

Response of Peripheral Blood Mononuclear Cells to Mitogenic Stimulation in Young Calves

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ABSTRACT

Sixteen Holstein bull calves were used to evaluate effects of increasing blood BHBA, as a consequence of ruminal development, on response of peripheral blood mononuclear cells to mitogenic stimulation. Calves were blocked by date of birth and assigned to weaning at 35 or 49 d of the study. Calves began the study at 4 d of age. Milk replacer was fed at 10% of BW to d 7 prior to weaning; thereafter, milk replacer offered was reduced by 50%. Calf starter was offered from d 1. Blood was sampled at wk 0, 2, 4, 6, 8, and 10 at 4 h after the a.m. feeding. Blood glucose declined, and blood BHBA increased, as calf starter intake increased. Blood BHBA increased at weaning in each treatment group and exceeded 1 mM during the week of weaning. Responses of peripheral blood mononuclear cells to stimulation with concanavalin A, pokeweed mitogen, or phytohemagglutinin were unaffected by increasing blood BHBA and increased with age. Increased concentration of blood BHBA in young calves from increased starter intake did not impair immune response.

(Key words: calves, immunity, immunoglobulins, ketones)

Abbreviation key: Con A = concanavalin A, PBMC = peripheral blood mononuclear cells, PHA = phytohemagglutinin, PWM = pokeweed mitogen, W35 = weaning at 35 d of the study, W49 = weaning at 49 d of the study.

INTRODUCTION

Weaning dairy calves at 4 to 6 wk of age is a common management practice. Calf starters fed with limited forage stimulate ruminal development by increasing ruminal VFA and reducing ruminal pH (1, 12). Low pH and high concentrations of ruminal VFA increase transport and metabolism of VFA, particularly butyrate, by ruminal epithelium (15). Butyrate is converted to BHBA by ruminal epithelium and released to portal blood. Blood ketones increase as a result of dry feed intake with early weaning (10, 11), and blood BHBA may reach or exceed 1 mM by 1 to 2 wk after weaning.

The ability of peripheral blood mononuclear cells (PBMC) to respond to mitogenic challenge was reduced when PBMC were incubated in vitro with BHBA at approximately .1, 1, and 5 mM (16) or when calves were fed 1,3-butanediol to increase blood BHBA (17). Reduced response of PBMC to mitogenic challenge may indicate immune suppression and increased susceptibility to disease. Conversely, Franklin et al. (5) reported no effect of ketones when PBMC were incubated with mitogens in vitro, although inhibition of PBMC stimulated by phytohemagglutinin (PHA) and pokeweed mitogen (PWM) occurred at 6.25 mM BHBA. Also, secretion of IgM by bovine blood lymphocytes in vitro was unaffected by ketones up to 6.25 mM (8). If BHBA concentrations, elevated as a consequence of developing ruminal function, inhibit PBMC response to mitogenic challenge, current feeding management may compromise the ability of calves to elicit an immune response. Therefore, our objective was to determine the effects of developing ruminal function and increasing blood BHBA on PBMC response to mitogenic stimulation. Ruminal development was effected by controlling age at weaning.

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MATERIALS AND METHODS

Experimental Design

Sixteen Holstein bull calves averaging 4 d of age (SE = .3) were blocked by date of birth and assigned randomly to weaning at 35 d (W35) or 49 d (W49) of the study. Shortly after birth, but before suckling the dam, calves were moved to the experimental unit and placed in individual wooden pens (3.3 m²) bedded with shavings. Calves were fed 2 L of colostrum (pooled within block) as soon as possible (within 4 h of birth) and 12 h later. Colostrum was pooled within block to minimize differences in acquisition of passive immunity. Commercial milk replacer (Maxi-Lac; Tennessee Farmers Cooperative, LaVergne, TN) (Table 1) was fed at 10% of BW (measured on d 0 or during the previous week) to d 7 prior to weaning; thereafter, the amount of milk replacer offered was reduced to 5% of BW. Commercial calf starter (Total Calf Ration; Tennessee Farmers Cooperative) (Table 1) was offered once daily for ad libitum consumption from d 1. Water was available at all times.

Sampling and Analysis

Body weights were measured at the initiation of the study (d 0) and every 7 d thereafter to wk 10. Blood was sampled approximately 24 h after birth, and serum IgG was measured by single radial immunodiffusion (VMRD Inc., Pullman, WA). Jugular blood (about 10 ml) was collected approximately 4 h after the a.m. feeding at 0, 2, 4, 6, 8, and 10 wk of the study. A 2-ml subsample was deproteinated immediately with 2 ml of 1 M HClO₄ and placed on ice prior to analysis of BHBA (19). A second blood sample (4 ml) was deproteinated in 4 ml of 8% HClO₄ prior to analysis of L(+)-lactate (lactate kit 826; Sigma Chemical Co., St. Louis, MO). Remaining blood was added to 400 µl of 6% EDTA prior to separation of plasma and analysis of glucose (glucose kit 510; Sigma Chemical Co.).

The PBMC were obtained as described by Torre and Oliver (18). Briefly, 10 ml of blood were collected into 100 µl of 15% EDTA and diluted 1:3 (vol/vol) in PBS (8 mM Na₂HPO₄, 1.5 mM KH₂PO₄; pH 7.3). Diluted blood was underlaid with 10 ml of Ficoll[®]-sodium di-

TABLE 1. Chemical composition of milk replacer and calf starter.

Item	Milk replacer ¹		Calf starter ²	
	\bar{X}	SE	\bar{X}	SE
DM, %	94.3	.3	91.5	.2
	(% of DM)			
CP	23.8	.3	20.9	.2
Ash	14.0	.1	7.1	.1
NDF	ND ³	...	20.5	.4

¹n = 7.

²n = 8.

³Not determined.

atrizoate (specific gravity, 1.083; Sigma Chemical Co.) and centrifuged (400 × g, 18°C) for 50 min. The PBMC were recovered and washed three times (300 × g for 15 min per washing at 4°C) in PBS. Residual red blood cells were lysed by hypotonic shock treatment using sterile phosphate-buffered water (.1 M phosphate). The PBMC were resuspended in RPMI-1640 (Whittaker Bioproducts, Walkersville, MD) containing 25 mM HEPES buffer (Sigma Chemical Co.), 2 mM L-glutamine (Whittaker Bioproducts), 100 U of penicillin G, 100 µg of streptomycin sulfate, .25 µg of amphotericin B/ml (Sigma Chemical Co.), 5 × 10⁻⁵ M 2-mercaptoethanol (Sigma Chemical Co.), and 10% heat-inactivated fetal bovine serum (Whittaker Bioproducts). Cell recovery and viability were determined by hemacytometer count using trypan blue exclusion. Viability of PBMC was 87.5 ± 5.0%.

Response of PBMC to mitogenic challenge was determined using T-lymphocyte mitogens concanavalin A (Con A; Sigma Chemical Co.) and PHA (Sigma Chemical Co.) and B- and T-lymphocyte mitogen PWM (Sigma Chemical Co.). Cells and mitogens for all assays were resuspended in complete RPMI-1640 containing 10% fetal bovine serum. One hundred microliters of PBMC resuspended at 2 × 10⁶ viable cells/ml were added into triplicate wells of a 96-well flat-bottom tissue culture plate (Corning Glass Works, Corning, NY). Mitogens (100 µl) were added to wells at final concentrations of 3.125, 3.125, and .78 µg/ml for Con A, PHA, and PWM, respectively. Control wells contained 2 × 10⁵ PBMC in 200

μ l of complete RPMI-1640 and 10% fetal bovine serum only. Tissue culture plates were incubated for 72 h at 37°C in an atmosphere of 95% air and 5% CO₂. Thereafter, 1 μ Ci of [methyl-³H]thymidine (ICN Radiochemicals, Irvine, CA) in 50 μ l of complete RPMI-1640 was added to each well and incubated for the remaining 20 h of total incubation. The PBMC were harvested on glass fiber filters using an automated cell harvester (PHD Cell Harvester; Cambridge Technology, Inc., Watertown, MA). Retained radioactivity was counted in a liquid scintillation counter (Mark V series liquid scintillation counting system; TM Analytical Inc., Elk Grove Village, IL).

Samples of calf starter were collected weekly, composited by shipment, and analyzed for DM, CP (2), and NDF (6).

Statistical Analysis

Data were analyzed as a repeated measures experimental design using a general linear mixed model algorithm (3). The model was

$$Y_{ijk} = \mu + B_i + T_j + BT_{(ij)} + W_k + WT_{(jk)} + e_{(ijk)}$$

where

- Y_{ijk} = observation,
- μ = overall mean,
- B_i = effect of block i ($i = 1..8$),
- T_j = effect of treatment j ($j = 1..2$),
- $BT_{(ij)}$ = effect of block \times treatment interaction,
- W_k = effect of week k ($k = 1..10$ for BW, 1..5 for blood data),
- $WT_{(jk)}$ = effect of week \times treatment interaction, and
- $e_{(ijk)}$ = error.

Block \times treatment interaction was used to test effects of treatment. Significance was at $P < .05$ unless otherwise noted. Because calves were treated similarly until wk 5 of the study, data also were grouped by preweaning (1 to 5 wk) and periweaning (6 to 10 wk) periods and analyzed as described; however, results by weaning periods were similar to results for all weeks.

RESULTS AND DISCUSSION

Calves were healthy throughout the study. Prevalence of respiratory and digestive tract diseases was minimal and unrelated to treatment. Concentration of IgG in colostrum averaged 42.6 mg/ml (SE = 1.2). Mean serum IgG concentration at 24 h was 8.5 mg/ml (SE = .6), ranged from 5.6 to 13.5 mg/ml, and was unaffected by treatment.

Chemical composition of calf starter and milk replacer (Table 1) generally was similar to NRC (7) recommendations for similar feeds, although CP was higher than recommendations in both feeds.

Calf BW was unaffected by treatment, although a week \times treatment interaction was significant for average daily gain (Table 2). Rates of gain were depressed during the week prior to weaning when milk replacer was limited to 50% of the intake of the previous week. However, by 1 wk after weaning, rates of average daily gain were similar to those prior to reduction in milk offered. Prior to and after weaning, rates of average daily gain did not differ between treatments and were typical of well-managed Holstein calves.

Starter intake (Table 2) and BHBA (Table 3) were significantly ($P < .01$) affected by treatment. Blood BHBA was correlated ($r = .65$) with starter intake and increased after weaning in both groups. Maximal BHBA was 1.17 and 1.21 mM in W35 and W49 treatment groups, respectively, at 1 wk postweaning. Rapid increases in blood BHBA have been reported (10, 11) and are associated with ruminal fermentation of high grain diets (13). Blood glucose declined at weaning and varied by treatment. Lactate was not influenced by treatment and decreased from 2.1 mM at wk 0 to 1.0 mM at wk 8. Neither blood glucose nor L(+)-lactate was correlated with PBMC response to all mitogens, although glucose was correlated ($r = -.21$) with PBMC response to PHA. These data indicate that moderate changes in concentration of glucose and lactate did not significantly affect PBMC response.

Response of PBMC to mitogenic challenge was unaffected by treatment (Table 4), although response to all mitogens changed as starter intake increased. Starter intake was correlated ($r = .38, .52, \text{ and } .38$) with PBMC response to Con A, PHA, and PWM, respec-

TABLE 2. Calf BW, average daily gain (ADG), and weekly intake of milk replacer and calf starter in calves weaned at 35 d (W35) or 49 d (W49) of the study.

Week	BW ^a		ADG ^b		Milk replacer ^b		Starter ^b	
	W35	W49	W35	W49	W35	W49	W35	W49
	(kg)				(kg of DM)			
0	38.1	40.1
1	40.0	41.5	.26	.19	3.0	3.2	.3	.8
2	43.1	44.6	.46	.45	3.2	3.3	1.0	2.2
3	47.2	49.4	.59	.68	3.4	3.5	2.2	2.4
4	53.6	55.4	.92	.87	3.7	3.9	3.8	3.6
5	55.2	61.5	.22	.87	1.9	4.4	6.5	5.6
6	61.8	68.8	.95	1.04	0	4.9	10.5	6.4
7	68.0	72.9	.88	.58	0	2.6	13.7	9.6
8	73.6	78.7	.80	.83	0	0	16.5	14.2
9	80.4	84.7	.97	.86	0	0	17.6	16.3
10	86.6	92.9	.88	1.18	0	0	19.9	19.3
SE	3.4		.12		.2		.8	

^aSignificant week effect ($P < .01$).

^bSignificant week \times treatment interaction ($P < .01$).

TABLE 3. Concentrations of blood BHBA and L(+)-lactate and plasma glucose in calves weaned at 35 d (W35) or 49 d (W49) of the study.

Week	BHBA ^a		Glucose ^a		L(+)-Lactate ^b	
	W35	W49	W35	W49	W35	W49
	(mM)					
0	.33	.39	4.63	5.72	2.3	1.9
2	.37	.35	4.83	4.79	1.1	1.2
4	.40	.42	5.65	4.95	1.2	.9
6	1.17	.53	4.09	5.32	1.0	1.0
8	1.14	1.21	4.46	4.76	.9	1.0
10	.89	1.05	4.56	4.56	1.5	1.3
SE	.09		.28		.2	

^aSignificant week \times treatment interaction ($P < .01$).

^bSignificant week effect ($P < .01$).

TABLE 4. Response (corrected for control) of blood mononuclear cells to stimulation with phytohemagglutinin (PHA), pokeweed mitogen (PWM), or concanavalin A (Con A) in blood of calves weaned at 35 d (W35) or 49 d (W49).

Week	PHA ^a		PWM ^a		Con A ^a	
	W35	W49	W35	W49	W35	W49
	(cpm $\times 10^{-3}$)					
0	46.8	48.1	65.2	84.0	106.6	148.2
2	42.0	42.2	55.7	62.2	143.1	128.7
4	46.4	67.0	57.6	66.1	130.4	151.7
6	55.6	73.7	60.9	97.7	181.0	197.4
8	119.3	103.1	114.9	107.5	226.0	215.7
10	125.0	113.8	113.9	105.3	208.3	201.3
SE	20.0		18.7		29.2	

^aSignificant week effect ($P < .01$).

tively ($P < .0001$). Blood BHBA was correlated ($P < .01$) positively with Con A ($r = .25$) and PHA ($r = .28$), but not with PWM ($r = .18$). Positive correlations indicated that BHBA did not have a negative impact on PBMC response to mitogens. Increases in PBMC activity were greatest during wk 4 to 6 (Con A) and wk 6 to 8 (PHA and PWM). Increased PBMC response may be attributable to maturation of cell-mediated immunity in young calves (9), although Reddy et al. (14) reported no effect of age on PBMC response to PHA stimulation.

Lack of decreased mitogenic response to increasing BHBA in this study conflicts with data of Targowski et al. (17), who reported reduced response of PBMC from calves during experimental ketonemia. Experimental ketonemia was induced by a diet with 1,3-butanediol, which caused blood BHBA to increase to 1.2 mM during the 2-wk feeding period (17). Targowski et al. (17) demonstrated that only high concentrations of 1,3-butanediol decreased PBMC response to mitogens *in vitro*; however, circulating concentrations were not measured. Possibly, 1,3-butanediol may have impaired PBMC response directly, although Nonnecke et al. (8) reported no effect of 1,3-butanediol on amount of IgM secreted by PBMC in response to mitogenic challenge *in vitro*. Also, 1,3-butanediol may have induced another physiological response, such as secretion of glucocorticoids, which inhibit PBMC response to mitogenic challenge (4). Franklin et al. (5) and Nonnecke et al. (8) also reported no effect of ketones on response of lymphocytes to mitogenic challenge.

Management practices to promote early weaning with rapid ruminal development and concomitant production of BHBA do not appear to affect PBMC response to mitogenic challenge negatively.

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