Influence of Substituting Two Types of Soybean Protein for Milk Protein on Gain and Utilization of Milk Replacers in Calves[1]

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ABSTRACT

Twenty-four male calves (8/treatment) were fed milk replacers containing 19% crude protein from: a) 100% milk protein; b) 66% modified soybean protein plus 34% milk protein; c) 66% heated soybean flour plus 34% milk protein as the only nutrients at 8, 9, 10, 11, 12, 12, 12, and 12% body weight from 1 to 8 wk of age, respectively. Milk protein resulted in faster gains and improved feed efficiencies. Organic matter and crude protein digestibilities for milk replacers containing all milk protein, modified soybean protein, and heated soybean flour at 5 wk of age were 90.8, 87.2, and 85.3%; 82.6, 72.1, and 64.1%, respectively.

Xylose absorption performed on d 50 by oral dosing of .5 g D-xylose/kg weight, and xylose disappearance performed on d 44 by intravenous injection of .25 g D-xylose/kg weight showed 16% greater xylose absorption for milk than modified soybean protein or heated soybean flour. Calves fed soybean protein cleared xylose faster from the blood.

Two calves per treatment were sacrificed for electron microscopic evaluation of intestinal tissue. Greater morphological variation in size and shape of villi was observed within animals than among treatments. These data suggest that the superior performance of calves fed milk compared with soy protein is related to a greater digestibility and absorptive capacity of digested nutrients.

INTRODUCTION

Substitution of soybean protein for milk protein in calf milk replacers should reduce the purchase price of the replacers and the subsequent cost of rearing calves. However, reduced body weight gains and decreased dry matter (DM) and crude protein (CP) digestibilities have been reported with increased soybean protein in milk replacers for young calves (1, 3, 22, 23). Negative results have been related to the presence of soybean trypsin inhibitor (SBTI) (10), residual carbohydrates such as arabinogalactans, acidic polyssacharides and arabinans (16), and more recently soybean antigenic globulins (17, 30). A decreased ability to absorb nutrients in the small intestine as determined by xylose absorption tests was associated with ingestion of milk replacers in which soy protein furnished a major portion of the protein (28). Moreover, severely damaged intestinal villi were shown in calves fed soy protein concentrate for an extended period (29).

Objectives of this experiment were to test performance, digestibility, and absorption in baby calves of two types of soybean protein compared with milk protein and to observe the effects of the dietary protein sources on morphology of intestinal villi.

MATERIALS AND METHODS

Twenty-four male Holstein calves purchased from a commercial dairy farm were transported to the Michigan State University Dairy Cattle Research and Teaching Center. Upon arrival,

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each calf received an injectable solution containing 500,000 IU of vitamin A, 75,000 IU of vitamin D, 50 mg of vitamin E, and 50 mg selenium. From 4 to 52 d of age each calf was fed its designated milk replacer containing 19% crude protein and diluted with water to 14% solids as the only source of nutrients at 8, 9, 10, 11, 12, 12, 12, and 12% body weight from 1 to 8 wk, respectively. Replacers were mixed with warm water immediately before feeding twice daily (12-h intervals) from open pails. Fresh, clean water was available at all times. Except for the digestibility period, animals were kept in tie stalls bedded with straw.

Protein sources for milk replacers especially prepared for this study were 100% milk protein (MP); 66% modified soybean protein plus 34% milk protein (MS); and 66% heated soybean flour plus 34% milk protein (HS).

Modified soybean protein was prepared by mixing defatted soybean flour with a solution of 84% ethanol and 16% water, placing in a closed reactor, and heating at 121°C for 30 min under pressure of 1.055 kg/cm^2. The flour was then sprayed into a drying chamber for evaporation of the alcohol and water. Ingredient and chemical composition of the ration are shown in Table 1.

To prepare the heated soybean flour, commerical soybean meal was gound and passed through a desolventizer, toaster apparatus to remove residual solvents and inactivate any anti-metabolites (such as SBTI) or urease still present. The protein dispersible index of the final product was 25 to 30.

On d 29 of treatment, calves were moved to metabolism cages to collect feces and urine for 5 d as described by Campos et al. (4). Food

TABLE 1. Ingredient and chemical composition of milk replacers[1] containing milk or soybean protein.

Item	Treatment[2]		
	MP	MS	HS
	(%)		
Ingredients			
Nonhydroscopic edible whey	65.58	55.38	55.74
Fat-milk concentrate[3]	23.03	22.22	22.45
Casein[4]	10.14	0	0
Modified soybean protein[4]	0	21.15	0
Heated soybean protein[4]	0	0	20.56
Vitamin-mineral premix[5]	1.25	1.25	1.25
Chemical analysis[6]			
Dry matter	96.92	96.66	96.79
	(% of DM)		
Crude protein	20.22	19.81	19.25
Ether extract	8.67	8.88	8.49
Nitrogen-free extract	63.43	63.58	64.53
Ash	7.68	7.73	7.73

[1] All milk replacers contained 250 g/ton neomycin base and 100 g/ton oxytetracycline.

[2] MP = Milk protein, MS = 66% modified soybean protein plus 34% milk protein, HS = 66% heated soybean flour plus 34% milk protein.

[3] 40% fat as homogenized white grease which was spray dried with 60% whey and contained 7% protein.

[4] Casein—90% protein; modified soybean protein—50% protein; heated soybean flour—50% protein.

[5] 44,000 USP units vitamin A/kg; 11,000 USP units vitamin D_3/kg; 44 USP units vitamin E/kg; 6.6 mg thiamine/kg; 6.6 mg riboflavin/kg; 2.6 mg niacin/kg; 13.0 mg D-pantothenic acid/kg; .11 mg biotin/kg; 110 mg ascorbic acid/kg; 6.6 mg pyridoxine hydrochloride/kg; .55 mg folic acid/kg; .07 mg vitamin B_{12}/kg; 2,650 mg choline chloride/kg; iron 100 ppm; copper 10 ppm; cobalt .1 ppm; zinc 40 ppm; manganese 40 ppm; iodine .25 ppm; and selenium .1 ppm.

[6] Association of Official Analytical Chemists (2).

intake was recorded daily. Calves were weighed on the first and last day of experiment and at weekly intervals. Fecal consistency was rated daily from 1 to 4 (18). Rectal temperatures were measured daily just before the morning feeding except during collection periods.

A xylose disappearance test was performed on d 41 to evaluate xylose clearance from the blood. Calves were fasted for 24 h and placed in metabolism cages before intravenous injection of 250 mg xylose/kg body weight in 20% aqueous solution. Jugular blood was sampled just before and 30, 60, 90, 120, 150, 180, 240, and 300 min after injection of xylose. Urine also was collected during blood sampling. Also, a xylose absorption test was performed on d 50 as described by Seegraber and Morrill (28).

After the test period, two calves on each treatment were sacrificed 5 h after feeding. Weights and pH were determined for rumen, abomasal, small intestine (divided in three equal length sections), cecal contents, and large intestinal contents. Samples of contents were kept at −20°C until analyzed for DM, CP, and ash as described by Association of Official Analytical Chemists (2).

Two segments 2.5 cm in diameter were removed from each section of the small intestine just after the animal was sacrificed and placed in an isotonic solution of sodium chloride of pH 6.0. Samples were fixed for 2 h in 4% glutaraldehyde buffered at pH 7.2 with .1 *M* sodium cacodylate buffer, rinsed twice for 15 min in the buffer alone, dehydrated in grade ethanol, and dried at the critical point in a Balyers Critical Point Drier using carbon dioxide as the transitional gas. Tissues were then mounted on stubs, coated with gold, and examined in a Jeol JSM-35C scanning electron microscope. Fields were chosen at random as representative of the tissue photographed, and villi morphology and integrity were evaluated by five evaluators and classified on a 1 to 5 scale with 1, normal; 2, normal but shorter; 3, slightly abnormal; 4, quite abnormal; and 5, abnormal or with an absence of villi.

Analysis of variance was as described by Gill (7). Data were analyzed as a completely randomized or a split-plot design.

Traits evaluated from the first model were body weight and gains, intake, nutrient digestibilities, nitrogen (N) retention, and xylose concentrations. The model included treatments (T), time of arrival [block, (B)], T × B (error term for T and B effects), age or period, and the two-way interaction. The subjective evaluations of villi were analyzed with a similar model that included T, evaluators (B), T × B, animal (A) within T (whole plot error), B × A/T, sample (S), and T × S.

RESULTS AND DISCUSSION

Initial body weights were not different among treatments, but final weights (Table 2) were higher for calves fed MP than MS or HS. Results were similar for body weight gains, gain as a percent of initial body weight (Table 2), and average daily gains (Table 3). Interaction of treatment by age was significant for body weight, total gain, and gain as a percent of initial body weight, suggesting that differences among treatments in weight gains became greater with increased age.

Calves on all treatments lost weight during the 1st wk. Average daily gains were diminished during the 5th wk due to stress on calves while they were kept in metabolic cages for collection of feces and urine.

Higher feed intake for MP than MS or HS was expected because calves were fed as a percent of body weight and the MS and HS groups did not grow as fast as MP. Feed efficiency (body weight gain/feed intake) was higher for MP than MS or HS and the interaction of treatment by age was significant with greater differences among MP and the soybean sources as age increased. These results show less utilization of soybean than milk protein and agree with previous data (4, 13, 14, 27).

At 5 wk, MP resulted in higher ($P<.05$) apparent digestibilities of DM, organic matter (OM), CP, and ether extract (EE) than HS; MS was intermediate (Table 4). Treatments did not differ for digestibility of N-free extract (NFE), but N retention tended to be greater for milk than soybean proteins (Table 4).

The significant increase ($P<.05$) in CP digestion by calves fed MS compared with HS indicates that processing improved soy flour as a protein source in milk replacers. However, it never reached values obtained for MP with digestibility of CP 22% lower for HS and 13% lower for MS than for MP. Others reported poor digestion of soybean proteins by the young calf during early life (11, 21, 24) and

TABLE 2. Influence of feeding milk protein (MP), modified soybean protein (MS), or heated soybean flour (HS) on body weight (BW), total weight gain (TG), and gain as a percent of initial body weight (% BW) to calves from 0 to 7 wk of age.

Age	BW[1]			TG[1]			% BW[1]		
	MP	MS	HS	MP	MS	HS	MP	MS	HS
(d)			(kg)						
0	43.26	42.35	41.13	...	...	...	...	...	...
8	42.98	42.48	40.76	−.29	−.13	.54	−.64	.21	−1.05
15	42.24	39.98	39.00	−1.03	−2.38	−1.95	−2.43	−5.99	−5.16
22	43.61	41.09	40.05	.35	−1.26	−1.31	.86	−3.24	−2.58
29	46.74	43.55	42.20	3.48	1.20	1.41	8.07	2.70	2.69
36	49.43	45.25	43.61	6.16^{g}	3.48^{h}	2.95^{h}	14.15^{g}	6.74^{h}	6.29^{h}
43	52.01	48.28	46.26	8.75^{g}	5.93^{h}	5.38^{h}	20.28^{g}	14.09^{h}	12.75^{h}
50	56.83	51.45	49.26	13.56^{c}	9.10^{d}	8.49^{d}	31.36^{c}	21.37^{d}	20.08^{d}
52	59.11^{c}	54.14^{d}	50.99^{d}	15.85^{c}	11.79^{d}	10.38^{d}	36.51^{c}	27.35^{d}	24.16^{d}
Means	48.47^{e}	45.39^{f}	43.70^{f}	5.85^{a}	3.50^{b}	3.10^{b}	13.52^{a}	7.91^{b}	7.15^{b}
SE	2.01	2.01	2.01	.78	.78	.78	1.74	1.74	1.74

$^{a,b;c,d;e,f;g,h}$Means in the same row with different superscripts are different ($P<.005$), ($P<.05$), ($P<.10$), ($P<.15$).

[1] Orthogonal contrasts for comparing means were MP vs. MS + HS, MS vs. HS.

TABLE 3. Influence of feeding milk protein (MP), modified soybean protein (MS), or heated soybean flour (HS) on average daily gain (ADG), feed intake (FI), and feed efficiency (G/I) of calves from 1 to 7 wk of age.

Ages	ADG[1]			FI[1]			G/I[1]		
	MP	MS	HS	MP	MS	HS	MP	MS	HS
(d)		(g)			(kg)				
8–14	−106[d]	−357[e]	−252[e]	3.24	2.86	3.22	−.259[a]	−.912[c]	−.574[b]
15–21	197	159	150	4.01	3.56	3.68	.357	.317	.289
22–28	447[h]	352[i]	307[i]	4.54[j]	3.91[k]	4.06[k]	.689[j]	.616[k]	.540[k]
29–35	384[f]	243[g]	202[g]	5.29[j]	4.81[k]	4.68[k]	.509[h]	.364[i]	.339[i]
36–42	370	432	379	5.07[j]	4.60[k]	4.41[k]	.514[k]	.677[j]	.598[k]
43–49	687[a]	453[b]	429[b]	5.55[j]	5.12[k]	4.92[k]	.869[d]	.602[e]	.615[e]
Means	330[a]	214[b]	202[b]	4.62[f]	4.15[g]	4.16[g]	.446[a]	.277[b]	.301[b]
SE	21	21	21	.17	.17	.17	.032	.032	.032

[a,b,c;d,e;f,g;h,i;j,k]Means in the same row with different superscripts are different ($P<.005$), ($P<.01$), ($P<.025$), ($P<.10$), ($P<.15$).

[1] Orthogonal contrasts for comparing means were MP vs. MS + HS, MS vs. HS.

attributed it to several causes. These were lack of coagulum in the abomasum (15, 26), reduction of pancreatic trypsin and chymotrypsin (10, 33), presence in soybean products of a SBTI (5, 8), and allergic reaction in the gastrointestinal tract (30, 32). Digestibility of soybean protein was improved by acid (5), alkali (9), and alcohol (6, 31) treatments. Heating also improved utilization of soybean products (22), but inclusion of pepsin or pancreatin in milk replacers containing soybean protein did not improve protein digestibility or calf performance (15).

Absorption and excretion tests of xylose have been used to measure intestinal absorption in calves (3, 28) as well as other species. In this study plasma xylose was higher for calves fed MP than the soybean proteins. Treatment differences were greatest at 120 to 180 min after oral xylose (Figure 1). Plasma xylose concentrations of this experiment were higher than those reported for calves of a similar age by Seegraber and Morrill (28) but lower than reported by Campos and Huber (3).

Peak concentrations of plasma xylose occurred 150 min after the oral dose for calves fed MP (Figure 1), but calves fed soybean proteins showed no distinguishable peak. The xylose tolerance curve for calves fed MP suggests an absorptive capacity of the intestine superior to that of calves fed soybean proteins and corroborates the faster weight gains and higher nutrient digestibilities observed for MP calves (Tables 2 to 4).

Cumulative oral xylose (O-XYL) excretion determined from the 5 h of urine collection taken after O-XYL feeding, expressed as percent of xylose intake, agrees with (28) for calves fed milk protein, but is about twice that for calves fed soybean protein in (28) (Table 5). The differences among treatments were not statistically significant, but the tendency for the higher xylose excretion for calves fed soybean protein in our study was associated with greater xylose disappearance from blood of these calves after intravenous injection of xylose (IV-XYL) (Figure 2).

Plasma xylose concentrations after calves received IV-XYL showed heterogeneous variance, but homogeneity was obtained by transformation to the log scale. Linear regressions of log IV-XYL concentration on time are in Figure 2. When variation among treatments was ana-

TABLE 4. Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), nitrogen-free extract (NFE), and ash digestibilities and nitrogen (N) retention in 5-wk-old calves fed milk protein (MP), modified soybean protein (MS), or heated soybean flour (HS).

Variable	Treatment[1]					
	MP		MS		HS	
No. calves	8		6		6	
	Digestibility (%)					
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
DM	89.56	1.03[a]	86.20	1.19[ab]	84.38	1.19[b]
OM	90.72	.99[a]	87.16	1.14[b]	85.30	1.14[b]
CP	82.53	1.85[a]	72.07	2.14[b]	64.09	2.14[c]
EE	94.06	.70[a]	92.19	.81[ab]	91.38	.81[b]
NFE	92.87	1.07	91.15	1.23	90.83	1.23
Ash	75.71	1.81	74.56	2.09	73.36	2.09
	Retention (g/d)					
N	58.08	6.79[d]	39.46	7.84[de]	29.47	7.84[f]

[a,b,c;d,e,f]Means in the same row with different superscripts are different ($P<.05$), ($P<.10$).

[1] Means were compared using the Bonferroni t test.

lyzed by the partitioned interaction method, IV-XYL clearance was higher for HS than MS or MP and was higher for MS than MP. These data suggest greater capacity for clearance of xylose from the blood of calves fed soybean protein than those fed milk protein with fastest clearance on the HS diet.

The faster IV-XYL clearance from the blood of calves fed soybean protein than those fed milk protein (Figure 2) might be explained by an increase in xylose metabolism or an increase of renal excretion of xylose. The tendency for a higher percentage of IV-XYL in urine of calves fed HS diets (Table 5) suggests an increase in renal excretion. The reason for such an effect is not clear.

In other species, Hill et al. (11, 12) suggested that D-xylose absorption reflects structural integrity of the small intestine. In this experiment, examination of two intestinal segments from each section was accomplished through use of a scanning electron microscope equipped

TABLE 5. Percentage of oral or intravenous xylose (XYL) excreted into the urine by calves fed milk protein (MP), modified soybean protein (MS), or heated soybean flour (HS) during the 5-h period subsequent to XYL administration.

Variable	Treatment[1,2]		
	MP	MS	HS
No. of calves	7	8	7
Oral XYL, %	12.74 ± 1.99	9.47 ± 1.86	10.34 ± 1.99
No. of calves	7	7	6
IV-XYL, %	21.86 ± 3.24	20.90 ± 3.24	28.02 ± 3.50

[1] Means in the same row are not significantly different ($P>.20$).

[2] Means were compared using Tukey's test.

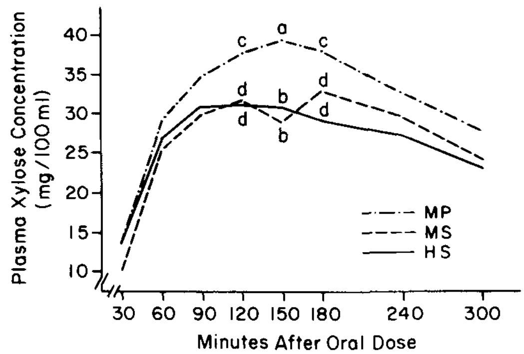

Figure 1. Effect of feeding milk protein (MP), modified soybean protein (MS), and heated soybean flour (HS) on calf plasma xylose concentrations at different times after oral ingestion of .5 g/kg body weight. For each time point different letters are different: a,b ($P<.05$); c,d ($P<.10$).

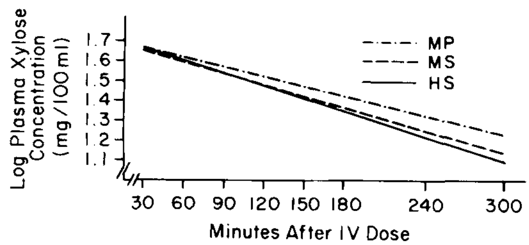

Figure 2. Effect of feeding milk protein (MP), modified soybean protein (MS), and heated soybean flour (HS) on calf plasma xylose concentration at different times after IV dose. Data presented as linear regression of the log of plasma xylose concentration on time.

with a Polaroid camera. Normal villi were similar to those described by Mebus et al. (19, 20) for the gnobiotic calf, and abnormal villi were similar to those described by Seegraber and Morrill (29) for a calf fed soybean protein concentrate or by Mebus et al. (20) for calves infected by virus of human infantile gastroenteritis. In this study no differences among treatments or evaluators were noted in villi morphology (Table 6); however, differences were significant for segments taken from different positions of the small intestine with morphology of distal segments more normal than proximal segments. Morphology of the first intestinal segment taken just proximal to the pylorus was less normal than all others for treatments MP and HS; whereas the first and second segments were different from third, fourth, and sixth segments on treatment MS. These results do not agree with those of Seegraber and Morrill (29) who associated impaired absorptive ability of the intestine of calves fed the soybean protein diets with morphological changes in intestinal structure. In this experiment calves fed soybean protein showed lower weight gains, feed efficiencies, nutrient digestibilities, and xylose absorption than calves fed milk protein, but differences in villi morphology were greater within animals than among treatments.

The pH of small intestinal contents increased from proximal to distal for calves fed MP and MS, but decreased for calves fed HS, was higher

TABLE 6. Influence of feeding milk protein (MP), modified soybean protein (MS), or heated soybean flour (HS) to calves on villi morphology.[1]

Treatment	Position of segment in small intestine[2] 1	2	3	4	5	6	Treatment $\bar{X}$	SE
MP	4.7^a	2.9^b	2.5^b	2.4^b	2.3^b	2.6^b	2.90	.27
MS	3.7^a	3.8^a	2.0^b	2.2^b	2.9^{ab}	1.9^b	2.75	.27
HS	4.4^a	1.9^b	2.9^b	1.7^b	2.4^b	2.1^b	2.57	.27

[1] Evaluated by five evaluators on a 1 (normal) to 5 scale from segments taken at six different positions (from proximal to caudal) in the small intestine.

[2] Means were compared using Tukey's test.

[a,b] Means in the same row with different superscripts are different ($P<.01$).

TABLE 7. Influence of feeding milk protein (MP), modified soy protein (MS), or heated soybean flour (HS) on pH and crude protein (%) of abomasal and intestinal tract contents.[1]

Item	Diet		
	MP	MS	HS
pH[2]			
Abomasum	2.95	3.70	2.80
Small intestine–1	6.18	5.96	5.80
Small intestine–2	6.93[d]	6.38[e]	5.80[f]
Small intestine–3	7.13[a]	6.18[b]	5.35[c]
Large intestine and cecum	5.23[d]	5.35[d]	4.75[e]
Crude protein (% of DM)[2]			
Abomasum	18.75	20.49	20.08
Small intestine–1	47.48[d]	38.74[e]	44.99[f]
Small intestine–2	45.41	47.92	42.35
Small intestine–3	31.54	33.21	28.54
Large intestine and cecum	18.61[a]	25.26[b]	26.63[b]

[1] From two calves per treatment. Small intestines were divided into three equal-length sections from distal to caudal. The Bonferroni *t* test was used to compare means.

[2] Standard errors of mean for pH, .25; for crude protein, 3.68.

[a,b,c;d,e,f] Means in the same row with different superscripts are different ($P<.10$), ($P<.15$).

for calves fed MP than either soybean protein, and was higher for MS than HS (Table 7). Fermentation of undegraded or partially degraded carbohydrates from soybean protein diets explains the increased acidity of small intestine (25), large intestine, and cecal contents (13, 25).

Dry matter averaged about 8.5% for the abomasal and small intestinal tract contents but increased to 10.8% for the large intestine and cecum with no difference among treatments. Crude protein of lower tract contents was higher for calves fed soybean than milk protein, which reflects the lower digestibility of soybean than milk proteins and agrees with (3).

Fecal scores were not different among treatments, averaging about 2.2 for the experiment. These data contrast with some previous reports where soybean and other nonmilk proteins resulted in more diarrhea (4, 10, 28) but not with others (3, 6). Stress on calves in metabolism stalls during the period of fecal collection may have masked treatment effects. A lower DM percent of intestinal contents for calves on the two soybean vs. milk diets (10.2 vs. 12.2%) suggest more fluid feces on soybean replacer, but differences were again nonsignificant. There was no difference among treatments in rectal temperatures; all were within normal range. No calf mortality was observed in this study that could be related to treatments.

In summary, lower digestibilities of DM, OM, CP, and EE were associated with inferior body weight gains, poorer feed efficiencies, and lower xylose absorption for calves fed milk replacers containing 66% of their protein from soybean sources than 100% from milk. Modified soybean protein tended to be superior to HS. No differences were observed among any treatments for villi morphology, fecal scores, or rectal temperatures. Greater xylose clearance from the blood during 5 h was shown for calves fed soybean than milk protein, suggesting that soybean diets increased xylose metabolism and excretion more than milk. Gastrointestinal tract contents showed a tendency to be more acid in the medium and distal portions of the small intestine, large intestine, and cecum of calves fed soybean protein. More CP was in the large intestine and cecum of calves fed the soybean protein, resulting in increased fermentation of undegraded or partially degraded nutrients on these diets.

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