Limestone in Milk Replacers Containing Soy Protein Concentrate[1]

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ABSTRACT

Sixteen male Holstein calves were used to study the buffering of limestone a in the small intestine of preruminant calves fed milk replacers containing 50% of the protein from soy protein concentrate. Experimental diets were: (A) 19% crude protein as milk protein; (B) same as A, but containing .8% limestone; (C) 19% crude protein, being 9.5% milk protein and 9.5% soy protein; and (D) same as C, but containing .8% limestone. Milk replacers were reconstituted with water to 14% solids and fed at 8, 9, 10, 11, 12 and 12% body weight from 1st to 6th week. Calves were fed twice daily and clean and fresh water was available at all times.

Soy protein concentrate in the replacer resulted in 20% lower weight gains, and significantly lower dry matter and protein digestibilities. Apparent crude protein retention was also reduced, but intake of nutrients, feed efficiency, fecal score and rectal temperature were not different between protein sources. Limestone resulted in no significant changes in the already mentioned parameters. Xylose absorption test performed when calves were 3 and 6 wk old showed differences due to age, but not treatment. Analysis of digesta obtained from different sections of the gut of 6 wk old calves sacrificed 6 h after feeding revealed that (a) abomasal pH was higher than previously reported for calves fed whole milk; (b) small intestinal pH was above 6 for both protein sources which may explain the ineffectiveness of adding limestone to the replacers; (c) the use of soy protein concentrate resulted in a higher pH in the large intestine and feces.

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INTRODUCTION

Among plant proteins, soybean products have been tested and improved the most and consequently have been successfully incorporated at higher levels in milk replacers for young calves. However, experimental results with preruminant calves varied widely depending upon the soybean product being used and its concentration in the replacer (2,4,9,19,26,35). In some cases, satisfactory growth was obtained when soybean protein concentrate supplied more than 50% of the total protein (12,13,31). On the other hand, Morrill et al. (24) concluded that soybean protein concentrate successfully replaced 25% but not 44% of total protein in calve's milk replacers.

Reduced digestibilities of protein and fat of milk replacers containing soy protein concentrate or soybean meal have been reported (5,27,29). Several hypotheses have been raised to explain the negative results obtained with soybean products: (a) presence of a soybean trypsin inhibitor which could act reducing secretion and/or enzyme concentrations of the pancreatic juice (13,14), or by formation of a trypsin inhibitor-protein complex which would resist proteolysis even in the presence of higher concentration of enzymes (23); (b) reduction in gastric acid secretion (45,48); (c) lack of coagulation of dietary protein in the abomasum (10) with reduction in retention time of the protein in the abomasum (6,41) and a decrease in proteolysis and possibly excessive quantities of undigested protein reaching the small intestine which tend to promote the proliferation of pathogenic organisms in the upper gut (34); (d) low levels of methionine and lysine in soybean products (33); (e) gastrointestinal allergy-type of response by calves fed soybean products (3,39,42,43), which could result in deleterious morphological modification of the intestinal mucosa surface (37).

There are some evidence suggesting that acid digesta entering the small intestine of adult ruminants is neutralized slowly. Harrison and Hill (15) found that pH of duodenal digesta in adult sheep increased slowly from 2.7 at the proxi-

mal duodenum to around 4.0 beyond the entry of the common bile and pancreatic duct. Low pH values caudal to this duct may suggest that duodenum secretions of ruminants have a limited neutralizing capacity. This conclusion is supported by other reports (22,30,40,47). Although there is some evidence that heat damaged milk protein (44) and soybean flour in milk replacers caused higher pH values in the pyloric outflow of young calves, the objective of this work was to test the potential buffer action of limestone at the small intestine of calves fed milk replacers containing half of dietary protein from soybean protein concentrate.

MATERIAL AND METHODS

This experiment was conducted at the Dairy Research and Teaching facilities of Michigan State University from November 23, 1981 to January 25, 1982.

Sixteen male Holstein calves were purchased from a large commercial herd when 3 to 4 days of age. The day calves started on experiment they were identified by ear tags, their navels were disinfected with iodine solution, each calf received an injectable solution containing 500,000 IU of vitamin A, 75,000 IU of vitamin D, and 50 IU of vitamin E, and they were randonly assigned to experimental diets, which were as follows:

Treatment 1: 19% crude protein (CP); 19% milk protein (MP)

Treatment 2: as 1, but milk replacer containing .8% limestone

Treatment 3: 19% CP; 9.5% MP; 9.5% soy protein concentrate (SPC)

Treatment 4: as 3, but milk replacer containing .8% limestone

One sample from each 22.7 kg sack of milk replacer used was taken and composited for dry matter (forced-air oven at 65°C), crude protein (macro-Kjeldahl), ether extract and ash determinations as described by A.O.A.C. (1). Ingredient and chemical compositions of milk replacer diets are shown in Table 1.

During 42 days each calf received its designated milk replacer as the only source of nutrients at 8, 9, 10, 11, 12 and 12% body weight for wk 1 to 6,

respectively. The solid of all milk replacers after mixing with water was 14%. Replacers were offered to calves twice daily (12 h apart) in open pails. Fresh and clean water was available at all times.

Animals were kept indoors in individual pens bedded with straw except during feces and urine collection periods when they were kept in metabolism cages.

Animals were weighed individually for 3 consecutive days at the beginning and at the end of the trial. Weights were also taken at weekly intervals during treatment.

Daily observations were made on each animal for degree of scouring by rating fecal consistency on an index of 1 to 4 (20). Rectal temperatures were taken daily just before the morning feed.

Calves were moved from individual pens to metabolism cages on days 14 and 35 of experiment. If a calf showed strong diarrhea or was under medication at the beginning of the 3rd wk, its transportation to the metabolism cage was postponed for 1 wk. For this reason, the average at the beginning of the first feces and urine collection period was respectively 14, 17, 16 and 17 days under experimental conditions for treatments 1 to 4. Total feces and urine were collected separately for 4 days, always at the same hour in the morning. At that time, samples of 10% of total feces and 5% of total urine were obtained. These samples were kept in freezer until laboratory analysis of dry matter (force-air oven at 65°C), crude protein (macro-Kjeldahl), and ash as described by A.O.A.C. (1). Daily individual samples of fresh feces were also collected throughout the fecal collection periods for pH determinations after dilution with distilled water.

Xylose absorption test was performed on all calves on days 21 and 40 exactly as described by Seegraber and Morrill (36). Jugular blood samples were taken from each calf 5 h after the morning feed on days 23 and 42 for plasma urea determinations as described by Fawcet (8).

At the end of the trial, two randomly assigned calves from each treatment were sacrificed (electrocuted). After removal of the entire digestive tract, weights were recorded for contents from rumen-reticulum-omasum (as a section), abomasum and large intestine. Small intestines were divided in three equal sections (from anterior to posterior) and weights were recorded separately for contents. Samples of all contents were obtained and kept in freezer until laboratory analysis for pH, dry matter (forced-air oven at 65°C), crude protein (macro-Kjeldahl) and ash as described by A.O.A.C. (1). Samples of the small intestine tissue at 1 and 2 m from the pylorus were taken from each calf for histological examinations of the mucosa surface. These samples were suspended with four pins to cardboards that were kept in 10% buffered neutral formalin solutions.

When a calf assigned to a replacer died prior to completion of treatment, the first calf available thereafter was a replacement. All dead animals were submitted to necropsy examinations.

Initial body weight was analyzed as a completely randomized design. All other variables were analyzed as split-plot designs. F-test was used when only two means were compared; tests involving more than two means were as indicated in table footnotes.

RESULTS AND DISCUSSION

The influence of protein source (milk or soy protein) and limestone (absence or presence) on daily weight gains, intake of nutrients, feed efficiency, fecal score, rectal temperature, apparent nutrient digestibilities, protein retention, and plasma urea nitrogen are shown in Table 2. Means are presented for main effects because the interaction protein source x limestone was not significant ($P>.10$) for any variable. Initial body weights were similar ($P>.10$) for treatment groups.

Replacement of 50% of the milk protein with SPC resulted in 20% lower average

daily gains. This difference was not statistically significant (P>.10) due to the large variation and to the small number of animals within treatments. Dry matter and crude protein intakes, as well as fecal scores and rectal temperatures, were not different (P>.10) between protein sources. Significant (P<.10) reductions in dry matter and crude protein digestibilities were observed when SPC was present. Organic matter digestibility was also reduced, although not significantly (P>.10). Similar results are reported in the literature (13,35,48) and may explain at least in part, the lower weight gains of calves fed SPC. Apparent crude protein retention, expressed either as g/day or as percent of the protein ingested, was 10% lower in calves fed SPC than only milk protein. Although this difference was not significant (P>.10), it is a reflection of the depressed protein digestibility. Plasma urea nitrogen was only slightly higher (P>.10) for calves fed SPC.

The addition of .8% limestone to milk replacers did not significantly (P>.10) affect any of the varaibles listed in Table 2, although apparent nutrient digestibilities and nitrogen retention tended to be slightly reduced when limestone was present.

The interaction treatment x calf's age was not significant (P>.10) for any variable in Table 2, suggesting that those were no specific ages at which effects of SPC or limestone, or both, were greater.

Age effects on weight gains, intake of nutrients, feed efficiencies, fecal consistency and pH, rectal temperature, apparent nutrient digestibilities, apparent protein retention, and plasma urea nitrogen are in Table 3. As expected most variables were affected (P<.10) by calve's age.

Weight losses during the first 2 wk of treatment emphasizes the importance of good management and nutrition for these young and sensitive animals in order to avoid high mortality. Gains imporoved (P<.10) with age, but the relatively low gain observed in the 6th wk was due to stress of keeping these animals in metabolism

cages. Feed intakes and efficiencies increased (P .10) with age. Since no significant weigh back of replacer was observed, intakes reflect the adopted feeding criterion. Nutrient intakes for wk 3 and 6 were slightly lower than expected because animals were fased for 24 h for the xylose absorption tests. The poorer efficiencies for wk 3 and 6 might also have been due to stress of the metabolism cages. Scour scores were not affected (P>.10) by age although they tended to decrease with age; they were higher during the collection periods. Rectal temperatures were not affected (P>.10) by age, all being within the normal range.

Apparent dry matter digestibility was lower (P<.10) in older animals (6 vs 3 wk of age). Instead of decreasing, dry matter digestibility was expected to remain the same or increase with age. Apparent crude protein retention was significantly (P<.10) higher for older animals, which supports the higher weight gains in older animals.

The higher plasma urea nitrogen (PUN) at 3 than 6 wk (P<.10) agrees with weight gains and crude protein retention. The higher PUN at 3 wk might be associated with greater catabolism of muscle protein as reported by Leibholz (21). As calves became older, protein intake increased and digestibility did not change, but crude protein retention increased, apparently resulting in less gluconeogenesis.

Fecal pH was higher (P<.10) in older calves and may be related to less fermentation in the large intestines due to more efficient nutrient absorption. The xylose absorption tests support this hypothesis.

As mentioned, a xylose absorption test was performed when calves were 3 and 6 wk old to evaluate absorption capability of experimental animals. Xylose concentrations in plasma tend generally to increase up to 1.5 to 2.5 h after ingestion and then decreases (5,36). In the present experiment, plasma xylose concentrations (mg %) were: 82.2, 143.7, 162.0, 166.8, 163.5, 153.0, 123.2 and 112.9, respectively for .5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 h after xylose ingestion. The

standard error for these means was ±8.2 mg %. The partial replacement of milk protein by soy protein (Figure 1) and the presence or absence of limestone (Figure 2) had no significant ($P>.10$) effect on plasma xylose curves.

The interaction treatment x calves' age on plasma xylose concentration was significant ($P<.05$). The interpretation of these significant interactions is, however, not simple. One possible explanation is shown in Figure 3. As animals become older, average plasma xylose increases ($P<.05$) in calves fed only milk protein but not those fed SPC. This observation, also made by Seegraber and Morrill (36), might lead to the conclusion that absorption in the small intestine does not improve with age in calves fed SPC but does when only milk protein is fed. However, a higher plasma xylose concentration might result from greater capacity for absorption or from an impairment in clearing of xylose from blood. Furthermore, data in Table 4 shows that when calves were 3 wk old, the replacement (50%) of milk protein by SPC resulted in higher ($P<.05$) plasma xylose, which exactly contradicts the data reported by Seegraber and Morrill (36). When calves are 6 wk old, SPC in the replacer tended to reduce plasma xylose concentration, although not significantly ($P>.05$).

Perhaps, the best way of interpreting plasma xylose concentrations is to consider responses during the first 2 to 3 h after xylose ingestion as an absorption pattern characteristic and the behavior during the last 2 to 3 h as an indication of xylose clearing from plasma.

Figure 4 suggests that xylose absorption capability is higher ($P<.05$) in older calves. A similar conclusion was made by Seegraber and Morrill (36) and agrees with higher weight gains, feed efficiencies and lower fecal pH at 6 than at 3 wk of age.

Table 5 shows pH values and grams of dry matter, crude protein and organic matter present in different sections of the digestive tract of 6 wk old calves

sacrificed 6 h after a meal. Mylrea (25) and Porter (32) observed that pH of abomasal contents 5 h after a meal reached prefeeding levels (1.0 to 2.0) in calves fed whole milk. In the present work, samples of abomasal contents obtained 6 h after feeding showed higher pH values(3.10) as observed by Gorrill and Thomas (13). Milk replacers containing skimmilk and whey (diets A and B) or SPC (diets C and D) may have resulted in less HCl production than whole milk, as proposed by Tagari and Roy (44) and Colvin et al. (6). The higher abomasal pH may in part explain the lower crude protein digestibilities obtained in this experiment (Table 2) than those reported by Noller et al. (28) and Jacobson et al. (18) with whole milk, since the optimum pH for pepsin activity was reported to be 2.0 by Tagari and Roy (44). Neither the substitution of milk protein by SPC nor the inclusion of limestone in the repalcer had significantly ($P<.05$) affected abomasal pH (Table 5).

Small intestinal pH increased from 6.28 to 6.72 from the proximal to the distal portion (Table 5). This result with studies of Huber (16) agrees with Huber and Moore (17). Harrison and Hill (15), Lennox and Garton (22) found that conditions in the upper jejunum of adult ruminants were notably acidic (pH of 2.0 or 3.0) and that pH did not reach values of 6.0 or 7.0 until the lower jejunum.

However, our studies were with calves fed only milk replacer and proximal section of the small intestine may have extended to the lower jejunum. Other workers (30,40,47) indicated a limit to the capacity of the small intestine of adult ruminants to neutralize acid digesta from the abomasum. Results obtained in the present experiment do not support these findings and tend to indicate that the neutralizing capacity of bile and pancreatic juice of preruminant calves is similar to monogastrics. As a result, the inclusion of .8% limestone in milk replacer might not be expected to cause significant change in small intestinal pH (Table 5). On the contrary, the presence of limestone resulted in a lower ($P<.05$)

pH of gastrointestinal contents, perhaps due to a stimulatory effect of limestone on HCl production by parietal cells of the abomasum in an effort to compensate for the added buffer. The replacement of half of the dietary milk protein by SPC resulted in a slightly lower small intestinal pH, but the change was not significant ($P>.05$).

The interaction protein source x presence or absence of limestone was significant ($P<.05$) since limestone addition to replacers containing only milk protein decreased ($P<.05$) overall gut pH, whereas no changes ($P>.05$) were observed for calves fed SPC (Table 6).

Large intestinal and fecal pH were lower ($P<.05$) in calves fed SPC than milk protein (Table 5). Lower digestibility of nutrients may have resulted in accumulation of more undigested nutrients in the large intestine (11), which created conditions for greater fermentation of the organic matter. Gastrointestinal disturbances have been associated with lower nutrient digestibility in calves fed milk replacers containing severely heated skimmilk or non-milk proteins (38,44, 48). Diarrhea in the present experiment, however, was not increased by feeding SPC (Table 2).

Dry matter and organic matter recovered at each site were not different ($P>.05$) for only milk protein or milk protein and SPC. However, the amount of dry matter and organic matter present in the abomasum of animals fed SPC tended to be slightly lower than for calves fed only milk protein. This might be due to a shorter retention time of ingesta in the abomasum of non-milk proteins in preruminant calves, as postulated by Colvin et al. (6) . However, quantity of protein in the abomasum of calves fed SPC was the same as those fed only milk protein (Table 5). According to Emmons et al. (7) and Ternouth et al. (46) milk protein (and milk fat) should remain in the abomasum a longer time than other nutrients.

The incorporation of .8% limestone in the replacers resulted in more ($P<.05$)

dry matter, organic matter and crude protein in the digestive tract of calves, probably due to the larger ($P<.05$) amount present in the rumen-reticulum-omasum section. This observation suggests that calves fed limestone probably had higher intakes of bedding (straw) perhaps in trying to compensate for the lower nutrient digestibilities and protein retention (Table 2).

The interaction of treatment x site in the gut was not significant ($P>.10$) for any digesta variable measured, suggesting that treatment effects followed the same trends throughout the different sections of the gut.

One calf from each treatment died during experiment and necropsy results did not indicate a diet relationship.

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TABLE 1. Ingredient and chemical composition of milk replacers (%)

	Diets			
	A	B	C	D
Ingredients				
Dried whey	19.50	19.34	19.50	19.34
Sodium caseinate	8.20	8.13	-----	-----
Skimmilk	20.30	20.14	14.00	13.89
7/60 high fat mixture[a]	33.30	33.03	33.30	33.03
Soy protein concentrate	-----	-----	14.20	14.09
Dextrose	17.40	17.26	17.40	17.26
Premix(minerals and vitamins)[b]	1.37	1.36	1.37	1.36
L-lysine	-----	-----	.09	.09
DL-methionine	-----	-----	.15	.15
Limestone	-----	.80	-----	.80
Total	100.07	100.06	100.01	100.01
Chemical analysis[c]				
Dry matter	96.39	96.58	96.43	95.64
Crude protein	18.53	18.15	18.42	18.33
Ether extract	13.24	14.21	14.17	14.25
Ash	6.52	7.06	6.44	7.12

[a]Spray-dried mixture prepared to contain 7% milk protein and 60% fat (lecithin included)

[b]Containing (per kg): 33,000 IU vitamin A, 6,600 IU vitamin D, and 22 mg of vitamin E. Diets 1 and 3 were calculated to contain 1% calcium, 17% phosphorus and .1 ppm of selenium.

[c]Crude protein, ether extract and ash are presented on dry matter basis.

TABLE 2. Influence of protein source and limestone on daily weight gain, intake of nutrients, feed efficiency, fecal score, rectal temperature, apparent dry matter (DM), organic matter (OM) and crude protein (CP) digestibilities, apparent CP retention and plasma urea nitrogen of young calves

Variables	Protein source		Limestone		SEM
	Milk	Milk+SPC	Present	Absent	
Initial body weight (kg)	38.0 [a]	40.7 [a]	40.7 [a]	38.0 [a]	1.7
Weight gain (g/an/day)	275 [a]	223 [a]	250 [a]	247 [a]	41
DM intake (g/an/day)	563 [a]	558 [a]	563 [a]	558 [a]	33
CP intake (g/an/day)	107 [a]	107 [a]	106 [a]	108 [a]	6
Weight gain/DM intake	.49[a]	.40[a]	.44[a]	.44[a]	.0
Fecal scores	2.2 [a]	2.0 [a]	2.2 [a]	2.0 [a]	.1
Rectal temperature (^{o}C)	38.9 [a]	38.9 [a]	38.9 [a]	38.9 [a]	.1
Apparent DM digestibility (%)	87.64[a]	85.04[b]	85.20[a]	87.48[a]	.9
Apparent OM digestibility (%)	87.80[a]	85.95[a]	86.35[a]	87.40[a]	1.1
Apparent CP digestibility (%)	85.24[a]	76.81[b]	79.53[a]	82.51[a]	1.8
Apparent CP balance (g/day)	66.37[a]	59.68[a]	59.99[a]	60.06[a]	6.
Apparent CP balance(% of ingested)	50.20[a]	44.95[a]	46.54[a]	48.61[a]	3.
Plasma urea N (mg/100 ml)	5.63[a]	6.06[a]	5.35[a]	6.33[a]	.

[ab] Means in rows (within protein sources and limestone) not sharing a common superscript are different at P< .10

TABLE 3. Effect of age on weight gain, intake of nutrients, feed efficiency, fecal score, fecal pH, rectal temperature, apparent nutrient digestibilities, apparent nitrogen retention and plasma urea nitrogen of young calves fed milk replacers as the only source of nutrients

Variables	Weeks						SEM
	1	2	3	4	5	6	
Weight gain (g/an/day)	-99^{d}	-39^{d}	206^{c}	446^{ab}	612^{a}	344^{bc}	44
DM intake (g/an/day)	403^{d}	445^{cd}	497^{c}	593^{b}	702^{a}	713^{a}	17
CP intake (g/an/day)	77^{d}	85^{d}	96^{c}	113^{b}	134^{a}	136^{a}	2
Weight gain/DM intake	$-.22^{c}$	$-.16^{c}$	$.45^{b}$	$.75^{ab}$	$.87^{a}$	$.49^{b}$	.10
Fecal scores	2.3^{a}	2.1^{a}	2.2^{a}	1.9^{a}	1.8^{a}	2.1^{a}	.2
Rectal temperature (^{o}C)	38.9^{a}	38.7^{a}	38.9^{a}	39.0^{a}	39.0^{a}	38.9^{a}	.1
Apparent DM digestibility (%)	----	----	87.59^{a}	-----	-----	85.09^{b}	.84
Apparent OM digestibility (%)	-----	-----	86.82^{a}	-----	-----	86.92^{a}	.92
Apparent CP digestibility (%)	-----	-----	80.03^{a}	-----	-----	82.02^{a}	1.55
Apparent CP balance(g/day)	-----	-----	42.59^{b}	-----	-----	82.34^{a}	3.91
Apparent CP balance (% of ingested)	-----	-----	40.83^{b}	-----	-----	54.32^{a}	1.90
Fecal pH	-----	-----	6.79^{b}	-----	-----	7.06^{a}	.07
Plasma urea N(mg/100 ml)	-----	-----	7.79^{a}	-----	-----	3.90^{b}	.85

^{abcd}Means on the same line not sharing the same superscript are different at $P<.10$ using Tukey's test.

TABLE 4. Average plasma xylose concentrations for treatment combination means of protein sources and limestone with calve's age[a]

Treatments	Calve's age	
	3 weeks	6 weeks
Milk protein only	115.5^{b2}	158.5^{c1}
Milk protein + SPC	141.5^{c1}	138.1^{c2}
No limestone	136.4^{c1}	142.8^{c1}
Limestone	120.7^{b2}	135.8^{c1}

[a]Standard error for treatment combination means = ±5.8.

[b,c]Means in the same row not sharing a common letter superscript are different at $P < .01$.

[1,2]Means in columns (within protein sources and limestone) not sharing the same numerical superscript are different at $P < .01$.

TABLE 5. Effect of protein source (milk or milk + SPC) and limestone incorporation in milk replacers on pH, dry matter, crude protein and organic matter in contents from different sections of the gastrointestinal tract of 6 week old calves.

Contents from	Protein source		Limestone		Mean	SEM
	Milk[k]	Milk+SPC[k]	Absent[k]	Present[k]		
			pH			
R.R.O.[a]	6.31	6.42	6.40	6.33	6.36[i]	.19
Abomasum	3.02	3.19	3.15	3.05	3.10[j]	.19
1st 3rd S.I.[b]	6.49	6.08	6.54	6.03	6.28[i]	.19
2nd 3rd S.I.[c]	6.86	6.59	6.87	6.58	6.72[hi]	.19
3rd 3rd S.I.[d]	7.44	7.24[f]	7.25	7.43	7.29[h]	.19
Large intestine[e]	6.68	5.92[f]	6.24	6.36	6.30[i]	.19
Mean	6.11	5.89[f]	6.07	5.95[g]		.04
Feces	7.35	6.50[f]	6.94	6.91	6.93	.20
			Dry Matter (g)			
R.R.O.[a]	230	263	187	307[g]	247[h]	16
Abomasum	72	46	48	70	59[i]	16
1st 3rd S.I.[b]	14	11	15	10	13[i]	16
2nd 3rd S.I.[c]	35	32	26	41	33[i]	16
3rd 3rd S.I.[d]	34	26	20	40	30[i]	16
Large intestine[e]	54	60	49	65	57[i]	16
Mean	73	73	57	89[g]		8
			Organic Matter (g)			
R.R.O.[a]	203	235	165	273[g]	219[h]	16
Abomasum	68	41	44	65	54[i]	16
1st 3rd S.I.[b]	13	10	13	10	11[i]	16
2nd 3rd S.I.[c]	30	28	23	35	29[i]	16
3rd 3rd S.I.[d]	28	22	17	33	25[i]	16
Large intestine[e]	46	49	40	55	47[i]	16
Mean	65	64	50	78[g]		7
			Crude Protein (g)			
R.R.O.[a]	23	24	18	29[g]	23[h]	2
Abomasum	12	12	10	15	12[i]	2
1st 3rd S.I.[b]	7	8	7	7	7[i]	2
2nd 3rd S.I.[c]	13	16	14	15	14[i]	2
3rd 3rd S.I.[d]	11	8	8	11	9[i]	2
Large intestine[e]	9	12	8	12	10[i]	2
Mean	12	13	11	15[g]		.1

[a]Contents from the rumen-reticulo-omasum section altogether.
[b]Contents from the first third of the small intestine (close to the pylorus)
[c]Contents from the second third of the small intestine.
[d]Contents from the third third of the small intestine(close to the cecum).
[e]Contents from the large intestine.
[f]Different from milk at $P<.05$.
[g]Different from absence of limestone at $P<.05$.
[hij]Means in columns within each variable measured not sharing the same superscript are different at $P<.05$.
[k]Standard error for treatment means in each of the different sites of the gastrointestinal tract were respectively .26, 23, 22 and 2.4 for pH, dry matter, organic matter and crude protein.

TABLE 6. Effect of limestone addition on overall gut pH of calves fed milk replacers containing only milk protein or half of dietary protein from SPC

Limestone	Protein source	
	Milk	Milk + SPC
Absent	6.28[a]	5.86[a]
Present	5.98[b]	5.91[a]

[ab]Means in the same column with unlike superscripts are different at P<.05.

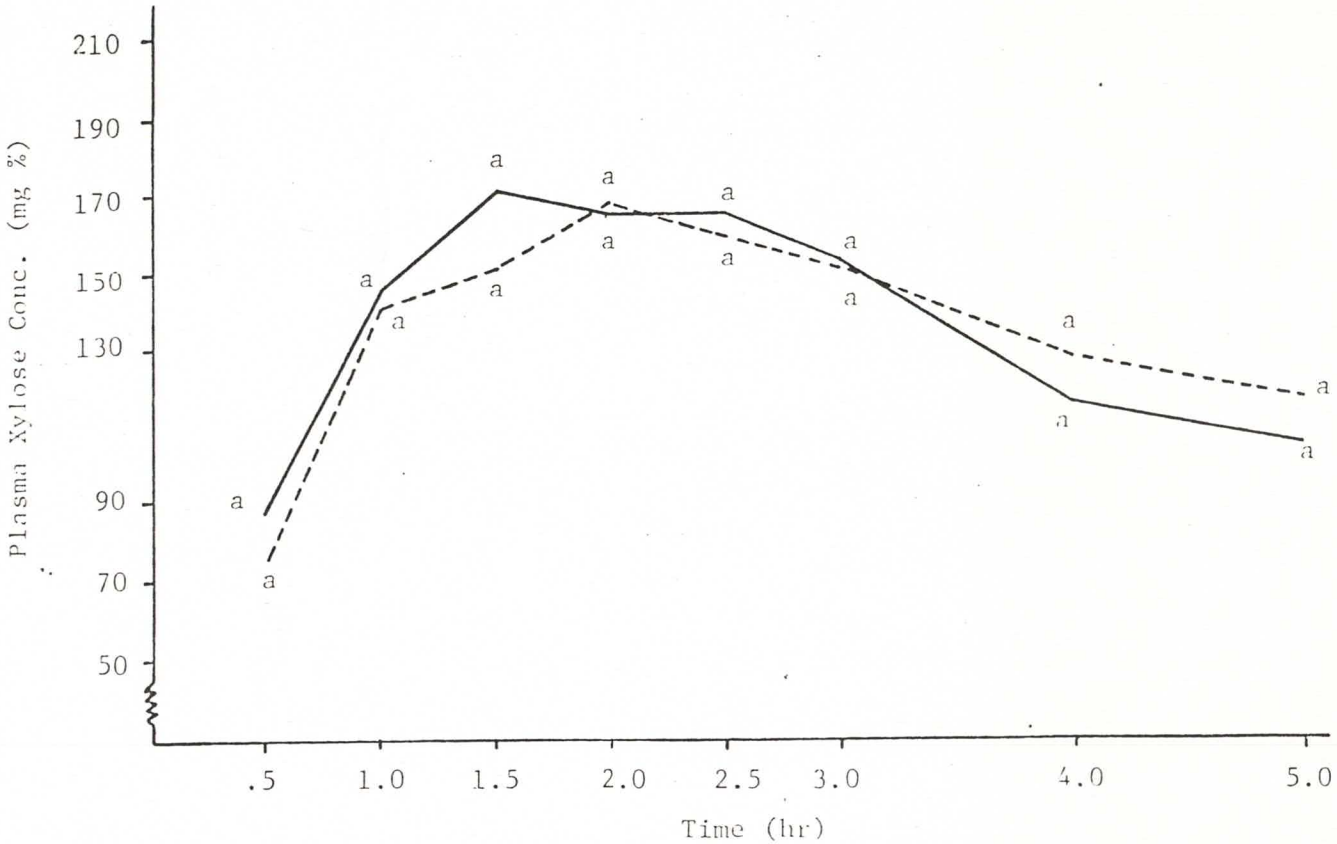

FIGURE 2. Mean xylose concentration in plasma of calves fed milk replacers with (---) or without (——) limestone. For each hour, points not showing the same letter are different ~~($P<.05$) using Duncan's test~~ (SEM = ± 11.58).

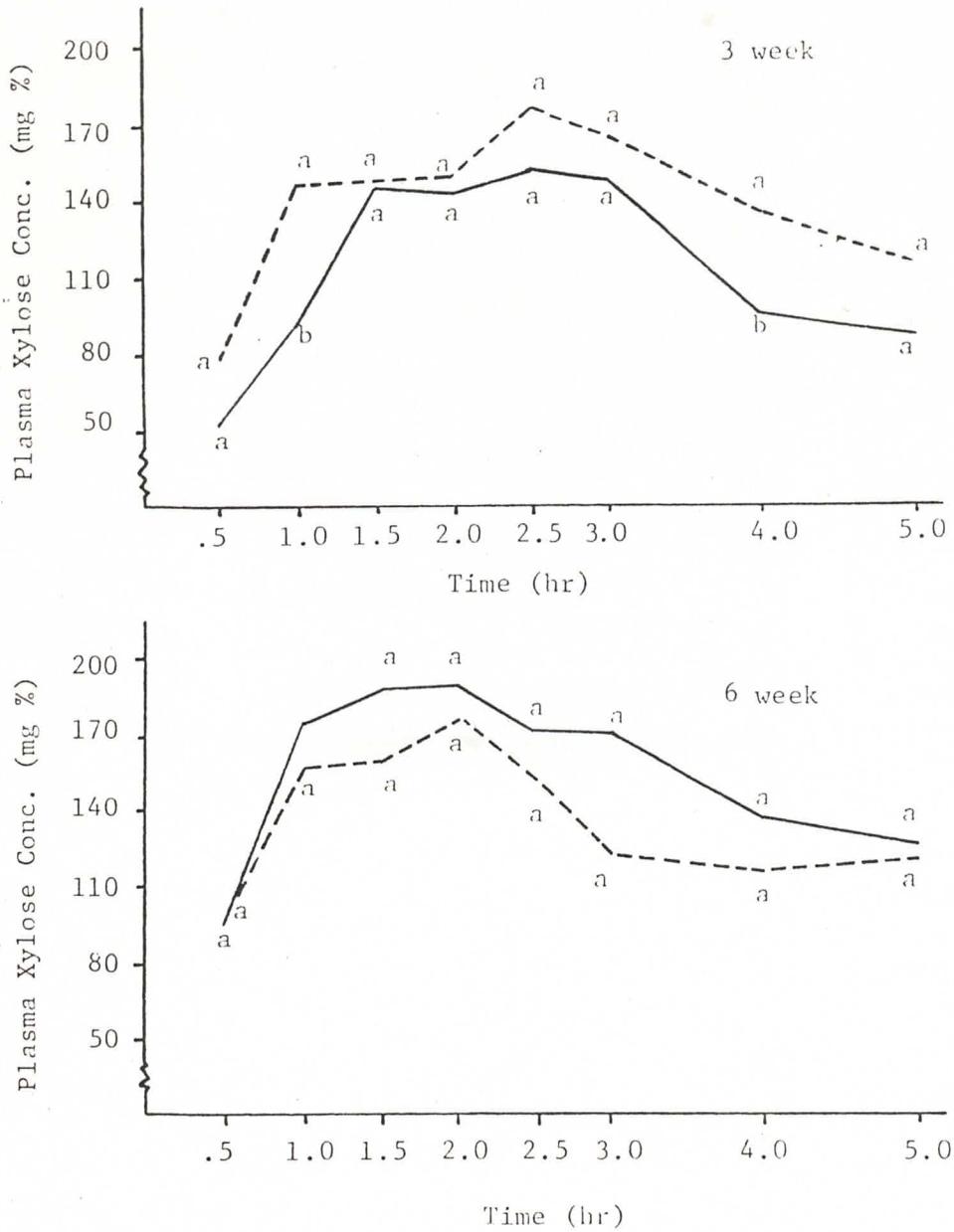

FIGURE 3. Mean xylose concentration in plasma of 3 and 6 weeks of age calves fed milk replacers containing milk (——) or soy (---) protein. For each hour, points in each graph not sharing the same letter are different ($P<.05$) ~~using Duncan's multiple t-test~~ (SEM = +16.37).

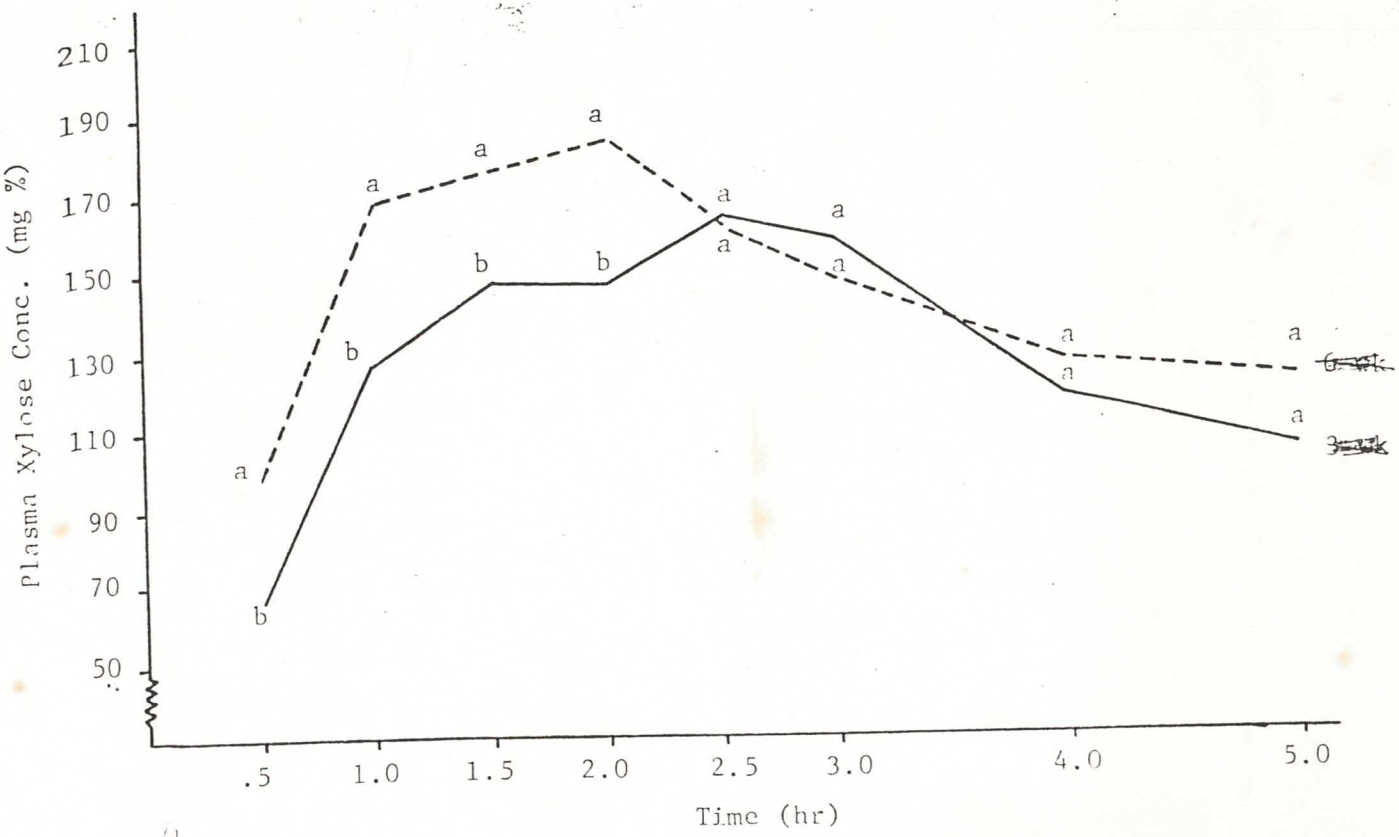

FIGURE 3. Mean xylose concentration in plasma of calves with 3 (——) or 6 (---) weeks of age. For each hour, points not showing the same letter are different [illegible] (SEM = ± 11.58).