Vegetable Proteins Enhance the Growth of Milk-Fed Piglets, Despite Lower Apparent Ileal Digestibility[1,2]

André R. Ebert, Adam S. Berman, Robert J. Harrell, Alexandre M. Kessler,* Steven G. Cornelius,† and Jack Odle[3]

*Interdepartmental Nutrition Program and Department of Animal Science, North Carolina State University, Raleigh, NC 27695; *Universidade Federal do Rio Grande do Sul, Porto Alegre-RS, Brazil 91540; and †Milk Specialties Company, Dundee, IL 60118*

ABSTRACT This experiment compared the replacement of whey protein with isolated soy protein (ISP), or 2 levels of a hydrolyzed vegetable protein mixture (Lo HVPM and Hi HVPM, containing a partially hydrolyzed blend of soy, wheat, and other proteins) in liquid milk-replacer diets fed to neonatal pigs from 2 to 19 d of age. Piglets fed the vegetable protein diets weighed 20% more (8179 ± 211 g, $P < 0.05$) at the end of the study than piglets fed the whey diet (6805 ± 244 g). Growth rates were 35% higher for piglets fed the Hi HVPM diet than for piglets fed the whey diet. Similarly, intakes of the vegetable protein diets exceeded that for the whey diet ($P < 0.05$). Although the apparent ileal digestibilities of most amino acids were greater for the whey diet, digestible amino acid intakes (especially Arg, Phe, Met, and His) were greater in pigs fed the Hi HVPM and ISP diets ($P < 0.01$). Furthermore, carcasses of piglets fed the whey diet contained a higher percentage of fat and ash, whereas piglets fed the vegetable protein–containing diets accreted protein 42% faster ($P < 0.01$). Villus height and area and leucine aminopeptidase activity in the small intestine were greater in piglets fed the Lo HVPM diet than in those fed the ISP diet. Collectively, these data support the conclusion that some processed vegetable proteins may be good alternatives to whey protein in liquid diets formulated for neonatal pigs and that an appropriate balance of amino acids is more important than the source of protein per se. J. Nutr. 135: 2137–2143, 2005.

KEY WORDS: • *soy protein* • *amino acid digestibility* • *milk replacer*

The piglet has gained much popularity as a pediatric research model (1,2). Most infant formula companies routinely use piglets as a preclinical model of infant nutrition. Indeed, in 2003, the NIH National Center for Research Resources established a National Swine Research and Resource Center because of the dramatic increase in the use of pigs as models of human disease. In particular, the gastrointestinal tract of pigs is more similar to that of humans than other domestic and research animal species (1). Our research efforts have thus focused on defining better the nutrient needs of piglets both to improve their utility as a biomedical model and to advance production in agriculture.

Several alternative ingredients have been evaluated as complete or partial replacements for milk-based ingredients to reduce diet costs. In terms of protein, soybean meal, isolated soy protein (ISP)[4] (3), hydrolyzed soy proteins (4), and wheat gluten (5) have been the more common alternatives. Overall, the use of vegetable proteins usually results in lower growth performance for young pigs (<14 d old). Poor performance has been explained by a temporary enzymatic limitation on digestion of vegetable nutrient sources and a transient intestinal hypersensitivity to soy proteins (glycinin and β-conglycinin) in young pigs (6). However, McCracken et al. (7) observed that neither intact nor hydrolyzed soy proteins elicit intestinal inflammation in neonatal pigs. Similarly, Moughan et al. (8) reported no difference in digestive enzyme activity in piglets fed ISP or bovine milk protein–based diets. Therefore, the objective of this experiment was to evaluate the partial replacement of milk protein by a hydrolyzed vegetable protein mixture (HVPM) in liquid diets fed to piglets from 2 to 19 d of age.

MATERIALS AND METHODS

Animals and diets. Animal procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University. A total of 66 crossbred (Pig Improvement Company genetics) 2-d-old mixed-gender piglets with a mean weight of 1.8 kg were drawn from 8 litters (supplied by Murphy Family Farms). Piglets received i.m. injections of iron-dextran within 24 h of birth. The experiment was conducted in 2 replicates. In each replicate, 6 piglets were used for initial body composition and intestinal measurements, and 6 piglets were randomly assigned to each dietary treatment. Piglets were housed individually in cages within an environmentally

[1] Previously presented at the National Animal Science meetings, July 2004, St. Louis, MO [Ebert, A., Berman, A. S., Harrell, R. H., Cornelius, S. G. & Odle, J. (2004) Liquid diets containing vegetable proteins accelerate piglet growth above milk-protein-based diets. J. Anim. Sci. 82 (suppl. 1): 24 (abs.)].

[2] Supported in part by the North Carolina Agricultural Research Service and by a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior educational fellowship to A.R.E.

[3] To whom correspondence should be addressed. E-mail: jack_odle@ncsu.edu.

[4] Abbreviations used: Hi HVPM, high hydrolyzed vegetable protein mixture; HVPM, hydrolyzed vegetable protein mixture; ISP, isolated soy protein; LAP, leucine aminopeptidase; Lo HVPM, low hydrolyzed vegetable protein mixture; ME, metabolizable energy; PUN, plasma urea nitrogen.

Manuscript received 21 February 2005. Initial review completed 11 March 2005. Revision accepted 14 June 2005.

controlled room (32°C). A gravity-flow feeding system (9) was employed, which consisted of a 2-L plastic bottle placed over the cage with a tube connecting the bottle to a nipple attached to the cage; a stir plate was used under each bottle. Using this feeding system, diet spillage and waste were negligible.

Antibiotic-free diets were prepared by Milk Specialties as dry powdered formulas, differing primarily in source of protein. In the first replicate, 4 diets were utilized: *1*) a positive control diet with whey as the only protein source (whey diet), *2*) a hydrolyzed vegetable protein mixture (HVPM) replacing 31% of the whey protein (Lo HVPM diet), *3*) a hydrolyzed vegetable protein mixture replacing 62% of the whey protein (Hi HVPM diet), and *4*) a negative control diet with isolated soy protein (ISP) replacing 62% of the whey protein (ISP diet). The HVPM contained soy, wheat, and other proteins that were subjected to partial enzymatic hydrolysis. Because the growth performance of piglets fed the positive control (whey) diet was lower than those fed the test diets in the 1st replicate, a 5th diet was included in the 2nd replicate of the experiment. In that diet, 47% of the whey protein was replaced by casein (casein diet). The data from piglets fed the casein diet were used only as a reference for the positive control (whey) diet and were not included in the statistical analyses. Diets were formulated (**Tables 1** and **2**) to contain similar metabolizable energy (ME) contents and total lysine levels to exceed the NRC requirements for 3- to 5-kg piglets (10). Cobalt-EDTA was prepared as described by Uden et al. (11) and was added (0.1%) to the diets 36 h before the end of the experiment to serve as an inert digestibility marker. Diets were reconstituted (180 g/L) on a daily basis and kept refrigerated at 4°C until feeding. Fresh liquid diet was added to the bottles 4 times/d (at 0800, 1300, 1800, and 2300 h) to ensure that piglets had free access to fresh feed.

On the final day of the experiment, all piglets (19 d old) were killed for sampling of gastric and ileal digesta and small-intestine mucosa; carcasses were frozen and ground for measurement of body composition. Heparinized blood was collected via jugular venipuncture and chilled on ice, and plasma was prepared (centrifugation at 824 × *g* for 10 min) and frozen at −20°C. Piglets were anesthetized with 5% (v:v) halothane gas, and the gastrointestinal tract was removed from the gastroesophageal junction to the distal rectum. Gastric contents were collected and the pH was measured immediately. The contents of the terminal ileum were collected and stored at −80°C. At 25, 50, and 75% of the small-intestine length (referenced from the pyloric end), 2 adjacent segments 3 and 10 cm in length were collected. The 10-cm segment was opened longitudinally and the mucosa was scraped with a glass slide and weighed (12). The mucosa was homogenized in 4 vol of ice-cold PBS and frozen at −80°C. The 3-cm segment was placed into a tube containing chilled fixative solution (formalin, 95% ethanol, and glacial acetic acid solution) and stored at 4°C for 24 h. The remaining digesta were removed from the intestines and the empty carcasses were stored at −20°C.

Plasma and intestinal analyses. Plasma urea nitrogen (PUN) was assayed by the quantitative urease/Berthelot procedure (Sigma Diagnostics) based on published methods (13,14). Leucine aminopeptidase (LAP) activity was measured in mucosal homogenates as described by Goldbarg and Ruttenburg (15). The protein content of the homogenates was determined by the biuret method (16). The 3-cm small-intestine segments were processed for histomorphology as previously described (17); 6 villus height and width and crypt depth measurements were made on each sample, and villus surface area was calculated as follows: Area = (width × 3.14) × (2 × height + radius).

Body composition, amino acid, and Co analysis. Carcasses were ground twice through a fixed-die meat grinder. Subsamples of the final mixture were stored at −20°C until analysis for dry matter, ash, protein (nitrogen × 6.25), and fat (18). Amino acid and Co analyses of diets and ileal digesta were performed by a commercial laboratory (Experiment Station Chemical Labs), utilizing Association of Official Analytical Chemists methods (18). Only the ileal digesta samples from piglets fed the whey, Hi HVPM, ISP, and casein diets were subjected to amino acid analysis. Digestibility values for the Lo HVPM diet were assumed to be intermediate between those for the whey and Hi HVPM diets. Tryptophan concentrations were not determined. Apparent ileal digestibility of nutrients was calculated by

TABLE 1

Experimental diet formulas

	Diet				
Ingredient	Whey	Lo HVPM	Hi HVPM	ISP	Casein
	g/kg				
Dried whey	518.8	527.0	504.5	439.5	496.8
Dried whey protein concentrate	215.7	131.3	57.0	59.3	100.3
Delactosed whey[1]	—	—	—	42.5	—
Edible lard[2]	217.9	199.6	198.3	221.0	218.6
HVPM[3]	—	92.1	184.3	—	—
Isolated soy protein	—	—	—	181.3	—
Sodium caseinate	—	—	—	—	128.5
Dicalcium phosphate	13.9	12.5	11.9	10.0	11.5
Calcium chloride	6.2	8.6	11.0	12.6	8.2
L-Lysine (HCl)	—	0.5	3.5	3.3	—
DL-Methionine	1.2	1.3	2.4	4.1	2.6
Sodium citrate	—	—	—	—	7.0
Other[4]	26.3	27.1	27.1	26.4	26.5

[1] Dried whey product with a portion of the lactose removed.

[2] Lard was added as a spray-dried, protein-encapsulated fat using either a whey plus whey protein concentrate mixture or HVPM as the protein source. The mixture also contained an emulsifier package that is reflected in this row.

[3] Hydrolyzed vegetable protein mixture, manufactured by Milk Specialties Company, contains a proprietary mixture of soy, wheat, and other proteins, subjected to partial enzymatic hydrolysis (Matrix Enhanced Protein, MEP) and processed to improve solubility and dispersal within liquid diets.

[4] Consisted of the following: mineral premix 0.5%, vitamin premix 0.12%, potassium sorbate 0.45%, artificial flavor 0.05%, and citric acid 1.0%. The mineral premix provided the following (g/kg premix): choline, 60; Zn, 23.5; Fe, 20.0; K, 20.0; S, 16.3; Ca, 10.0; P, 5.5; Mn, 5.0; Na, 2.8; Cu, 1.8; Mg, 1.0; I, 0.4; Cl, 0.4; Co, 0.2; Se, 0.06. The vitamin premix provided the following (per kg premix): vitamin A, 33,000,000 IU; cholecalciferol, 6,600,000 IU; Vitamin E, 55,000 IU; vitamin K, 5.12 g; vitamin C, 117 g; niacin, 33.07 g; pantothenate, 29.98 g; riboflavin, 8.38 g; vitamin B-6, 4.00 g; folic acid, 2.76 g; thiamin, 2.04 g; biotin 66 mg; cobalamin, 44 mg.

TABLE 2

Nutrient composition of experimental diets[1]

Item	Dietary protein source				
	Whey	Lo HVPM	Hi HVPM	ISP	Casein[2]
ME, *MJ/kg*	18.6	18.6	18.6	18.6	18.6
			%		
Crude protein	21.7	24.6	27.1	26.8	23.3
Fat	22.8	21.5	21.0	22.4	21.8
Lactose	39.6	37.7	35.8	35.2	38.6
Ash	6.4	8.1	7.8	8.3	8.4
Amino acids					
Lysine	1.58	1.54	1.56	1.50	1.74
Tryptophan	0.35	0.35	0.35	0.35	0.35
Threonine	1.05	1.35	0.91	0.73	0.90
Methionine	0.52	0.55	0.61	0.65	0.71
Leucine	2.03	2.26	1.91	1.81	2.30
Isoleucine	1.22	1.31	1.14	1.03	1.17
Valine	1.13	1.28	1.13	1.04	1.27
Phenylalanine	0.63	0.91	0.92	0.96	0.91
Tyrosine	0.48	0.66	0.61	0.60	0.75
Arginine	0.49	0.90	0.90	1.28	0.66
Histidine	0.37	0.44	0.47	0.47	0.52
Aspartate[3]	2.00	2.12	1.98	2.22	1.72
Glutamate[4]	3.17	3.54	4.32	4.06	4.35
Serine	0.62	1.12	0.74	0.70	0.73
Proline	1.02	1.58	1.38	1.01	1.72
Glycine	0.28	1.32	0.52	0.60	0.31
Alanine	0.87	1.01	0.83	0.76	0.70

[1] Values were calculated for ME, fat, lactose, and tryptophan content of all diets, and all amino acid concentrations were calculated for the Lo HVPM diet. Values for crude protein and ash were analyzed for all diets, and amino acid concentrations (except Trp) were analyzed for the whey, Hi HVPM, ISP, and casein diets.
[2] The casein diet was tested only in the second replicate of the experiment.
[3] Sum of aspartate and asparagine.
[4] Sum of glutamate and glutamine.

comparing the ratio of nutrient to Co in the diet to the ratio in the ileal digesta (11).

Statistical analyses. Piglets were assigned to treatments according to a completely randomized design, without regard to gender or litter of origin. Two piglets were excluded from the trial because they developed severe diarrhea and had very low feed intake. The effects of dietary protein were assessed by ANOVA using the General Linear Model procedure of SAS (version 8.1, SAS Institute); treatment means were separated using Tukey's test. For growth performance variables, initial body weight was included in the model as a covariable, and the reported values are the least-square (covariate-adjusted) means ± SEM. Differences were considered significant when $P < 0.05$.

RESULTS

Piglet growth performance. At the end of the trial, piglets fed the whey diet (6805 ± 244 g, SEM) weighed only 83% as much as piglets fed the vegetable protein–containing diets (8180 ± 210 g). The body weights of piglets fed the casein diet (in the second replicate) were similar (7265 ± 703 g) to those of piglets fed the whey diet. Overall, piglets fed the whey diet gained 100 g/d less than piglets fed the Hi HVPM diet (**Fig. 1**), and the growth of piglets fed the ISP diet exceeded by 33% the growth of those fed the whey diet. Even during wk 1 of the experiment (data not shown), piglets fed the Hi HVPM diet gained 60% more than piglets fed the whey diet. However, piglets fed the Hi HVPM and ISP diets did not differ in growth ($P > 0.1$). The overall growth rate of piglets fed the casein diet (305 ± 44 g/d) was similar in magnitude to that of piglets fed the whey diet (291 g/d).

Piglets fed either the Hi HVPM or the ISP diet consumed

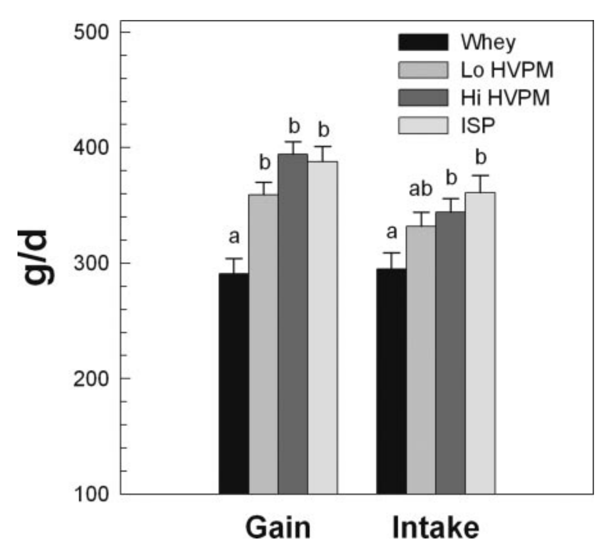

FIGURE 1 Body weight gain and feed intake (dry matter) of suckling piglets fed diets containing various sources of protein from 2 to 19 d of age. Bars represent means ± SEM, n = 9–12. Means for a variable without a common letter differ, $P < 0.05$.

~22% more feed than those fed the whey diet (Fig. 1, $P < 0.05$). Piglets fed the casein diet consumed 283 ± 27 g/d. Collectively, the Hi HVPM diet increased efficiency (weight gain/feed intake) by 16%, compared to the whey diet ($P < 0.05$, data not shown).

Apparent ileal dry matter and amino acid digestibility and intake. The diets did not differ in apparent ileal dry matter digestibility (**Table 3**). Apparent ileal digestibility of most amino acids was 7–10% greater for the whey diet than for the Hi HVPM or ISP diets. Nearly all essential amino acids were more digestible in the whey diet than in either the Hi HVPM or ISP diet. The casein diet dry matter digestibility was 89.7 ± 1.3% and amino acid digestibilities were 90.3 ± 1.3% and 87.1 ± 2.9% for essential and total amino acids, respectively. Daily intakes of apparent ileal digestible lysine (5.1 g/d), leucine (6.4 g/d), isoleucine (3.8 g/d), or valine (3.7 g/d) did not differ among the treatments (data not shown). However, piglets fed the whey diet had a lower intake of apparent ileal digestible methionine, histidine, and phenylalanine than piglets fed the Hi HVPM and ISP diets (**Fig. 2**, $P < 0.01$). Piglets fed the ISP diet had a lower intake of threonine, but the intake of arginine was 3-fold that of piglets fed the whey diet. Similarly, intake of apparent ileal digestible arginine by piglets fed Hi HVPM was 2-fold that of piglets fed the whey diet. Collectively, total essential amino acid intake was ~20% greater for piglets fed the Hi HVPM and ISP diets than for piglets fed the whey diet (data not shown). The intakes of digestible nonessential amino acids (i.e., serine, glycine, glutamine, and tyrosine) also were greater for piglets fed the Hi HVPM and ISP diets than for those fed the whey diet (data not shown). The intake of total analyzed amino acids (not shown) was ~25% greater for piglets fed the Hi HVPM and ISP diets than for those fed the whey diet. Piglets fed the casein diet had an intake of essential amino acids and total analyzed amino acids of 29.5 ± 2.9 and 59.4 ± 5.6 g/d, respectively.

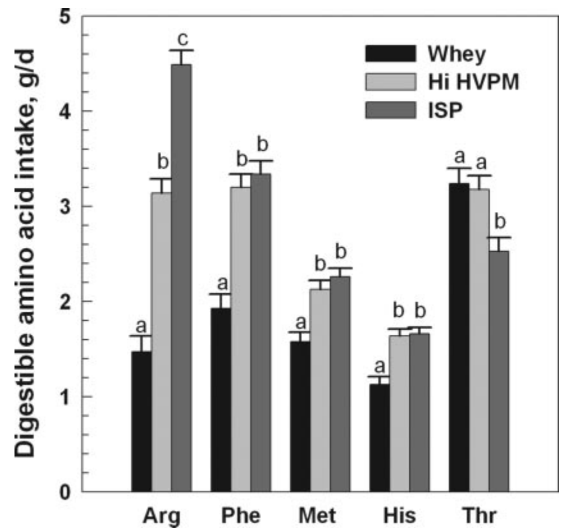

FIGURE 2 Apparent ileal digestible intakes of indispensable amino acids by suckling piglets fed diets containing various protein sources from 2 to 19 d of age. Bars represent means ± SEM, $n = 9$–12. Means for a variable without a common letter differ, $P < 0.01$.

TABLE 3

Apparent ileal dry matter and amino acid digestibilities of diets containing various sources of protein fed to suckling piglets[1]

Item	Dietary protein source			$P > F$
	Whey	Hi HVPM	ISP	
		%		
Dry matter	85.5 ± 1.0	86.1 ± 1.0	85.1 ± 1.0	0.732
Lysine	85.6 ± 1.5[a]	78.9 ± 1.4[b]	77.2 ± 1.4[b]	<0.001
Methionine	91.3 ± 1.0[a]	87.8 ± 0.9[b]	86.4 ± 0.9[b]	0.001
Threonine	77.2 ± 1.7[a]	70.9 ± 1.5[b]	66.7 ± 1.6[b]	<0.001
Leucine	91.5 ± 1.4[a]	81.5 ± 1.3[b]	80.8 ± 1.3[b]	<0.001
Histidine	86.1 ± 1.7[a]	81.3 ± 1.5[ab]	77.2 ± 1.5[b]	0.001
Valine	86.0 ± 1.6[a]	78.5 ± 1.4[b]	75.4 ± 1.4[b]	<0.001
Phenylalanine	87.2 ± 1.6[a]	82.0 ± 1.4[b]	79.3 ± 1.4[b]	0.001
Isoleucine	90.8 ± 1.5[a]	82.0 ± 1.4[b]	77.3 ± 1.4[c]	<0.001
Arginine	85.3 ± 1.5	82.0 ± 1.4	84.6 ± 1.4	0.210
Total essential[2]	86.9 ± 1.4[a]	80.1 ± 1.3[b]	78.7 ± 1.3[b]	<0.001
Tyrosine	86.4 ± 1.9[a]	80.9 ± 1.7[ab]	77.0 ± 1.7[b]	0.001
Proline	81.6 ± 1.8[a]	79.5 ± 1.6[a]	73.5 ± 1.6[b]	0.004
Glutamate[3]	87.4 ± 1.4[a]	82.8 ± 1.2[b]	81.9 ± 1.3[b]	<0.010
Aspartate[4]	87.7 ± 1.5[a]	78.3 ± 1.3[b]	76.7 ± 1.3[b]	<0.001
Alanine	84.7 ± 1.7[a]	75.9 ± 1.6[b]	71.4 ± 1.6[b]	<0.001
Glycine	63.6 ± 2.6	67.6 ± 2.3	67.1 ± 2.5	0.453
Serine	75.0 ± 1.9	71.4 ± 1.7	70.1 ± 1.7	0.131
Total analyzed	85.7 ± 1.4[a]	79.5 ± 1.3[b]	77.9 ± 1.4[b]	<0.001

[1] Values are means ± SEM, $n = 9$–12. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] Without tryptophan (not analyzed).
[3] Sum of glutamate and glutamine.
[4] Sum of aspartate and asparagine.

Body composition and tissue deposition rates. Body composition of the initial group (2 d of age) was 78.51 ± 0.56% water, 12.69 ± 0.19% protein, 3.94 ± 0.52% fat, and 2.82 ± 0.04% ash. After 17 d of feeding, the body composition of piglets fed the whey diet contained ~7% less protein and 23% more fat, compared to piglets fed the Hi HVPM or ISP diets (**Table 4**). Carcasses from piglets fed the casein diet were 70.3 ± 0.9% water, 13.6 ± 0.1% protein, 12.7 ± 0.9% fat, and 2.3 ± 0.03% ash. The accretion rates (g/d) of water and protein were 37 and 42% greater, respectively, in piglets fed the Hi HVPM, Lo HVPM and ISP diets compared to piglets fed the whey diet. Ash deposition rates were higher in piglets fed the Hi HVPM and ISP diets than in piglets fed the whey diet, whereas rates in piglets fed the Lo HVPM diet were intermediate. The nutrient accretion rates for piglets fed the casein diet were 204 ± 29 g/d of water, 42 ± 6 g/d of protein, 50 ± 8 g/d of fat, and 6.3 ± 1 g/d of ash.

PUN, gastric pH, LAP, and intestinal morphology. Concentrations of PUN were lower in piglets fed the whey diet than in piglets fed the other diets (**Table 5**). The PUN concentration measured in piglets fed the casein diet was 3.24 ± 0.26 mmol/L. There was no effect of diet on gastric pH measured in the second replicate of the experiment. The gastric content pH for piglets fed the casein diet was 4.02 ± 0.18. LAP activity measured at 75% of the length of the small intestine was greater in piglets fed the Lo HVPM diet than in piglets fed the ISP diet. LAP activity did not differ at 25 and 50% of the intestinal length or in the mean values (data not shown). Villus height was greater for piglets fed the Lo HVPM diet than the ISP diet (Table 5), but crypt depth did not differ. The calculated villus:crypt ratios and villus surface areas were higher for piglets fed the Lo HVPM diet than for those fed the ISP diet.

DISCUSSION

The overall growth rate during the 17 d test period was 358 ± 12 g/d, which is similar to our previous experiments (19,20);

TABLE 4

Body composition and protein, fat, water, and ash accretion rates in suckling piglets fed diets containing various sources of protein from 2 to 19 d of age[1]

Item	Dietary protein source				$P > F$
	Whey	Lo HVPM	Hi HVPM	ISP	
Body composition, %					
Water	68.1 ± 0.7	69.9 ± 0.6	70.4 ± 0.6	70.3 ± 0.7	0.083
Protein	12.8 ± 0.2[a]	13.5 ± 0.2[ab]	13.7 ± 0.2[b]	13.9 ± 0.2[b]	0.016
Fat	15.4 ± 0.7[a]	13.1 ± 0.6[ab]	12.5 ± 0.6[b]	12.5 ± 0.7[b]	0.010
Ash	2.34 ± 0.03[a]	2.15 ± 0.02[b]	2.10 ± 0.02[b]	2.12 ± 0.03[b]	<0.001
Accretion rate, *g/d*					
Water	187 ± 8[a]	242 ± 7[b]	268 ± 7[b]	260 ± 8[b]	<0.001
Protein	37 ± 2[a]	49 ± 1[b]	55 ± 2[b]	54 ± 2[b]	<0.001
Fat	58 ± 4	56 ± 3	58 ± 3	57 ± 4	0.983
Ash	6.2 ± 0.3[a]	7.1 ± 0.3[ab]	7.7 ± 0.3[b]	7.4 ± 0.3[b]	0.012

[1] Values are means ± SEM, $n = 9$–12. Means in a row with superscripts without a common letter differ, $P < 0.05$.

it is ~130% greater than the rate observed in sow-reared piglets (17) and up to 180% greater than that reported in other artificial piglet-rearing studies (21).

The poorest performance was exhibited by piglets fed the whey diet. There is an apparent contradiction between this result and the widely held idea that newborn piglets have unique nutritional needs that include high-quality sources of nutrients, especially proteins, with milk-based proteins being considered of very high quality (22); however, this applies to animals in transition onto dry feed, and extrapolation to liquid-fed piglets should be done with caution. Newport (23) observed no effect on piglet performance from 2 to 28 d of age with partial replacement of dried skim milk by a mixture of ISP and dried whey when soy protein provided 37% of dietary protein. However, very poor growth was observed when soy protein provided 74% of dietary protein, with only 4 of 21 piglets surviving. This mortality was related to severe diarrhea. Jones et al. (24) reported that piglet performance was not affected when 25% of dried skim milk was replaced by soy flour as the protein source in a liquid diet fed to piglets from 21 to 36 d of age. Mateo and Veum (3) reported a decline of ~50% in growth rates of piglets fed an ISP-based diet compared with piglets fed a dried skim milk– or casein-based diet from 1 to 15 d of age, but piglet performance did not differ from 15 to 29 d of age. McCracken et al. (7) reported an intermediate growth rate for piglets fed a hydrolyzed soy protein–based diet (109 g/d) compared with a casein/whey (121 g/d) and an intact soy protein–based diet (85 g/d) from 7 to 17 d of age. From these studies, it seems clear that the response to inclusion of a vegetable protein source depends on the percentage of inclusion, how it is processed, and the age of the piglets. Indeed, improved efficiency of utilization of vegetable proteins has been attributed to an adaptation of the digestive enzyme system of the piglets to the protein sources that occurs with age (25). Nonetheless, we found no literature showing better performance for any vegetable protein compared with milk-protein-based diets fed to such young pigs as we report in this experiment.

As expected, the apparent ileal digestibility of amino acids was greater for the whey diet. Apparent ileal digestibility of lysine was 85.55, 78.87, and 77.15% for the whey, Hi HVPM, and ISP diets, respectively. Overall, our data showed that the apparent ileal digestibility of essential amino acids and total analyzed amino acids was 8% lower for the Hi HVPM diet and 10% lower for the ISP diet, compared with the whey diet. Mavromichalis et al. (26) reported values of true ileal digestibility of sow's milk as follows: lysine, 88%; methionine, 99%; threonine, 84%; leucine, 91%; histidine, 100%; valine, 87%; and arginine, 90%. All of these values are greater than those found in our research, even in the milk-based diets. However, our data were not adjusted for endogenous amino acid contamination; thus, this comparison is crude.

Despite the better apparent ileal amino acid digestibility observed for the whey diet, the daily intake of apparent ileal

TABLE 5

PUN, gastric pH, LAP activity, and intestinal morphology of suckling, 19-d-old piglets fed diets containing various sources of proteins for 17 d[1]

Item	Dietary protein source				$P > F$
	Whey	Lo HVPM	Hi HVPM	ISP	
PUN, *mmol/L*	2.9 ± 0.3[a]	3.8 ± 0.2[b]	4.6 ± 0.2[b]	4.1 ± 0.2[b]	<0.001
Gastric pH	4.56 ± 0.15	4.31 ± 0.15	4.30 ± 0.16	4.22 ± 0.15	0.462
LAP, *μmol/(g protein · min)*	278 ± 32[ab]	346 ± 32[a]	263 ± 30[ab]	219 ± 30[b]	0.045
Villus height, *mm*	0.94 ± 0.05[ab]	1.02 ± 0.04[a]	0.86 ± 0.04[ab]	0.81 ± 0.04[b]	0.019
Crypt depth, *mm*	0.17 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.563
Villus area, *mm*^{2}	0.72 ± 0.06[ab]	0.87 ± 0.05[a]	0.67 ± 0.04[ab]	0.60 ± 0.04[b]	0.006
Villus:crypt ratio	5.71 ± 0.42[ab]	6.56 ± 0.32[a]	5.15 ± 0.35[b]	5.09 ± 0.33[b]	0.022

[1] Values are means ± SEM, $n = 9$–12 except for pH, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$.

digestible amino acids was greater for most of the amino acids in piglets fed the Hi HVPM and ISP diets, and this is congruent with the superior growth performance observed in piglets consuming these diets. Although there was no difference for lysine intake, piglets fed the vegetable protein–containing diets consumed 114–205% more arginine, 69% more phenylalanine, 39% more methionine, and 46% more histidine than the whey-fed piglets (Fig. 2). Essential and total amino acid intakes were ~20 and 28% lower, respectively, for the whey diet.

These differences in amino acid intake were reflected in both carcass composition and tissue accretion rates. Piglets fed the whey diet deposited 32–48% less protein than other piglets; however, the lower growth rates of piglets fed the whey diet were compensated by the greater percentage of carcass fat in these piglets, resulting in no net difference in fat deposition rates. Disregarding treatment effects, piglet carcasses were fatter (13.4% fat) and had a lower protein percentage (13.5% protein) in this experiment compared with data reported by Oliver et al. (27) who studied piglets of similar age (11.6% fat and 15.6% protein). Total lysine per unit of metabolized energy in our diets was 1.03 g/MJ, which is slightly below NRC (10) recommendations for piglets from 3 to 5 kg (1.08 g/MJ) and much lower than that used by Oliver et al. (27) (1.48 g/MJ). This might explain the different body composition between the 2 experiments. However the lysine:ME ratio among the diets used in this experiment did not change significantly: 0.84, 0.84, and 0.79 g apparent ileal digestible lysine/MJ ME for the whey, Hi HVPM, and ISP diets, respectively. Thus, the differences among treatments are much more likely related to an imbalance of some amino acids (other than lysine) rather than the source of protein per se. An amino acid imbalance may result when diets contain a complement of amino acids well in excess of the limiting amino acid. A reduction in feed intake is common in most of these situations (10). Manufactured milk replacers can be especially subject to this imbalance because amino acid requirements are not yet well established for very young pigs consuming liquid diets. For example, one of the amino acids that had the highest variation among the experimental diets and higher difference in daily intake was arginine. The total arginine level in sow's milk is 1.37% (26), ~65% of the lysine level in the milk, whereas the NRC (10) suggests an arginine level of 40% of total lysine for 3- to 5-kg piglets. The level of total arginine in the experimental diets was 30, 55, and 78% of total lysine for the whey, Hi HVPM, and ISP diets, respectively. Consistent with our results, Kim et al. (21) recently reported a linear increase in piglet growth when arginine was supplemented incrementally (up to 0.4%) into a whey-based milk replacer.

Soybean meal was reported to induce a transient intestinal hypersensitivity in early-weaned piglets. Li et al. (6) reported that piglets fed a diet containing soy protein had depressed weight gain, lower villus height, and greater crypt depth from 3 to 4 wk of age compared with piglets fed milk protein–based diets. Dreau et al. (28) investigated the development of local and systemic immune responses to antigenic or nonantigenic (no immunoreactive glycinin or β-conglycinin) proteins in early weaned piglets. Piglets fed nonantigenic soy proteins had greater villus height, villus perimeter, villus area, and villus height:crypt depth ratio. On the other hand, McCracken et al. (7) tested liquid diets based on intact or hydrolyzed soy proteins against a whey/casein–based diet in neonatal pigs and concluded that the lack of substantial dietary effects on intestinal variables demonstrates that neither hydrolyzed nor intact soy proteins result in significant intestinal inflammation when fed to neonatal pigs. In the present study, piglets fed the ISP diet had lower intestinal morphology measures than piglets fed the Lo HVPM diet (Table 5), but the effects were apparently insufficient to have a negative effect on the piglets' growth (Fig. 1; Table 4).

Despite lower amino acid digestibility, both the HVPM and ISP were shown to be excellent alternatives to milk proteins used in liquid diet formulations for neonatal pigs. Even if the comparison with a milk protein–based diet was prejudiced by the low performance of our positive control, the growth performance data from the alternative protein sources were proven to be very similar to those found in current literature for piglets of same age. Data from this study also underscore that a good amino acid balance may be more important than the source of protein per se. Clearly, additional work is warranted to better define the nutritional (amino acid) requirements of very young liquid-fed piglets. In particular, these data suggest that arginine may be limiting in milk-based proteins. In conclusion, there is a valuable niche for alternative vegetable proteins in liquid diets for very young piglets. Exploitation of this fact will help to make liquid feeding technology more economically attractive to the swine industry.

ACKNOWLEDGMENTS

The authors thank Lori Gatlin, Ryan Odle, Oulayvanh Pillips, Simone Pophal, and Lin Xi for their assistance.

LITERATURE CITED

1. Moughan, P. J., Birtles, M. J., Cranwell, P. D., Smith, W. C. & Pedraza, M. (1992) The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. In: Nutritional Triggers for Health and in Disease (Simopoulos, A. P., ed.), vol. 67, pp. 40–113. Karger, Basel, Switzerland.
2. Tumbleson, M. E. & Schook, L. B. (1996) Advances in Swine in Biomedical Research, Plenum Press, New York, NY.
3. Mateo, J. P. & Veum, T. L. (1980) Utilization of casein or isolated soybean protein supplemented with amino acids and glucose or lactose by neonatal piglets reared artificially. J. Anim. Sci. 50: 869–876.
4. Zijlstra, R. T., Mies, A. M., McCracken, B. A., Odle, J., Gaskins, H. R., Lien, E. L. & Donovan, S. M. (1996) Short-term metabolic responses do not differ between neonatal piglets fed formulas containing hydrolyzed or intact soy proteins. J. Nutr. 126: 913–923.
5. Chae, B. J., Han, I. K., Kim, J. H., Yang, C. J., Hancock, J. D., Kim, I. H. & Anderson, D. A. (1999) Effects of dietary protein sources on ileal digestibility and growth performance for early-weaned pigs. Livest. Prod. Sci. 58: 45–54.
6. Li, D. F., Nelssen, J. L., Reddy, P. G., Blecha, F., Hancock, J. D., Allee, G. L., Goodband, R. D. & Klemm, R. D. (1990) Transient hypersensitivity to soybean meal in the early-weaned pig. J. Anim. Sci. 68: 1790–1799.
7. McCracken, B. A., Zijlstra, R. T., Donovan, S. M., Odle, J., Lien, E. L. & Gaskins, H. R. (1998) Neither intact nor hydrolyzed soy proteins elicit intestinal inflammation in neonatal piglets. J. Parenter. Enteral Nutr. 22: 91–97.
8. Moughan, P. J., Pedraza, M., Smith, W. C., Williams, M. & Wilson, M. N. (1990) An evaluation with piglets of bovine milk, hydrolyzed bovine milk, and isolated soybean proteins included in infant milk formulas. I. Effect on organ development, digestive enzyme activities, and amino acid digestibility. J. Pediatr. Gastroenterol. Nutr. 10: 385–394.
9. McClead, R. E., Jr., Lentz, M. E. & Vieth, R. (1990) A simple technique to feed newborn piglets. J. Pediatr. Gastroenterol. Nutr. 10: 107–110.
10. National Research Council (1998) Nutrient Requirements of Swine. National Academy Press, Washington, DC.
11. Uden, P., Colucci, P. E. & Van Soest, P. J. (1980) Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agric. 31: 625–632.
12. Dahlqvist, A. (1964) Method for assay of intestinal disaccharidases. Anal. Biochem. 57: 18–25.
13. Fawcett, J. K. & Scott, J. E. (1960) A rapid and precise method for the determination of urea. J. Clin. Pathol. 13: 156–159.
14. Chaney, A. L. & Marbach, E. P. (1962) Modified reagents for determination of urea and ammonia. Clin. Chem. 8: 130–132.
15. Goldbarg, J. A. & Rutenburg, A. M. (1958) The colorimetric determination of leucine aminopeptidase in urine and serum of normal subjects and patients with cancer and other diseases. Cancer 11: 283–291.
16. Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) Determination of serum protein by means of the biuret reaction. J. Biol. Chem. 177: 751–756.
17. Mathews, S. A., Oliver, W. T., Phillips, O. T., Odle, J., Diersen-Schade, D. A. & Harrell, R. J. (2002) Comparison of triglycerides and phospholipids as

supplemental sources of dietary long-chain polyunsaturated fatty acids in piglets. J. Nutr. 132: 3081–3089.

18. Association of Official Analytical Chemists (1997) Official Methods of Analysis, 15th ed. AOAC, Gaithersburg, MD.

19. Zijlstra, R. T., Whang, K. Y., Easter, R. A. & Odle, J. (1996) Effect of feeding a milk replacer to early-weaned pigs on growth, body composition, and small intestinal morphology, compared with suckled littermates. J. Anim. Sci. 74: 2948–2959.

20. Kim, J. H., Heo, K. N., Odle, J., Han, K. & Harrell, R. J. (2001) Liquid diets accelerate the growth of early-weaned pigs and the effects are maintained to market weight. J. Anim. Sci. 79: 427–434.

21. Kim, S. W., McPherson, R. L. & Wu, G. (2004) Dietary arginine supplementation enhances the growth of milk-fed young pigs. J. Nutr. 134: 625–630.

22. Hansen, J. A., Nelssen, J. L., Goodband, R. D. & Weeden, T. L. (1993) Evaluation of animal protein supplements in diets of early-weaned pigs. J. Anim. Sci. 71: 1853–1862.

23. Newport, M. J. (1980) Artificial rearing of pigs. 11. Effect of replacement of dried skim-milk by an isolated soya-bean protein on the performance of the pigs and digestion of protein. Br. J. Nutr. 44: 171–182.

24. Jones, E. E., Coalson, J. A. & Lecce, J. G. (1977) Soy flour as a source of protein for baby pigs. J. Anim. Sci. 45: 1073–1078.

25. Kidder, D. E. & Manners, M. J. (1980) The level of distribution of carbohydrates in the small intestine mucosa of pigs from 3 weeks of age to maturity. Br. J. Nutr. 43: 141–153.

26. Mavromichalis, I., Parr, T. M., Gabert, V. M. & Baker, D. H. (2001) True ileal digestibility of amino acids in sow's milk for 17-day-old pigs. J. Anim. Sci. 79: 707–713.

27. Oliver, W. T., Mathews, S. A., Phillips, O., Jones, E. E., Odle, J. & Harrell, R. J. (2002) Efficacy of partially hydrolyzed corn syrup solids as a replacement for lactose in manufactured liquid diets for neonatal pigs. J. Anim. Sci. 80: 143–153.

28. Dreau, D., Lalles, J. P., Philouze-Rome, V., Toullec, R. & Salmon, H. (1994) Local and systemic immune responses to soybean protein ingestion in early-weaned pigs. J. Anim. Sci. 72: 2090–2098.