were sampled daily and composited monthly for analysis of DM and CP (AOAC, 1980), ADF and NDF (Goering and Van Soest, 1970), and minerals (atomic absorbance spectrophotometry). Refused calf starter was weighed back daily and reported every 14 d. Calves were weighed at initiation of the study and every 7 d thereafter to 112 d.

Blood Sampling. Approximately 10 mL of blood was taken from calves on d 0 and every 14 d thereafter by jugular venipuncture into evacuated tubes at 0 and 2 h after the morning feeding. A 2-mL subsample was immediately deproteinated by addition to 2 mL of 1 *M* perchloric acid for analysis of blood βHBA (Williamson and Mellanby, 1965). A second 2-mL subsample was deproteinated in 4 mL of 8% perchloric acid before analysis of L(+) lactate (Sigma Chemical, St. Louis, MO). Remaining blood was added to 400 μL of 6% EDTA and plasma was harvested by centrifugation (3,000 × *g*). All tubes were placed on ice and transported to the laboratory by next-day air mail. Analysis of βHBA commenced immediately upon receipt of samples. Plasma was stored at -20°C before analysis for VFA (Quigley et al., 1991b), urea nitrogen (PUN), NEFA, and glucose as described by Quigley et al. (1991a).

Statistical Analysis. Blood measurements at 0 and 2 h after feeding and changes in blood concentration were analyzed using the model $Y_{ijk} = \mu + T_i + C_{(i)j} + P_k + (TP)_{ik} + e_{(ijk)}$, where Y_{ijk} = dependent variable; μ = overall mean; T_i = effect of the i^{th} treatment; $C_{(i)j}$ = effect of the j^{th} calf within the i^{th} treatment; P_k = effect of the k^{th} day of study; $(TP)_{ik}$ = effect of treatment × day interaction; and $e_{(ijk)}$ = residual.

The term $C_{(i)j}$ was used as error term to test differences due to treatment. 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 using initial BW as covariable. Probability of $P < .05$ was used throughout unless otherwise noted. Several samples of deproteinated blood were damaged in shipping, resulting in missing observations. Therefore, least squares means are presented.

Results and Discussion

Chemical composition of calf starter and milk replacer is given in Table 1. Although calf starter contained less CP and more NDF than recommended (NRC, 1988), the starter contained high-fiber byproducts to stimulate development of ruminal function and was designed to be fed as the sole feed. Milk replacer contained 23.7% CP, which is similar to recommended CP for milk replacers containing nonmilk protein.

Table 2. Least squares means of body weight, intake, daily gain, and feed efficiency in calves fed diets to promote early grain intake (Grain) or extended milk intake (Milk)

Item	Grain	Milk	SE	*P* <
BW, kg				
0 d	37.4	37.7	1.4	NS[e]
28 d	50.2	43.9	2.9	NS
56 d	69.9	53.2	4.2	.01
84 d	94.2	75.8	3.1	.01
112 d	117.4	103.8	3.1	.01
ADG, kg				
1 to 56 d	.58	.28	.11	.01
57 to 84 d	.87	.91	.13	NS
85 to 112 d	.90	.94	.06	NS
Intake, kg/d				
Dry matter	2.3	1.4	.1	.01
Milk[a]	.2	.6	.1	.01
Grain[bc]	2.1	1.0	.1	.01
Protein, g/d[c]	461.3	297.0	16.3	.01
ME, Mcal/d[cd]	7.9	5.5	.3	.01
Gain:feed				
1 to 56 d	.405	.431	.08	NS
57 to 84 d	.306	.535	.06	.01
85 to 112 d	.232	.293	.07	NS

[a]Milk DM intake from 1 to 84 d.
[b]Grain DM intake from 1 to 112 d.
[c]Significant week × treatment interaction.
[d]Calculated from NRC (1988).
[e]NS = not statistically significant.

Body weight (Table 2) increased with time of the study ($P < .01$). Calves fed Grain were heavier ($P < .05$) by 56 d and remained heavier to the end of the study than calves fed Milk. However, daily gain indicated that after calves fed Milk were offered grain at 56 d, their rate of daily gain (.90 kg/d) was similar to that of calves fed Grain (.84 kg/d). Greater intake of energy and protein (Table 2), as well as increased gut fill, was responsible for increased rate of gain in calves on the Grain treatment. Energy intake was approximately 40% greater when calves were fed Grain than when they were fed Milk over the 16-wk study.

Intake of feed ingredients and nutrients (Table 2) was influenced by treatment application and, therefore, differed ($P < .01$). Feed efficiency tended to be greater in calves fed Milk ($P < .10$), a result of greater energy and protein density and availability of milk replacer compared with calf starter. However, when calves on Milk treatment were offered grain (57 to 84 d), feed efficiency was markedly increased, indicating compensatory gain in these calves. After weaning, however, there was little difference in feed efficiency.

Concentrations of blood metabolites averaged across all days of the study (Table 3) indicated that most blood metabolites were affected by treatment at both 0 and 2 h postfeeding; however, in most cases, there were treatment × day of study interactions ($P < .05$). Also, the changes from 0 to 2 h postfeeding differed from zero ($P < .05$), except for L(+) lactate, and were affected by treatment (Table 2).

Blood βHBA concentrations at 0 and 2 h postfeeding (Figure 1) were affected both by treatment ($P < .10$ and $P < .01$, respectively) and by day of study ($P < .01$). Before weaning, blood βHBA was generally < .2 m*M* in all calves. Milk consumption reduced βHBA, and 2 h concentrations in both groups were consistently lower than those at 0 h during the preweaning period. This was likely a consequence of insulin secretion (Kronfeld, 1970) with milk consumption.

Table 3. Least squares means of blood and plasma metabolites in calves fed diets to promote early grain intake (Grain) or extended milk intake (Milk)

Item	Grain	Milk	SE	$P <$
0 h postfeeding				
β-hydroxybutyrate, m*M*[a]	.362	.304	.023	.10
Glucose, m*M*	5.02	4.43	.13	.01
Nonesterified fatty acids, m*M*[a]	.205	.280	.024	.03
Plasma urea N, mg/dL[a]	11.9	8.8	.3	.01
L(+) lactate, m*M*	1.70	1.60	.23	NS[b]
VFA, m*M*	.87	.74	.05	.08
Acetate, mol/100 mol[a]	94.0	95.0	.3	.05
Propionate, mol/100 mol[a]	4.5	3.9	.2	.05
Butyrate, mol/100 mol[a]	1.5	1.1	.1	.10
2 h postfeeding				
β-hydroxybutyrate, m*M*[a]	.534	.314	.025	.01
Glucose, m*M*	4.69	5.07	.17	NS
Nonesterified fatty acids, m*M*	.153	.215	.016	.01
Plasma urea N, mg/dL[a]	11.6	8.8	.3	.01
L(+) Lactate, m*M*	1.49	1.46	.16	NS
VFA, m*M*[a]	1.14	.78	.07	.01
Acetate, mol/100 mol[a]	92.2	94.5	.3	.01
Propionate, mol/100 mol[a]	5.5	4.3	.2	.01
Butyrate, mol/100 mol[a]	2.3	1.2	.2	.01
Change, 0 to 2 h				
β-hydroxybutyrate, m*M*[a]	.172	.011	.034	.01
Glucose, m*M*[a]	-.33	.63	.16	.01
Nonesterified fatty acids, m*M*	-.054	-.065	.018	NS
Plasma urea N, mg/dL	-.3	.1	.2	.10
L(+) lactate, m*M*	-.21	-.14	.16	NS
VFA, m*M*	.28	.04	.07	.03
Acetate, mol/100 mol[a]	-1.9	-.4	.4	.01
Propionate, mol/100 mol[a]	1.0	.4	.2	.03
Butyrate, mol/100 mol[a]	.9	.1	.2	.01

[a]Significant day of study × treatment ($P < .05$) interaction.
[b]NS = not statistically significant.

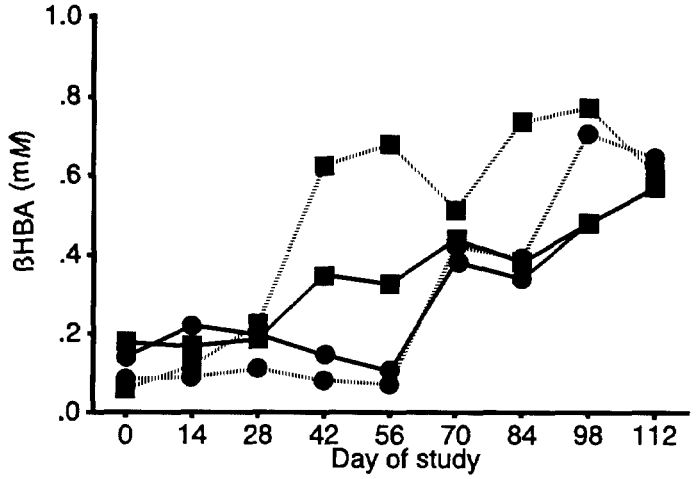

Figure 1. Concentration of blood β-hydroxybutyrate (βHBA) in calves fed diets for early grain consumption (■) or extended milk consumption (●) at 0 (solid lines) or 2 h (dashed lines) after feeding. Standard error = .05 and .05 at 0 and 2 h, respectively.

Concentration of βHBA in blood of calves fed Grain increased sharply by 42 d, particularly at 2 h postfeeding, when concentration increased from .22 to .62 m*M* between 28 and 42 d. A rapid increase in βHBA indicates a close relationship between grain consumption and blood βHBA; the correlation between these two traits was .63 and .81 at 0 and 2 h postfeeding, respectively. Calves fed Milk experienced an increase in blood βHBA by 70 d, 14 d after calf starter was initially offered. Blood βHBA also increased at 98 d, when concentration reached .7 m*M* at 2 h postfeeding. Postweaning increases with grain intake were similar to those of calves fed Grain, further suggesting that βHBA was of alimentary origin.

Increased concentration of blood ketones with increasing dry feed intake in young calves has been reported (Quigley et al., 1991a). Increased βHBA from 0 to 2 h postfeeding indicates rapid

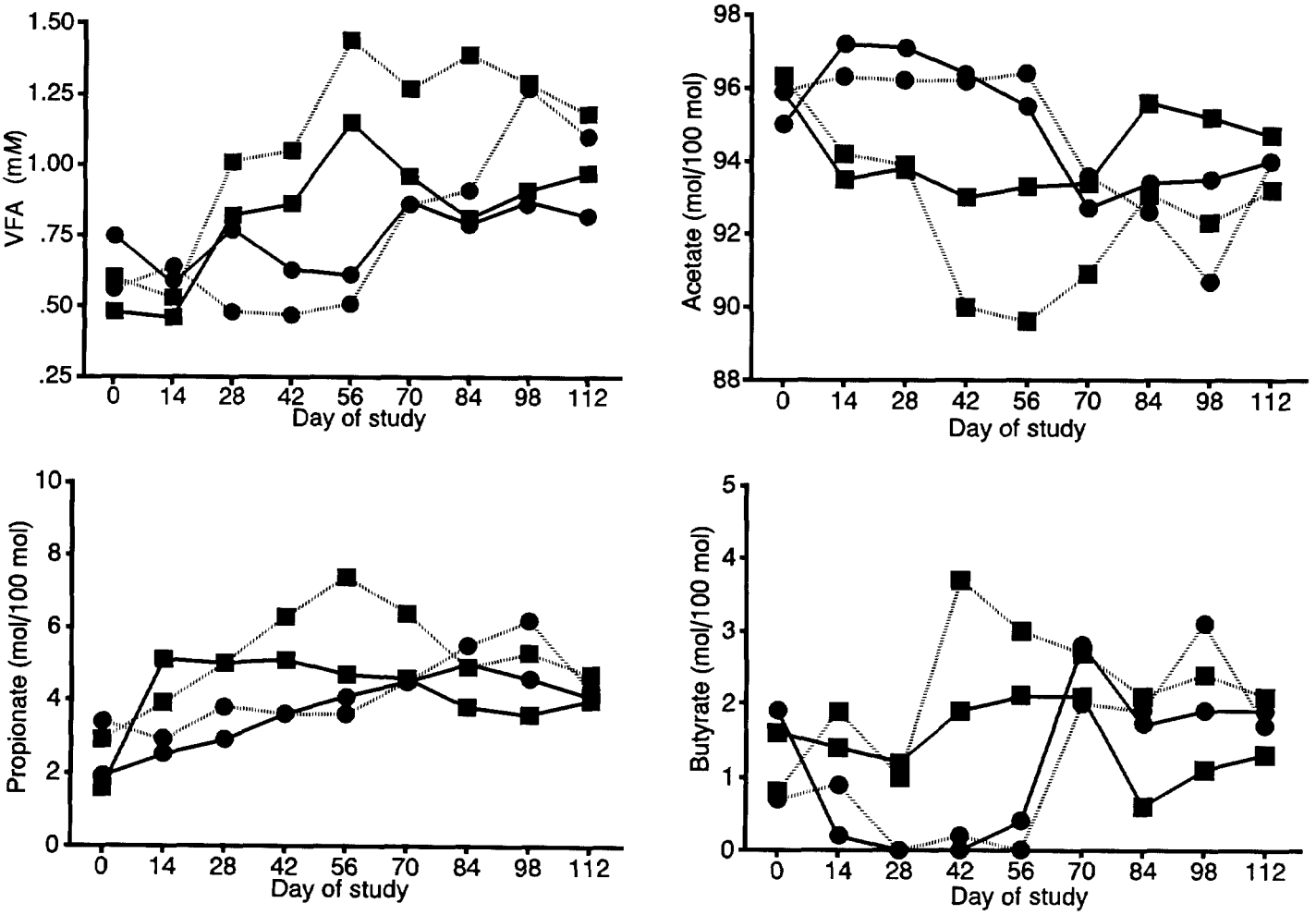

Figure 2. Concentration of plasma volatile fatty acids (VFA) and molar proportions of plasma acetate, propionate, and butyrate in calves fed diets for early grain consumption (■) or extended milk consumption (●) at 0 (solid lines) or 2 h (dashed lines) after feeding. Standard errors at 0 and 2 h were as follows: VFA, .12 and .13; acetate, .7 and .9; propionate, .5 and .6; butyrate, .4 and .5, respectively.

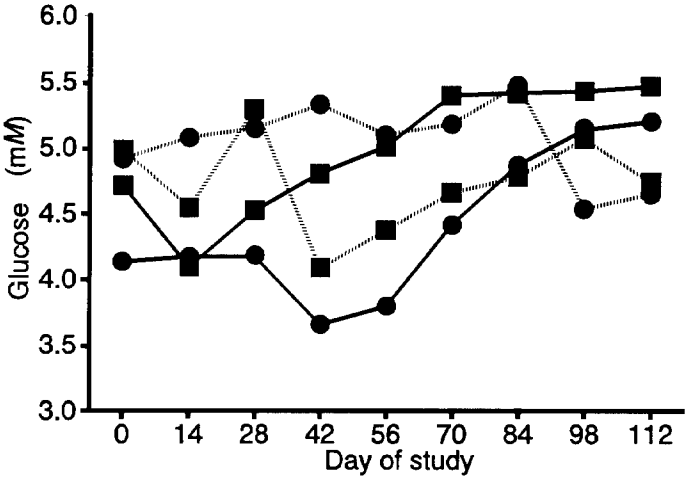

Figure 3. Concentration of plasma glucose in calves fed diets for early grain consumption (■) or extended milk consumption (●) at 0 (solid lines) or 2 h (dashed lines) after feeding. Standard error = .33 and .40 at 0 and 2 h, respectively.

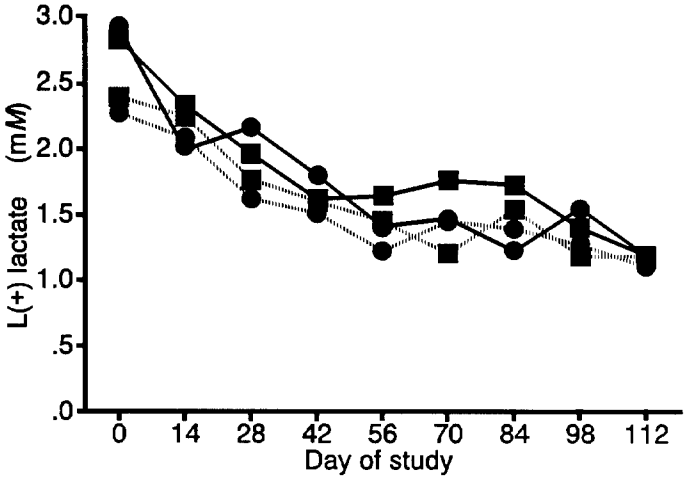

Figure 4. Concentration of plasma L(+) lactate in calves fed diets for early grain consumption (■) or extended milk consumption (●) at 0 (solid lines) or 2 h (dashed lines) after feeding. Standard error = .35 and .25 at 0 and 2 h, respectively.

ruminal fermentation of ingested feed, with subsequent metabolism of butyrate to βHBA by ruminal epithelium. Because ruminal epithelium develops the ability to metabolize absorbed butyrate (and to a lesser extent, acetate) from an early age (Walker and Simmonds, 1962), it seems that blood ketones are a function of ruminal butyrate production and absorption. That early-weaned calves tend to have higher proportions of ruminal butyrate (Anderson et al., 1987) further supports the hypothesis that alimentary ketogenesis is responsible for observed blood ketone concentrations.

Concentrations of plasma VFA at 0 and 2 h postfeeding ranged from .4 to 1.44 m*M* (Figure 2) and were affected by treatment ($P < .10$ and $P < .01$, respectively) and day of study ($P < .01$). Plasma VFA in blood of calves on the Grain treatment at 2 h postfeeding increased from 14 to 56 d, when VFA reached 1.44 m*M*. Thereafter, plasma concentrations declined slightly to the end of the study. Prefeeding VFA concentrations followed a pattern similar to VFA concentrations at 2 h, although increases were less marked. Plasma VFA in calves fed Milk did not increase until significant dry feed was consumed, between 56 and 70 d. By 14 d after weaning (98 d), VFA had increased to levels similar to those in calves fed Grain at 0 and 2 h postfeeding. Correlations between βHBA and plasma VFA were .43 and .71 at 0 and 2 h postfeeding, respectively.

As expected, acetate made up 90 to 97% of total plasma VFA (Figure 2). Propionate and butyrate (Figure 2) contributed 2.5 to 7.4 and 0 to 3.7% of total VFA, respectively. No other VFA were observed in measurable quantities. Molar proportions of VFA changed with day of study, time after feeding, and treatment. Molar percentage of butyrate increased with increasing feed intake, particularly at 2 h postfeeding. Propionate increased with grain feeding as well, but to a lesser extent than butyrate. Increases in molar percentages of propionate and butyrate were at the expense of acetate, which declined in both groups after weaning.

Concentrations of plasma glucose at 0 and 2 h postfeeding (Figure 3) were affected by treatment and day of study ($P < .01$). Before weaning, concentrations in both groups at 2 h were greater than prefeeding values; however, by 14 d after weaning, concentrations declined by .9 to 1.2 m*M*, then remained lower than prefeeding values to the end of the study. In contrast, plasma glucose at 0 h increased with increasing grain intake ($r = .58$), to maximum concentration of 5.4 (Grain) and 5.2 (Milk) m*M* at 70 and 112 d. Results of the current study support previous results (Quigley et al., 1991a) in calves sampled 2 h after feeding, particularly during the periweaning period.

Concentrations of blood L(+) lactate (Figure 4) at 0 and 2 h postfeeding were unaffected by treatment ($P > .05$) and declined ($P < .01$) from 3 m*M* at 0 d to 1.1 m*M* by the end of the study. Bartley et al. (1966) reported that plasma lactate was unaffected by developing ruminal function and ranged from 1.8 to 5.8 m*M*. Increased jugular lactate concentrations at an early age may be a result of lactate produced in the rumen of preweaned calves (Anderson et al., 1988) and decreased glycolysis by peripheral tissues associated with ruminal development (Howarth et al., 1968).

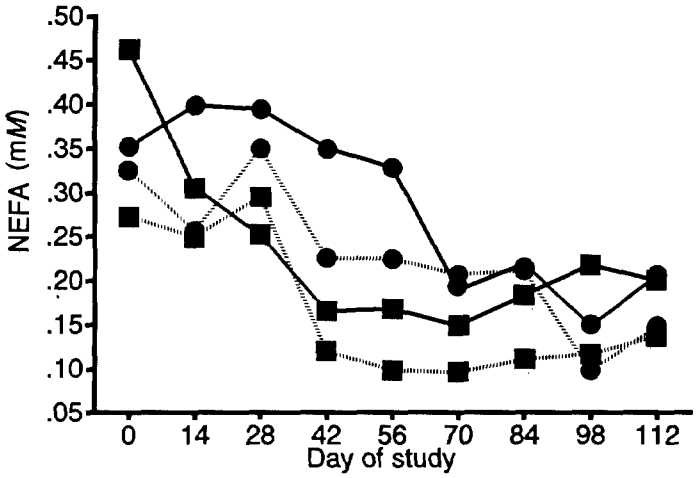

Figure 5. Concentration of nonesterified fatty acids (NEFA) in calves fed diets for early grain consumption (■) or extended milk consumption (●) at 0 (solid lines) or 2 h (dashed lines) after feeding. Standard error = .04 and .03 at 0 and 2 h, respectively.

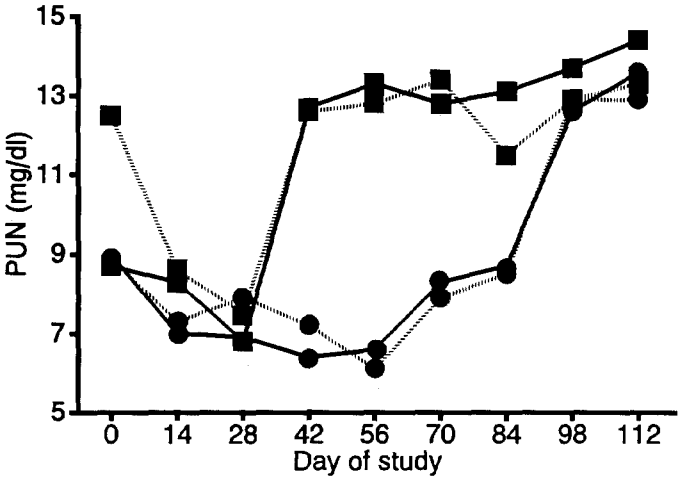

Figure 6. Concentration of plasma urea N (PUN) in calves fed diets for early grain consumption (■) or extended milk consumption (●) at 0 (solid lines) or 2 h (dashed lines) after feeding. Standard error = .7 and .7 at 0 and 2 h, respectively.

Elevated initial concentrations of plasma NEFA at 0 and 2 h postfeeding (.27 to .46 m*M*; Figure 5) declined to constant levels by 14 d after weaning in each group. Calves fed the Milk diet maintained higher NEFA concentrations from 42 to 84 d, when calves fed Grain were weaned but calves fed Milk were not. Feeding tended to cause an overall reduction in NEFA concentrations, although the change from 0 to 2 h was not always different from 0 ($P > .05$). Reduction in NEFA with feeding is consistent with insulin secretion in response to feeding (Trenkle and Kuhlemeier, 1966).

Plasma urea N (Figure 6) increased ($P < .01$) from 7 to 8 mg/dL at 28 d to 13 mg/dL by 2 wk after weaning in each treatment group without effect of time after feeding ($P > .05$). Plasma urea was related to grain intake (r = .71 and .79 at 0 and 2 h, respectively), indicating extensive ruminal degradation of dietary protein and carbohydrate, metabolism of absorbed amino acids, and, possibly, urea recycling.

Results reported herein indicate that increasing grain intake and developing ruminal function had a marked effect on all blood metabolites measured, except L(+) lactate. Changes in concentrations of blood metabolites were generally most evident 2 h after feeding, when rapid ruminal fermentation of ingested feed increased production of ruminal VFA with concomitant changes in other blood metabolites. Of particular interest was the increase in blood βHBA. Although levels reported were not as high as those reported previously (Quigley et al., 1991a), peak concentration approached .8 m*M* in calves fed the Grain treatment. Although these elevated levels indicate considerable ketogenesis, low NEFA concentrations and normal concentration of plasma glucose suggest that the contribution of hepatic ketogenesis to total ketone production was minor. Therefore, most ketones were probably a result of ruminal fermentation and alimentary ketogenesis.

Metabolic effects of increasing βHBA with weaning and dry feed intake are not clear. Targowski et al. (1985) reported that 1,3 butanediol (a potent ketogenic agent) inhibited mitogenic response of lymphocytes in calves when blood βHBA reached approximately 1.2 m*M*. Calves on the study developed respiratory infections indicative of depressed immune response. Further data are required to determine whether postprandial changes in concentration of blood βHBA in ruminants fed high-grain diets can impair immune response. Finally, use of βHBA as an indicator of overall energy status (Russell, 1984; Thomas et al., 1988) may depend on time after feeding, as our data suggest.

Implications

Rapid development of ruminal function is essential to allow early weaning and rapid growth in young dairy calves. Blood metabolites, particularly β-hydroxybutyrate and volatile fatty acids, increased rapidly with increasing consumption of calf starter. Samples taken 2 h after feeding showed that consumed calf starter is fermented rapidly in the rumen, leading to large changes in blood metabolite concentrations. Additional research is needed to determine effects, if any, of

these changes on animal health, energy efficiency, and overall performance.

Literature Cited

Anderson, K. L., T. G. Nagaraja, and J. L. Morrill. 1987. Ruminal metabolic development in calves weaned conventionally or early. J. Dairy Sci. 70:1000.

Anderson, K. L., T. G. Nagaraja, J. L. Morrill, P. G. Reddy, T. B. Avery, and N. V. Anderson. 1988. Performance and ruminal changes of early-weaned calves fed lasalocid. J. Anim. Sci. 66:806.

AOAC. 1980. Official Methods of Analysis (13th Ed.). Association of Official Analytical Chemists, Washington, DC.

Bartley, J. C., R. A. Freedland, and A. L. Black. 1966. Effect of aging and glucose loading on the activities of glucose-6-phosphatase and phosphorylase of livers of cows and calves. Am. J. Vet. Res. 27:1243.

Bergman, E. N. 1984. Disorders of carbohydrate and fat metabolism. In: M. J. Swenson (Ed.) Dukes' Physiology of Domestic Animals. pp 412–422. Cornell University Press, Ithaca, NY.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook 379. ARS, USDA, Washington, DC.

Howarth, R. E., R. L. Baldwin, and M. Ronning. 1968. Enzyme activities in liver, muscle, and adipose tissue of calves and steers. J. Dairy Sci. 51:1270.

Kronfeld, D. S. 1970. Ketone body metabolism, its control, and its implications in pregnancy toxaemia, and feeding standards. In: A. T. Phillipson (Ed.) Physiology of Digestion and Metabolism in the Ruminant. pp 566–583. Oriel Press, Newcastle Upon Tyne, UK.

NRC. 1989. Nutrient Requirements of Dairy Cattle (6th Ed.). National Academy Press, Washington, DC.

Quigley, J. D., III, L. A. Caldwell, G. D. Sinks, and R. N. Heitmann. 1991a. Changes in blood glucose, nonesterified fatty acids, and ketones in response to weaning and feed intake in young calves. J. Dairy Sci. 74:250.

Quigley, J. D., III, Z. P. Smith, and R. N. Heitmann. 1991b. Changes in plasma volatile fatty acids in response to weaning and feed intake in young calves. J. Dairy Sci. 74:258.

Russel, A.J.F. 1984. Means of assessing the adequacy of nutrition of pregnant ewes. Livest. Prod. Sci. 11:429.

Targowski, S. P., W. Klucinski, E. T. Littledike, and D. A. Hoy. 1985. Reduction in mitogenic response of bovine lymphocytes by ketone bodies. Am. J. Vet. Res. 46:1378.

Thomas, V. M., M. J. McInerney, and R. W. Kott. 1988. Influence of body condition and lasalocid during late gestation on blood metabolites, lamb birth weight and colostrum composition and production in Finn-cross ewes. J. Anim. Sci. 66: 783.

Trenkle, A., and K. V. Kuhlemeier. 1966. Relationship of rumen volatile acids, blood glucose and plasma nonesterified fatty acids in sheep. J. Anim. Sci. 25:1111.

Walker, D. M., and R. A. Simmonds. 1962. The development of the digestive system of the young animal. VI. The metabolism of short-chain fatty acids by the rumen and caecal wall of the young lamb. J. Agric. Sci. 59:375.

Williamson, D. H., and J. Mellanby. 1965. Acetoacetate and D-(-)-β-hydroxybutyrate. In: H. V. Bergmeyer (Ed.) Methods of Enzymatic Analysis. pp 454–461. Academic Press, New York.