Supplementation of 1% L-Glutamine to Milk Replacer Does Not Overcome the Growth Depression in Calves Caused by Soy Protein Concentrate¹

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ABSTRACT

Glutamine, an important fuel and biosynthetic precursor in intestinal epithelial cells, helps maintain intestinal integrity and function when supplemented to the diet of many species. The hypothesis tested here was that glutamine supplementation would overcome the decreased average daily gain (ADG) and altered intestinal morphology caused by milk replacer containing soy protein concentrate (SPC). Holstein calves (9 male and 1 freemartin female per treatment) were assigned to diets of 1) all-milk-protein (from whey proteins) milk replacer, 2) milk replacer with 60% milk protein replacement from SPC, and 3) SPC milk replacer as in diet 2 plus 1% (dry basis) L-glutamine. Milk replacers were reconstituted to 12.5% solids and were fed at 10% of body weight from d 3 to 10 of age, and at 12% of body weight (adjusted weekly) from d 10 through 4 wk of age. No dry feed (starter) was fed, but water was freely available. Glutamine was added at each feeding to reconstituted milk replacer. Five calves from each treatment were slaughtered at the end of wk 4 for measurements of intestinal morphology. The ADG was greater for calves fed the all-milk control than for those fed SPC; glutamine did not improve ADG (0.344, 0.281, and 0.282 kg/d for diets 1 to 3, respectively). Intake of protein was adequate for all groups and did not explain the lower growth for calves fed SPC. Villus height and crypt depth did not differ among treatments in the duodenum. In the jejunum, villus height (713, 506, and 464 μm , for diets 1 to 3, respectively) and crypt depth (300, 209, and 229 μm , respectively) were greater for calves fed all milk protein than for either SPC group. In the ileum, villus height was greater for calves fed all milk

than for either soy group (532, 458, and 456 μm), whereas crypt depth tended to be greater (352, 301, and 383 μm for diets 1 to 3, respectively), and the villus to crypt ratio was lower for calves supplemented with glutamine than for those fed SPC alone. Urea N concentration in plasma was greater for calves supplemented with glutamine than for those fed SPC alone, indicating that glutamine was at least partially catabolized. Supplemental L-glutamine did not improve growth or intestinal morphology of calves fed milk replacer containing SPC.

Key words: calf, soy protein, intestinal morphology, glutamine

INTRODUCTION

Milk proteins, principally whey proteins, remain the “gold standard” for use in milk replacers for young calves (Davis and Drackley, 1998). Extensive research has been conducted to identify alternative proteins that would provide performance and health equivalent to milk proteins but at lower cost. Soy protein sources such as soy protein concentrate (SPC) and soy protein isolate have been studied extensively (Ramsay and Willard, 1975; Dawson et al., 1988; Lallès et al., 1995). Although soy proteins are widely used in alternate-protein milk replacer formulas in which soy replaces 50% or less of the milk protein (Davis and Drackley, 1998), calf growth and feed efficiency usually are less than with all-milk-protein formulas (Lallès, 1993, 2000).

Responses to SPC depend on the age of the calves, with adverse effects more prevalent in calves less than 3 wk of age than in older calves (Davis and Drackley, 1998). When SPC replaced approximately 50% of milk protein in milk replacer, average daily gain (ADG) and feed conversion decreased by 32.5 and 33.3%, respectively, when fed during d 1 to 14 of age but decreased by only by 7.1 and 5.9%, respectively, over the entire 42-d feeding period, indicating that calves greater than 2 wk of age tolerated SPC well (Tomkins et al., 1994). Reasons for the negative impact of SPC on calves are

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not entirely clear, although intestinal abnormalities are common (Lallès, 2000).

Glutamine (Gln) is a major fuel for intestinal enterocytes in mammals (Reeds and Burrin, 2001), including ruminants such as cattle (Okine et al., 1995) and sheep (Beaulieu et al., 2001), and is involved in maintenance of intestinal mucosal integrity (Potsic et al., 2002). The unique roles of Gln have stimulated interest in its potential ability to modulate intestinal injury, promote healing, or lessen the impact of diarrhea (Reeds and Burrin, 2001). In pigs, addition of 1% Gln to a soy protein-based weaning diet prevented the decrease of jejunal villus height observed in unsupplemented controls, and improved gain:feed in newly weaned pigs (Wu et al., 1996). Although soy proteins are higher in Gln content than whey proteins (Baxter et al., 2004), it is possible that absolute supply of Gln might be limiting for optimal intestinal function in the presence of other negative aspects of SPC in very young calves. Alternately, provision of additional Gln might prevent some of the negative aspects of SPC on intestinal structure and function in young calves. Consequently, our hypothesis was that supplementation of free L-Gln to a milk replacer in which SPC replaced a portion of whey proteins would prevent the growth depression and intestinal alterations expected from SPC intake in young calves.

MATERIALS AND METHODS

All procedures involving animals were approved by the University of Illinois Laboratory Animal Care Advisory Committee. Thirty Holstein calves born between June and December 1998 were allocated randomly to 1 of 3 treatments, resulting in 9 male calves and 1 freemartin female assigned to each treatment. Calves received their dam's colostrum and transition milk for the first 2 d of life and were housed in hutches bedded with straw. Treatments, initiated on d 3 of age, were: 1) an all-milk-protein milk replacer that served as positive control, 2) a milk replacer in which SPC replaced 60% of the whey protein, which served as negative control, and 3) the same milk replacer as treatment 2 but supplemented with 1% L-Gln (Sigma Chemical Co., St. Louis, MO). The number of calves per treatment was selected by use of power tests to be sufficient to detect statistical differences in growth rates expected between the positive and negative control treatments. The amount of Gln supplemented was based on experiments in young pigs by Wu et al. (1996), in which graded additions of Gln at 0.6% or more of dietary DM resulted in maximal response in growth rates, and addition of 1.0% Gln restored villus height in the jejunum relative to unsupplemented pigs.

Milk replacers were formulated to contain 20% CP and 15% fat. Formulations were proprietary (Land O'Lakes Animal Milk Products, St. Paul, MN) but in general were based on whey protein concentrate, dried whey, lard, and bleachable fancy tallow, with appropriate mineral and vitamin supplementation. Contents of Met and Lys were equalized between the control and SPC-containing milk replacers by addition of crystalline L-Lys and D,L-Met. Milk replacers were reconstituted to 12.5% solids with warm tap water and fed at rates of 10% of BW from d 3 through 9, and 12% of BW from d 10 through the end of the experiment at approximately d 28. The daily amount was divided into 2 feedings at 0730 and 1500 h. The amount fed was adjusted weekly as calves grew. Glutamine was stored in a desiccator and added to reconstituted milk replacer at each feeding. Water was available at all times but no starter was fed.

Calves were weighed and measured (height at withers, body length, and heart girth) before the afternoon feeding on the same day each week. A sample of jugular blood was obtained by venipuncture before the a.m. feeding during wk 2 and wk 4. Blood was collected into evacuated tubes containing heparin. Plasma was obtained by centrifugation and stored at -20°C until analyses for concentrations of urea N (kit number 535-A, Sigma-Aldrich Chemical Co., St. Louis, MO), glucose (kit number 315-500, Sigma-Aldrich Chemical Co.), and total protein (kit number 541-2, Sigma-Aldrich Chemical Co.) as described by Blome et al. (2003).

Milk replacers were analyzed for CP content by Kjeldahl (AOAC, 1995) and gross energy by bomb calorimetry (1261 Isoperibol Calorimeter; Parr Instrument Co., Moline, IL). Total fatty acids were determined by gas chromatography (Shimadzu GC-17A, Shimadzu Scientific Instruments, Inc., Columbia, MD) of methyl esters formed by acid-catalyzed transesterification (Sukhija and Palmquist, 1988). Total fat content was estimated as total fatty acid content divided by 0.9 to correct for glycerol content (Blome et al., 2003). Metabolizable energy was calculated as $0.90 \times$ gross energy content, based on conversion of gross energy to ME measured in calves of similar age that were fed similar milk replacers in a previous study in our laboratory (Blome et al., 2003).

Five male calves from each treatment group were selected randomly for euthanasia and necropsy on the Wednesday closest to d 28 of age. Calves were humanely euthanized using barbiturate overdose. After opening the body cavity, 10-cm segments of duodenum (20 cm distal to the pylorus), jejunum (at the midpoint), and ileum (20 cm proximal to the ileocecal junction) were removed within 5 to 10 min after death, and placed in 10% buffered formalin. All organs were examined for

Table 1. Chemical analysis of control (all-milk-protein) and soy protein concentrate (SPC) milk replacer without or with supplemental glutamine (Gln)

Variable	Milk replacer		
	Control	SPC	SPC + Gln
CP, %	20.5	19.6	20.6
Fat, %	15.0	15.1	15.0
ME, Mcal/kg	4.37	4.36	4.36

gross structure, and histopathology was conducted on lung, liver, kidney, spleen, heart, and colon to rule out concurrent disease. After 48 h in formalin, intestinal sections were embedded in paraffin and stained with hematoxylin and eosin. Five individual villi and their crypts in cross-section were identified and measured in duodenum, jejunum, and ileum of each calf using standard histomorphometric methods. The pathologist was blind to treatment assignments. The 5 measurements were averaged for each intestinal segment for each calf before statistical analysis.

All data were subjected to ANOVA for a balanced randomized design using the GLM procedure of SAS (release 8.2, SAS Institute Inc., Cary, NC). The repeated measures data for blood metabolites were analyzed using the MIXED procedure of SAS (Littell et al., 1996). Calf was designated as a random term and the covariance structure was modeled with the default variance components procedure. Treatment means were separated using preplanned orthogonal contrasts of 1) control vs. SPC treatments, and 2) SPC vs. SPC with Gln. Least squares means were calculated and are presented throughout. Significance was declared when $P < 0.05$.

RESULTS

Analyzed composition of milk replacers was similar to formulated values (Table 1). Initial BW and days on experiment did not differ among treatment groups (Table 2). Differences among groups for final BW did not achieve statistical significance ($P > 0.40$), but ADG was decreased 18.2% ($P < 0.05$) when SPC replaced 60% of the whey protein in the control milk replacer (Table 2). Addition of SPC decreased ($P < 0.05$) the gain to feed ratio by 14.5%. Mean intake of supplemental Gln was 6.3 ± 0.8 g/d. Addition of Gln did not improve ADG or gain:feed over that for SPC alone (Table 2). Final measurements for withers height, body length, and heart girth were not significantly different among treatments but in general were numerically less for calves receiving either SPC treatment (Table 2).

Villus height and crypt depth in the duodenum were not different among treatments (Table 3). In the jeju-

Table 2. Growth, DMI, and efficiency for calves fed an all-milk-protein control milk replacer or milk replacer containing soy protein concentrate (SPC) without or with supplemental glutamine (Gln)

Variable	Diet			SE
	Control	SPC	SPC + Gln	
Initial BW, kg	42.4	41.1	41.6	1.9
Days on experiment	28.3	29.1	28.8	0.7
Final BW, kg	52.1	49.3	49.9	2.4
Average daily gain, ¹ kg	0.344	0.281	0.282	0.025
DMI, kg	0.625	0.597	0.608	0.031
Gain:feed ¹	0.55	0.47	0.47	0.03
Final withers height, cm	82.4	80.8	81.4	0.9
Final body length, cm	69.2	67.6	69.4	1.0
Final heart girth, cm	86.8	84.6	84.5	1.3

¹Control vs. SPC and SPC + Gln, $P < 0.05$.

num, however, both villus height and crypt depth were decreased by the SPC-containing diets compared with the all-milk control. Addition of Gln to the SPC diet did not prevent these changes. Villus height in the ileum was lower for calves fed the SPC diets and was not affected by Gln supplementation. In contrast, crypt depth tended ($P = 0.08$) to be greater for calves fed SPC + Gln than for those fed SPC alone (Table 3). The ileal ratio of villus height to crypt depth was lower for Gln-supplemented calves than for calves fed SPC alone.

The concentration of urea N in plasma was greater for calves supplemented with Gln than for those fed SPC without Gln (Table 4). Concentrations of total protein in plasma did not differ among treatment groups. Mean glucose concentrations were lowest for calves fed SPC + Gln and highest for calves fed the control diet, but differences among treatments did not approach statistical significance ($P > 0.38$).

Table 3. Villus height, crypt depth, and ratio of villus height to crypt depth in sections of duodenum, jejunum, and ileum from calves fed an all-milk-protein control milk replacer or milk replacer containing soy protein concentrate (SPC) without or with supplemental glutamine (Gln)

Site and variable	Diet			SE
	Control	SPC	SPC + Glut	
Duodenum				
Villus height, μm	400	372	395	66
Crypt depth, μm	240	247	275	24
Villus:crypt	1.68	1.59	1.50	0.27
Jejunum				
Villus height, ¹ μm	713	506	464	66
Crypt depth, ¹ μm	300	209	229	23
Villus:crypt	2.46	2.46	2.04	0.26
Ileum				
Villus height, ¹ μm	532	458	456	26
Crypt depth, ² μm	352	301	382	29
Villus:crypt ³	1.54	1.64	1.27	0.11

¹Control vs. SPC and SPC + Gln, $P < 0.05$.

²SPC vs. SPC + Gln, $P < 0.08$.

³SPC vs. SPC + Gln, $P < 0.05$.

Table 4. Concentrations of urea N, total protein, and glucose in plasma from calves fed an all-milk-protein control milk replacer or milk replacer containing soy protein concentrate (SPC) without or with supplemental glutamine (Gln)

Variable	Diet			SE
	Control	SPC	SPC + Gln	
Urea N, ¹ mg/dL	5.2	4.3	6.1	0.6
Total protein, ² g/dL	5.45	5.48	5.46	0.20
Glucose, mg/dL	82.8	80.3	76.1	4.3

¹SPC vs. SPC + Gln, $P < 0.05$.²Values at wk 2 were higher ($P < 0.001$) than those at wk 4 (5.60 vs. 5.33 g/dL), but the treatment $\times$ week interaction was not significant ($P = 0.63$).

DISCUSSION

The lower growth performance for calves fed milk replacers containing SPC is consistent with previous studies (Dawson et al., 1988; Tomkins et al., 1994; Lallès, 2000). The NRC (2001) model was used to evaluate calf growth relative to predicted requirements for ME and apparently digestible protein (ADP). At the mean BW and DMI of calves fed the control milk replacer, model-predicted ME-allowable ADG (0.35 kg/d) was similar to actual ADG (0.34 kg/d; Table 5). For calves fed SPC without or with Gln, however, actual ADG was approximately 16% less than model predictions for ME-allowable growth. For control calves, predicted ADP-allowable growth (0.38 kg/d) was slightly greater than actual ADG (0.34 kg/d), indicating that ME supply may have limited growth. For calves in either SPC group, predicted ADP-allowable growth was 17.6% (for SPC) to 24.3% (for SPC + Gln) higher than actual ADG (Table 5).

Reasons for the poorer than predicted (by NRC, 2001) performance for calves fed SPC remain unclear. Antinutritional factors and antigenic proteins present in raw soybeans have received much attention. Most antinutritional factors are inactivated by heat treatment and antigenicity is greatly decreased by the hot aqueous ethanol treatment involved in production of SPC (Lallès, 1993), yet adverse effects on growth and intestinal function still occur when SPC is fed to calves (Lallès,

2000). Changes in intestinal histomorphology for calves fed SPC were consistent with previous results showing that intestinal villus height is decreased when soy flour (Kilshaw and Slade, 1982) or SPC are included in milk replacers fed to young calves (Seegraber and Morrill, 1982, 1986; Dawson et al., 1988; Grant et al., 1989). The cellulose and hemicellulose present in SPC may increase villus abrasion and cell desquamation and also increase mucus loss in the terminal small intestine (Leterme et al., 1998). In addition to alterations in villus size, a variety of other intestinal abnormalities have been observed in calves fed low-antigenic soy protein products, including decreases in protein synthetic capacity (Grant et al., 1989), mucosal digestive enzyme activities (Grant et al., 1989; Montagne et al., 1999), and absorptive capacity (Seegraber and Morrill, 1982, 1986; Grant et al., 1989), and increases in mucin secretion (Montagne et al., 2000), immune activation (Lallès, 2000), and specific endogenous protein loss (Montagne et al., 2001).

The NRC (2001) model assumes that only milk proteins are fed. Plant-based proteins have high true digestibilities but lower apparent digestibilities because specific endogenous protein losses at the ileum are increased (Montagne et al., 2000, 2001). Montagne et al. (2003) suggested that resistant dietary oligopeptides might interact with intestinal mucosa to stimulate endogenous protein secretion. In a previous study, SPC increased ileal flow of diet-specific host protein by 3 g/kg of DMI (Montagne et al., 2001), which would increase dietary protein requirements by no more than 2 g/d for calves fed SPC in the present study. Consequently, intake of ADP did not appear to limit growth of calves fed SPC in our study. Similar concentrations of total protein in plasma also indicate that overall protein status was adequate; total protein concentrations were lower in calves fed milk replacer diets that were deficient in ADP (Bartlett et al., 2006). Although the AA composition of diets was not determined, contents of Lys and Met were formulated to be similar between diets. It is possible, however, that another indispensable AA such as Thr (Kanjapaputhipong, 1998) was

Table 5. Comparison of actual average daily gain (ADG) with NRC (2001) model predictions for ME-allowable ADG and apparently digestible protein (ADP)-allowable ADG

Variable	Diet		
	Control	SPC	SPC + Gln
Actual ADG, kg	0.34	0.28	0.28
ME intake, Mcal/d	2.73	2.60	2.65
ME-allowable ADG, kg	0.35	0.33	0.34
CP intake, g/d	128	117	125
ADP-allowable ADG, kg	0.38	0.34	0.37
CP required for ME-allowable ADG, kg/d	120	115	117

limiting in SPC relative to whey proteins in the control. Regardless of mechanism, ADG and gain:feed were lower than for controls and lower than predicted by the NRC (2001) model when calves were fed the SPC-containing milk replacer.

Supplemental Gln was not able to prevent the lower growth performance for calves fed SPC. Glutamine is metabolized in intestinal tissue by an initial deamidation reaction catalyzed by glutaminase, with resultant release of ammonia (Reeds and Burrin, 2001). Nappert et al. (1999b) found that the portal-drained viscera of milk-fed calves released from 0.24 to 0.88 moles of ammonia for every mole of Gln utilized by those tissues. The ammonia would be removed from portal blood by the liver and converted to urea. Intravenous or dietary supplementation of Gln increased uptake of Gln by the portal-drained viscera of milk-fed calves, which increased ammonia production from the gut and tended to increase uptake of indispensable AA, presumably for enhanced protein synthesis (Nappert et al., 1999a,b). Increased urea N concentration in plasma of calves supplemented with Gln in our study (Table 4) indicates, therefore, that Gln was bioavailable and was metabolized to some degree by the intestine. However, this Gln metabolism did not translate into improved calf growth or gain:feed.

Glutamine is also needed for nucleic acid synthesis in rapidly proliferating cells (Souba, 1993) and is an important substrate for conversion to ornithine, arginine, proline, and polyamines in intestinal mucosa (Reeds and Burrin, 2001). Changes in intestinal villus and crypt size during diarrhea were lessened by administration of a Gln-supplemented oral rehydration solution compared with solutions without Gln (Brooks et al., 1998). Glutamine exerts other positive effects on intestinal structure and function. Because of its role in amino sugar synthesis, Gln may promote maintenance of tight junctions and thereby improve mucosal structure (Panigrahi et al., 1997; Potsic et al., 2002). We postulated, therefore, that Gln might stimulate proliferation of intestinal epithelial cells, which would be reflected in increased villus height in jejunum or ileum, and might counteract other intestinal alterations caused by SPC. Increases in crypt depth in the ileum (Table 3) would be consistent with this hypothesis, but ileal villus height was not altered and no changes in morphology of duodenum or jejunum were observed. Our results stand in contrast to results from weanling pigs fed corn and soybean meal diets, in which supplementation of similar (1% of dietary DM; Wu et al., 1996) or smaller amounts (0.5% of dietary DM; Domeneghini et al., 2004) of Gln prevented the loss of villus height and maintained growth performance relative to unsupplemented pigs. Differences between studies could re-

late to the fact that the pigs had lower total protein intake and received no milk proteins after weaning, whereas our calves were not weaned, had similar ADP intake to controls, and continued to receive some milk proteins in the diet.

The tendency for increased crypt depth and the lower villus to crypt ratio in the ileum (Table 3) in the absence of greater villus height may indicate an enhancement of secretory or immune activity relative to calves fed SPC alone. Glutamine is important for the synthesis of glycoprotein moieties in mucin (Khan et al., 1999). Similar to our results, Domeneghini et al. (2004) found that supplementation of 0.5% L-Gln to newly weaned pigs increased crypt depth and decreased the villus height to crypt depth ratio in the terminal ileum; they also reported increased villus height, increased mitotic to apoptotic rate, and enhanced immune cell abundance in the intestine. Whether the possible stimulation of secretory or immune activity suggested by greater crypt depth might translate to improvements in health or disease resistance cannot be determined from our experiment. Oral rehydration solutions containing Gln were more effective in preventing BW loss, hyponatremia, and metabolic acidosis in diarrheic calves than were solutions without Gln (Brooks et al., 1997).

Only one supplementation rate for Gln was tested in this experiment. Although it is unknown whether larger amounts might produce improvements in calves fed SPC-containing milk replacer, the amount used in this experiment is at the upper end of dosages reported in the other published studies cited herein. Addition of 1% Gln to milk replacer containing 20% protein represents supplementation at 5% of total protein supply. Because Gln is already extremely abundant in both milk and soy proteins (Baxter et al., 2004), it seems unlikely that larger amounts would be more effective in overcoming limitations of SPC; however, this could be tested in future research.

CONCLUSIONS

Replacing 60% of the whey proteins in an all-milk-protein milk replacer with SPC decreased growth rates and feed efficiency and decreased villus height and crypt depth in the jejunum and villus height in the ileum. Supplementation of Gln to the SPC-containing milk replacer did not prevent these decreases and resulted in no improvements in calf performance relative to the unsupplemented SPC milk replacer. Addition of Gln tended to increase crypt depth and decreased the villus to crypt ratio in the ileum without altering villus height, which might indicate increased secretory activity.

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