Changes in Plasma Volatile Fatty Acids in Response to Weaning and Feed Intake in Young Calves

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ABSTRACT

Effects of weaning age on plasma VFA were examined using 16 Holstein heifer calves. Animals entered the study at 6 ± 3.5 d of age and were fed 1.8 kg milk twice daily to 28 (early weaning) or 56 d (late weaning) and a commercial pelleted calf starter from 0 (early) or 28 (late) d. Blood was sampled once weekly for 14 wk. Total blood concentrations of VFA, acetate, propionate, and butyrate were higher in calves weaned early. Difference between treatments was greatest during wk 5 to 8, after early calves had been weaned. Total VFA and acetate were both highly correlated with grain intake (r = .77), whereas propionate (r = .47) and butyrate (r = .56) were less highly correlated. Data indicate that blood VFA responded rapidly to dry feed intake, and adaptation to high grain diets was complete by 1 to 2 wk postweaning. (Key words: calves, ruminal development, volatile fatty acids)

Abbreviation key: ACAC = acetoacetate, βHOB = β-hydroxybutyrate, TVFA = total VFA.

INTRODUCTION

Development of ruminal function in young calves causes fundamental changes in metabolites available for maintenance and growth. During the transition to the ruminant state, calves, originally reliant upon glucose as primary energy substrate, must adapt to end-products of ruminal fermentation for energy. Decreased glucose tolerance (10), increased ruminal concentration of VFA (1, 15), and absorptive (20) and metabolic activity (21) of ruminal epithelium that occur with increased feed intake indicate greater use of VFA during transition to ruminant digestion. However, calves appear capable of utilizing significant VFA prior to weaning and intake of solid feed (11, 23). Thus, it appears that production of ruminal VFA or absorption of VFA from the rumen mediate transition to the ruminant state.

Blood VFA generally increase with age and dry feed intake in calves (12) and lambs (16). Murdock and Wallenius (13) suggested that feeding high grain rations to promote increased ruminal butyrate and concomitant ruminal development may allow early weaning. Impact of high grain rations on blood VFA concentrations, particularly butyrate, a major ketogenic precursor, and propionate, a gluconeogenic precursor, have not been established. Stobo et al. (19) reported that high grain rations may depress blood VFA in ruminating calves compared to high forage rations. Therefore, objectives of this study were to measure changes in blood VFA in young calves fed high grain diets during the periweaning period and to determine if early weaning alters concentrations of blood VFA.

MATERIALS AND METHODS

Animal assignments, feeding regimen, feed management, and statistical procedures have been described (14). Briefly, 16 Holstein heifer calves were assigned alternately at birth to a completely randomized 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) and fed 1.8 kg whole milk, or waste milk, or both twice daily to weaning. Commercial pelleted calf starter was offered from 0 (early) or 28 d (late). Alfalfa hay was offered for ad libitum

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consumption when starter consumption reached 2.7 kg/d.

Blood samples were taken weekly and plasma harvested for analysis of VFA by a modification by Reynolds et al. (17). Plasma (2 ml) was deproteinated by centrifugation (15,000 × *g* for 15 min) following addition of 4 ml .15 *M* $Ba(OH)_2$, 4 ml .175 *M* $ZnSO_4$ and 6 ml H_2O containing .008% Triton X-100. Internal standard (2-ethyl-butyric acid) was added to supernatant liquid and poured onto cation (AG 50W-X 8 resin, BioRad, Richmond, CA) and anion (Bio-Rex 5, BioRad, Richmond, CA) exchange columns. After rinsing with H_2O, VFA were collected into beakers using 10 m*M* NaOH poured onto anion column. Samples were dried in a forced-air oven (50°C) and reconstituted in 1 ml phosphoric acid (16%). Samples were analyzed for VFA by gas chromatography (10 m × .53 mm × 1 μm i.d. capillary column HP-FFAP; flame ionization detector; and N carrier at 11 ml/min) using a Hewlett-Packard Model 5890 gas chromatograph (Hewlett-Packard, Avondale, PA). Acids were corrected for recovery of internal standard.

Concentrations of total VFA (TVFA = acetate + propionate + butyrate) and individual acids in plasma were analyzed as a split-plot design. Standard errors of treatment means were calculated by Gill et al. (6). Probability of ($P<.05$) was used throughout unless otherwise noted.

RESULTS AND DISCUSSION

Intake, BW gain, and concentrations of blood ketones, NEFA, and glucose have been reported (14). Volatile fatty acids other than acetate, propionate, and butyrate were not in sufficient quantities to be integrated, although chromatograms occasionally showed minute peaks with retention times similar to those of isobutyrate, valerate, and isovalerate. Huntington et al. (9) reported low concentrations of these VFA in plasma of mature cattle.

Mean TVFA were greater ($P<.02$) in plasma of calves weaned early and averaged 485 and 399 μ*M*. Week of study had a significant effect ($P<.01$) on TVFA (Figure 1). From 0 to 3 wk, little change was observed in either treatment group, although calves weaned early had access to grain and consumed small amounts. After

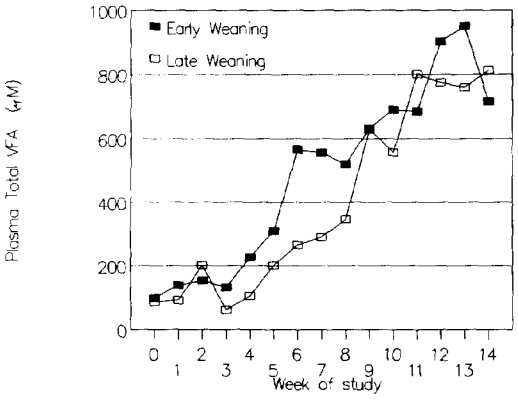

Figure 1. Least squares means of plasma total VFA concentration in calves weaned early (28 d) or late (56 d). Effect of week was significant ($P<.01$).

weaning at 4 wk, however, TVFA increased rapidly in calves weaned early and reached maximal concentration (951 μ*M*) at 13 wk. In calves weaned late, TVFA rose from approximately 100 to 814 μ*M* at 11 wk.

Data were analyzed by weaning periods to evaluate effects of treatment during preweaning (0 to 4 wk), during the time when the early group had been weaned, but the late group had not (5 to 8 wk), and postweaning (9 to 14 wk). During preweaning and postweaning periods, plasma TVFA numerically were greater in calves weaned early, although these trends were not significant. Concentrations of TVFA were markedly higher in calves weaned early during the 5- to 8-wk period and averaged 488 and 280 μ*M* in calves weaned early and late, respectively. Increased grain intake that occurred immediately postweaning probably was responsible for increased TVFA. Grain intake averaged 1.5 and 1.0 kg/d in calves weaned early or late, respectively, during the 5- to 8-wk period.

The high correlation (r = .77) between TVFA and grain intake (Table 1) indicates that most of the increase in TVFA was associated with intake of grain, resulting in fermentation of substrate in the rumen.

Acetate constituted 90% of VFA measured during the study. Thus, most changes in TVFA were a result of changes in acetate concentration. Molar proportion of acetate varied with week ($P<.01$), however. During the first few weeks of the study, acetate declined from ap-

proximately 100 to 86% of TVFA by 7 to 9 wk, then increased slightly to the end of the study. Molar proportions of TVFA as propionate and butyrate averaged 4% and tended to be greater in calves weaned early, although this trend was not significant. Propionate and butyrate were affected significantly by week ($P<.01$), and both increased from 0 to 10% by 6 to 7 wk of the study; thereafter, molar percentage did not vary significantly and averaged 4 to 6% of TVFA.

Plasma acetate was low prior to weaning (0 to 4 wk), averaging 121 μM, and did not differ between treatments. Marked increase occurred in both groups during 5 to 8 wk, although increase was greater ($P<.01$) in calves weaned early. Mean plasma acetate during wk 5 to 8 was 422 and 244 μM in calves weaned early and late, respectively. From 9 to 14 wk, no difference in plasma acetate, averaging 677 μM, was observed between treatments, which was similar to mature ruminants (9).

Absorption of VFA from the rumen depends on development of metabolic activity of ruminal mucosa (20, 21), which in turn is dependent upon intake of dry feed and production of ruminal VFA (5, 18). Small amounts of dry feed consumed by calves weaned early from 1 to 3 wk did not result in increased acetate production. Rather, acetate and butyrate produced in the rumen may have been metabolized to β-hydroxybutyrate (βHOB), which increased during the first 3 wk of the study (14). Although acetate is not a major substrate for ketogenesis (4), significant metabolism of acetate to ketones in young animals has been reported (23). However, when calves in the present study consumed increasing amounts of grain, acetate increased from approximately 100 μM to 700 to 800 μM by the end of the study.

Plasma propionate was affected by treatment ($P<.04$), averaging 27 and 20 μM in calves weaned early or late, respectively, during the 14-wk study. Propionate increased ($P<.01$) with week of study (Figure 2). A sharp increase was observed in calves weaned early from 4 to 7 wk, when concentration of propionate increased from 14 to 55 μM; thereafter, propionate ranged from 30 to 50 μM. After feed was offered at 4 wk to calves weaned late, plasma propionate increased rapidly to 50 μM by 11 wk of the study.

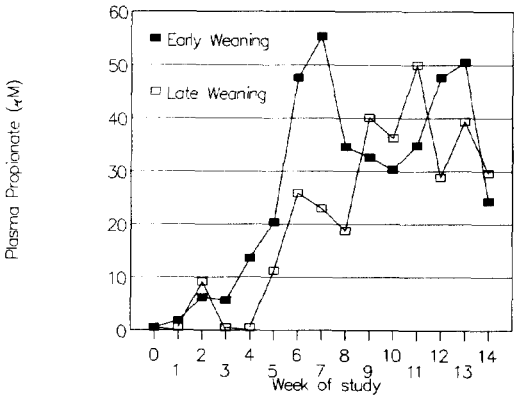

Figure 2. Least squares means of plasma propionate concentration in calves weaned early (28 d) or late (56 d). Effect of week was significant ($P<.01$).

When analyzed by weaning period, greatest differences were observed during 5 to 8 wk, when propionate in calves weaned early was twice that in calves weaned late and averaged 40 and 20 μm, respectively. Prior to weaning (0 to 4 wk), propionate was low; in many animals no propionate was detected. During wk 9 to 14, concentration of propionate reached levels similar to those of mature cows (9) with no difference between treatments.

Because propionate is a major gluconeogenic precursor in ruminants, its availability to calves weaned early is critical for the successful transition to a fully functional rumen. Rapid increase in plasma propionate immediately after weaning (Figure 2) suggests increased contribution of glucose derived from hepatic gluconeogenesis to total blood glucose in peripheral circulation. Hepatic gluconeogenesis develops rapidly in response to changing substrate (2, 3). Plasma glucose declined only slightly in both treatment groups in the week after weaning, although most glucose in the diet, provided as lactose in milk, was removed.

Changes in plasma butyrate in response to treatment closely resembled those observed with propionate (Figure 3). Throughout the study, butyrate was higher in calves weaned early, with most of the difference between treatments occurring at 5 to 8 wk ($P<.03$). Prior to (0 to 4 wk) and after weaning (9 to 14 wk), no difference between treatments was significant.

TABLE 1. Significant ($P<.01$) coefficients of correlation of plasma concentrations of total VFA (TVFA), acetate (A), propionate (P), and butyrate (B), and intakes of grain (GI), dry feed (DFI), hay (HI), and total DM (TDMI).

	A	P	B	GI	DFI	HI	TDMI
TVFA	.99	.69	.73	.77	.77	.57	.76
A		.62	.68	.77	.78	.58	.76
P			.71	.47	.44	.22	.41
B				.56	.54	.33	.51
GI					.97	.64	.96
DFI						.79	.99
HI							.81

It is of interest to note that during the first 2 to 3 wk of the study, prior to dry feed being offered low concentrations of butyrate were detected in calves weaned late. Because little or no endogenous release of butyrate occurs into the peripheral circulation (4), it is assumed that ruminal or caecal fermentation (8) of milk probably contributed to the butyrate found.

Most of the ruminal butyrate is metabolized by ruminal epithelium to ketones (4) in mature ruminants. In a study with lambs fed milk only or milk plus pasture, Walker and Simmonds (22) reported increased ketogenic activity when dry feed was consumed. Metabolic activity typical of mature ruminants was observed by 3 wk of age. These results were supported by data of Sutton et al. (21) using calves fed milk or milk plus hay and grain to 16 wk of age. Additionally, ability of liver to metabolize butyrate is influenced by age (7, 10) and ruminal fermentation. Therefore, it appears that metabolism of butyrate to ketones by ruminal epithelium and liver is maximal shortly after significant amounts of dry feed are consumed.

Assuming that most butyrate is metabolized prior to release to peripheral circulation, rapid increases in butyrate concentration at 4 to 5 wk should have been accompanied by similar increases in blood ketones. As reported (14), blood βHOB and acetoacetate (ACAC) increased twofold in calves weaned early from 4 to 5 wk; however, no significant increase in blood ketones was observed in calves weaned late, although plasma butyrate increased from 4 to 16 μM. Significance of this observation is unclear, but it is possible that the ability of ruminal epithelium and liver to metabolize butyrate was low from 4 to 5 wk in calves weaned late. Increased grain intake provided excess ruminal butyrate, which subsequently was absorbed without metabolism to ketones. Confirmation of this hypothesis awaits further experimentation.

Table 1 contains coefficients of correlation of plasma VFA and intake measurements. As expected, correlation between TVFA and acetate was very high (r = .99), as most TVFA was acetate. Correlations between acetate and intake of grain, dry feed, hay, and total DM intake were higher than those for propionate and butyrate. This was expected, as extensive utilization of propionate by liver and butyrate by ruminal epithelium (4) would alter the relationship between intake of substrate and plasma profiles. Little acetate, however, is used by these tissues (4), and thus, circulating acetate would relate most closely to intake.

Correlation coefficients of plasma VFA and other blood metabolites reported by Quigley et al. (14) and in Table 2 generally reflect changes

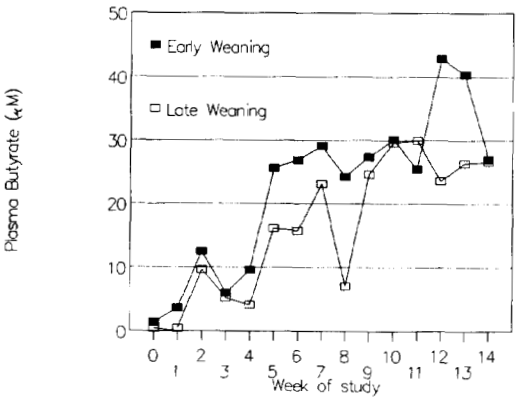

Figure 3. Least squares means of plasma butyrate concentration in calves weaned early (28 d) or late (56 d). Effect of week was significant ($P<.01$).

TABLE 2. Coefficients of correlation[1] of concentrations of plasma total VFA (TVFA), acetate (A), propionate (P), and butyrate (B), and blood β-hydroxybutyrate (βHOB), acetoacetate (ACAC), NEFA, and glucose (GLUC).

	A	P	B	ACAC	βHOB	NEFA	GLUC
TVFA	.99	.69	.73	.61	.74	−.21	−.37
A		.62	.68	.61	.74	−.20	−.35
P			.71	.33	.42	−.18	−.31
B				.53	.60	−.26	−.33
ACAC					.89	−.15[b]	−.37
βHOB						−.17	−.41
NEFA							.05[a]

[1]All coefficients were significant at ($P<.01$), except [a] ($P>.05$), and [b] ($P<.05$).

in plasma metabolites with increased intake of feed. Correlation of acetate with βHOB (r = .74) and ACAC (r = .61) were greater than butyrate, although butyrate serves as the major ketogenic acid produced in the rumen. Apparently, blood ketone and acetate concentrations were most closely associated with grain intake. Negative correlations between VFA and NEFA reflect decrease in NEFA during the course of the study; generally low correlation coefficients suggest that the relationship may be only indirect. Similar conclusions are apparent for glucose and VFA.

CONCLUSIONS

Plasma VFA increased from low concentrations to reach levels typical of mature ruminants by 1 to 2 wk postweaning. Increasing VFA concentrations were closely related to increasing dry feed intake associated with treatment application in this study. Changes in blood VFA concentration suggest that calves adapted rapidly to early weaning program in this study.

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