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EFFECTS OF ETHANOL EXTRACTION AND HEAT TREATMENT OF SOYBEAN FLAKES ON FUNCTION AND MORPHOLOGY OF PIG INTESTINE[1,2]

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ABSTRACT

Digesta and tissue samples were collected from the intestinal tracts of 27 pigs to determine the relationship between intestinal morphology and the utilization of nutrients from soybean flakes. Soybean flake treatments were under-, intermediate- and over-processed (i.e., 5, 20 and 60 min of autoclaving) either without extraction or with heating before or after extraction with a 55% ethanol-water mixture. Final BW was greatest ($P < .001$) for pigs fed soybean flakes given 20 min of heat treatment. There was a trend ($P < .09$) for plasma lysine concentrations to be reduced when the unextracted soybean flakes were over-processed (60 min of heat). Differences in the flow rate of DM and N through the ileum and colon reflected differences in DM and N intake, rather than differences in intestinal function. The soybean flake treatments had no effect ($P > .08$) on pH of the contents of the stomach, duodenum, ileum or colon. The ethanol extraction process increased ($P < .001$) N digestibility of the soybean flakes, especially when the soybean flakes were underprocessed (interaction, $P < .02$). Villus size (area, height and perimeter length) tended to be greater in pigs fed the soybean flakes heated after extraction and(or) exposed to the intermediate level of heat treatment. Indicators of villus shape (villus area/villus height) and proliferative activity (crypt depth and villus height/crypt depth) were not affected by soybean flake treatment ($P > .08$). Ethanol extraction and heat treatment affected the utilization of nutrients from soybean flakes. These effects presumably were mediated through changes in villus size, nutrient digestibility and availability, rather than from changes in villus shape, conditions reflecting an allergic response.

(Key Words: Soy Protein, Ethanol, Extraction, Small Intestine, Morphology, Pigs.)

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Introduction

Researchers have identified and overcome many of the factors that limit the utilization of nutrients from unprocessed soybeans. Improved utilization of nutrients resulting from heat treatment of soybeans has been credited to inactivation of protease inhibitors that both limit digestibility (Borchers et al., 1948; Rackis et al., 1975; Vandergrift et al., 1983) and induce hypersecretion of pancreatic zymogens (Chernick et al., 1948; Booth et al., 1960). Yet the inability of the young pig to utilize nutrients from soybeans has led swine nutritionists to recommend that more expensive milk products be used instead of soybean products in diets for young pigs.

Smith and Sissons (1975), Barratt et al. (1978), Kilshaw and Sissons (1979) and Seegraber and Morrill (1982) reported that feeding milk replacers containing soybean protein to preruminant calves caused digestive

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disturbances and morphological changes in the intestine and elevated serum antibodies. Sissons et al. (1979, 1982) suggested that the allergenicity of soybean proteins is eliminated by extracting it with aqueous ethanol. Hancock et al. (1989, 1990a,b) reported previously that alcohol extraction improved the utilization of protein from heated soybean flakes. This experiment was conducted to determine the effects of ethanol extraction and duration of heat treatment of soybean flakes on changes in function and morphology of pig intestine.

Materials and Methods

Defatted raw soybean flakes (52-kg samples) were autoclaved at 121°C and 1.1 kg/cm^2 steam pressure for 5, 20 or 60 min either before (B) or after (A) extraction with ethanol (OH) or with no ethanol treatment (W/O). The soybean flakes were spread onto stainless steel pans (60 × 70 × 3 cm) that were stacked in a steam sterilizer[7], with spacers to hold the pans approximately 10 cm apart. The soybean flakes were autoclaved for 5, 20 or 60 min after the sterilizer chamber reached 100°C. After the allotted time, the steam was rapidly exhausted from the sterilizer and the soybean flakes were promptly removed and spread onto a cool concrete floor. Ethanol extraction involved placing the soybean flakes in a 90-cm × 56-cm metal drum filled with a 55% ethanol-water mixture (v/v) at 0700. The soybean flakes were stirred manually at 0700, 1200 and 1800. After the stirring at 1800, the spent ethanol-water mixture was allowed to drain from the soybean flakes and fresh ethanol-water mixture was added the next morning at 0700. This process was repeated daily for 4 d. Upon completion of extraction, the soybean flakes were spread onto plastic sheets and dried under forced air at 23°C for 48 h. All soybean flake preparations were ground[8] through a 1.5-mm screen before use. The soybean flake preparations were incorporated into a basal diet containing 76.4% corn (Table 1). Treatments were duration of heat (5, 20 and 60 min) without ethanol extraction (W/O-OH) or with heat treatment before or after extraction (B-OH and A-OH) in a 3 × 3 factorial arrangement. The corn contributed 7.3% CP to all diets, and the soybean flake preparations were added at the expense of cornstarch to bring the total CP content of each diet to 15%.

TABLE 1. BASAL DIET

Ingredient	Amount, %
Soybean flakes[ab]	
Corn	76.40
Cornstarch	18.90
Salt	.30
Tallow	3.00
Limestone[b]	
Dicalcium phosphate[b]	
Vitamin and mineral mix[c]	1.15
Chromic oxide	.25

[a]Soybean flakes were prepared by autoclaving for 5, 20 and 60 min either without ethanol extraction, or with heat treatment either before or after extraction with a 55% ethanol-water mixture.

[b]Soybean flakes, limestone and dicalcium phosphate were added at the expense of cornstarch to bring the diets to 15% CP, .8% Ca and .7% P.

[c]Provided the following in mg/kg complete diet: Zn, 75; Fe, 87.5; Mn, 30; Cu, 8.8; I, 1; Se, .1; and the following per kg complete diet: vitamin A, 5,500 IU; vitamin D_3, 550 IU; vitamin E, 22 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 5.5 mg; d-pantothenic acid, 19.8 mg; niacin, 33 mg; choline chloride, 551 mg; vitamin B_{12}, 16 μg; ethoxyquin, 4.4 mg.

Protein quality of the soybean flake preparations was evaluated in a growth assay and N balance experiment; those results have been reported previously (Hancock et al., 1990b). Upon completion of the 24-d growth assay, three pigs fed each dietary treatment were chosen at random and fed their respective experimental diet plus .25% chromic oxide for 4 d. These pigs were weighed and anesthetized with nitrous oxide and trifluoroethane gas. Approximately 10 ml of blood were collected from the brachial region with heparinized vacuum tubes. The abdominal cavity was opened and samples of the duodenum (10 cm distal to the pyloric valve) and ileum (10 cm proximal to the ileo-cecal juncture) were collected. Tissue samples were rinsed with physiological saline and quickly plunged into a 10% formalin solution. These samples were stored in the formalin solution at room temperature until they were examined histologically. The remainder of the digestive tract was removed and samples (60 to 80 ml) of digesta from the stomach, proximal duodenum, distal ileum and distal colon were collected prompt-

[7]Steam Sterilizer, Model No. 57 CR, American Sterilizer Co., Erie, PA.

[8]Jacobson Pulverator, No. 66 B, Jacobson Machine Works, Inc., Minneapolis, MN.

TABLE 2. EFFECTS OF ETHANOL EXTRACTION AND HEAT TREATMENT OF SOYBEAN FLAKES ON FINAL BODY WEIGHT AND PLASMA CONSTITUENTS OF PIGS

	W/O[a], min of autoclaving			B[a], min of autoclaving			A[a], min of autoclaving			
Item	5	20	60	5	20	60	5	20	60	CV
Initial wt, kg[b]	8.7	8.8	8.8	8.8	9.0	9.0	9.2	9.2	9.1	6.5
Final wt, kg[c]	10.5	19.9	15.4	15.8	21.4	16.2	18.3	22.0	21.7	7.4
Plasma urea, mg/dl[d]	44.0	38.2	43.2	34.2	35.4	44.2	31.5	28.4	39.6	12.4
Plasma lysine, %[e]	1.24	1.13	.69	1.11	1.09	.92	1.17	1.16	1.32	25.8

[a]W/O = heat treatment without ethanol extraction; B = heat treatment before ethanol extraction; A = heat treatment after ethanol extraction.

[b]No heat or extraction effect ($P > .27$).

[c]Effect of heat treatment was linear ($P < .04$) and quadratic ($P < .001$); W/O vs B+A ($P < .001$); B vs A ($P < .001$); heat treatment linear × B vs A ($P < .005$); heat treatment linear × W/O vs B+A ($P < .05$); heat treatment quadratic × W/O vs B+A ($P < .02$).

[d]Effect of heat treatment was linear ($P < .005$); W/O vs B+A ($P < .005$); B vs A ($P < .05$).

[e]No heat or extraction effect ($P > .08$).

ly. The pH of the samples was determined and the samples were frozen in a dry ice-methanol bath. These samples were stored frozen until they were analyzed for DM, N and Cr content.

Blood samples were centrifuged under refrigeration at 1,400 × *g* for 30 min. A 2-ml aliquot of plasma was mixed vigorously with 60 mg sulfosalicylic acid and centrifuged. The deproteinized plasma was decanted into a plastic tube, capped and frozen along with a 2- to 3-ml aliquot of the original plasma. Plasma urea concentration was determined using the automated procedure described by Marsh et al. (1965). Plasma lysine concentration was determined on the deproteinized plasma samples using ion exchange chromatography.

Two subsamples of the duodenum and ileum tissues were embedded in paraffin wax; sections were cut at 4 μm thickness, mounted on slides and stained with hematoxylin and eosin by the periodic acid Schiff method (Luna, 1968). Fifteen to twenty villi and crypts were selected from each sample of duodenum and ileum. Area, height and length of perimeter were measured on each villus; depth was measured on each crypt using a computer-integrated microscope, digitizer pad[9] and high-resolution monitor.

Digesta samples were lyophilized and analyzed for DM and CP content by the Kjeldahl procedure (AOAC, 1984). Chromium content was determined by atomic absorption spectroscopy[10].

Statistical analyses were conducted as a completely randomized design with a 3 × 3 factorial arrangement of treatments. Orthogonal polynomials were used to compare the shapes of the response curves resulting from 5, 20 and 60 min of autoclaving of the soybean flakes heated W/O-OH, B-OH and A-OH. The error mean square for the three-way interaction (block × heating time × extraction regimen) was used as the error term to test all main effects and interactions and to draw treatment comparisons. All statistical analyses were conducted using SAS (1982).

Results and Discussion

The quadratic response to duration of heat treatment on final weight was more pronounced in pigs fed the W/O-OH soybean flakes than in pigs fed B-OH and A-OH soybean flakes (i.e., heat treatment × extraction regimen interaction, $P < .02$). Hancock et al. (1990b) suggested that the extraction time × heat interaction for pigs fed soybean flakes extracted with ethanol occurred because ethanol reduced trypsin inhibitor activity of under-processed (i.e., 5 min autoclaved B-OH and A-OH) soybean flakes. Also, protein quality was maintained by removing the reducing sugars with ethanol before exposing the soybean flakes to extreme heat treatment (60 min autoclaving of A-OH).

[9]Bioquant Systems IV Hipad Digitizer, Houston Instruments, Austin, TX.

[10]Model 30, Varian Techtron Pty. Ltd., Springvale, Australia.

Duration of heat treatment had a quadratic effect ($P < .001$) on final weight of pigs fed the soybean flake preparations (Table 2). As heat treatment increased from 5 to 20 min, final pig weight was increased (21.1 vs 14.9 kg), and as heat treatment was increased further to 60 min, final weight was decreased to 17.8 kg. This quadratic effect of heat treatment is consistent with responses reported previously from experiments with rats (Klose et al., 1946; Borchers, 1965; Hancock et al., 1990b), chickens (Clandinin et al., 1948; McNaughton et al., 1981) and pigs (Heinz and Poppe, 1975; Garren et al., 1982).

There was a trend ($P < .06$) for plasma urea concentration to respond in a quadratic manner to heat treatment (36.6, 34.0 and 42.3 mg/dl for 5, 20 and 60 min of autoclaving, respectively); pigs fed soybean flakes autoclaved for 20 min tended to have the lowest values. Pigs fed B-OH and A-OH soybean flakes had plasma urea concentrations that were 15% lower than those of pigs fed the W/O-OH soybean flakes ($P < .005$). The within-treatment variation for plasma lysine concentration was high, with a CV of 25.8%. However, there was a trend ($P < .09$) for lower plasma lysine concentrations in pigs fed W/O-OH and B-OH soybean flakes heated for 60 min. Plakas et al. (1985) reported that plasma lysine concentration of rainbow trout declined when they were fed fish protein hydrolysates that had been allowed to react with glucose. A decline in plasma lysine concentration is of particular interest in the present study because the diets were formulated to be equal but limiting in lysine; therefore, lysine content of the free amino acid pool would determine rate of protein accretion and lean tissue growth.

The pH of the contents of the stomach and small intestine was not affected ($P > .05$) by the soybean flake treatment (Table 3). Decuypere et al. (1981a,b) suggested that, compared to milk proteins, soybean proteins impair clot formation in the stomach. The lack of clot formation increases buffering of stomach contents and attenuates the decline in gastric pH after feeding, which reduces the extent of peptic digestion of ingested proteins. The lack of pH changes in the present study casts doubt on the likelihood that ethanol extraction or heat treatment of the soybean flakes affected performance of pigs by altering the pH of digesta.

DM and N flow in the pig ileum and colon responded in a quadratic manner ($P < .002$) to duration of heat treatment of soybean flakes (Table 4). These responses corresponded closely to the quadratic responses in DM and N intakes presented in Table 3. Other differences in DM and N flow, such as the increases with ethanol extraction of soybean flakes, mimicked differences in DM and N intakes. Smith and Sissons (1975) reported that source of dietary protein had a large effect on the rate of flow of digesta from the abomasum of the preruminant calf. Feeding milk replacers containing casein or alcohol-extracted soyflour resulted in a slow, steady release of N from the abomasum. They suggested that feeding heated or unheated soyflour greatly inhibited the flow of digesta for some hours after feeding, followed by a rapid outflow of digesta through the small intestine. Smith and Sissons (1975) proposed that the aberrant abomasal emptying resulted from gastrointestinal allergy. Asche (1987) reported that flow rates of DM, total N and the particulate fraction of digesta was higher in the stomach and small intestine of pigs fed diets containing dried skim milk than in those of pigs fed diets containing soybean meal. In the present experiment, differences in flow of DM and N may have been due to differences in feed intake rather than to differences in digestive tract function in response to alcohol extraction and(or) duration of heat treatment of the soybean flake preparations.

Apparent DM and N digestibility coefficients for the soybean flake preparations from samples taken at the terminal ileum and colon are given in Table 5. DM digestibilities generally were not as responsive as N digestibilities to treatment. Duration of heat treatment did not affect DM digestibility ($P > .18$), although the underprocessed, unextracted soybean flakes (W/O + 5) had the lowest percentage DM digestibility at the ileum (57.8%) of any treatment. Alcohol extraction (i.e., B-OH and A-OH) increased DM digestibility ($P < .001$) compared with the W/O-OH soybean flakes. N digestibility was greater in the B-OH and A-OH soybean flakes than in W/O-OH soybean flakes ($P < .001$) and responded in a quadratic manner to duration of heat treatment at the ileum ($P < .006$) and colon ($P < .05$). Although the ileal DM and N digestibility coefficients were consistently lower than the digestibility coefficients at the

TABLE 3. EFFECTS OF ETHANOL EXTRACTION AND HEAT TREATMENT OF SOYBEAN FLAKES ON NUTRIENT INTAKE AND pH OF DIGESTA IN PIGS

Treatment[a]	DM intake, g/d[b]	N intake, g/d[c]	pH of contents			
			Stomach[d]	Duodenum[d]	Ileum[d]	Colon[d]
W/O + 5	334	8.7	3.5	6.4	6.6	6.6
W/O + 20	1,062	27.3	3.2	6.3	6.5	6.6
W/O + 60	725	18.6	3.1	6.2	6.4	6.4
B + 5	704	18.4	2.9	6.1	6.6	6.9
B + 20	1,102	29.1	4.0	6.2	6.6	6.5
B + 60	820	21.7	3.6	6.3	6.2	6.5
A + 5	805	20.7	3.0	6.3	6.5	6.5
A + 20	1,204	31.8	2.8	6.3	6.5	6.3
A + 60	1,292	34.5	3.5	6.1	6.7	6.4
CV	16.5	16.6	17.4	3.1	3.3	2.8

[a]W/O = heat treatment without ethanol extraction; B = heat treatment before ethanol extraction; A = heat treatment after ethanol extraction; 5, 20 and 60 = min of autoclaving.

[b]Effect of heat treatment was linear ($P < .006$) and quadratic ($P < .001$); W/O vs B+A ($P < .001$); B vs A ($P < .005$); heat treatment linear × B vs A ($P < .02$).

[c]Effect of heat treatment was linear ($P < .004$) and quadratic ($P < .001$); W/O vs B+A ($P < .001$); B vs A ($P < .005$); heat treatment linear × B vs A ($P < .02$).

[d]No heat or extraction ($P > .08$).

colon (18% for DM and 10% for N), the differences between treatments were consistent. Only with the soybean flake preparation considered to have the lowest nutritional value (i.e., W/O + 5) was the digestibility value affected to a markedly greater extent at the ileum than at the colon. These results compare favorably to those of Walker et al. (1986a,b), in which trends for ileal digestibility values were similar to total tract digestibility values between different soybean protein preparations.

Tables 6 and 7 contain measurements of villus size (area, height and perimeter length), villus shape (villus area/villus height) and indicators of mucosal epithelial cell prolifera-

TABLE 4. EFFECTS OF ETHANOL EXTRACTION AND HEAT TREATMENT OF SOYBEAN FLAKES ON FLOW OF DRY MATTER AND NITROGEN IN PIGS

Treatment[b]	DM flow, g/d[a]		N flow, g/d[a]	
	Ileum[c]	Colon[d]	Ileum[e]	Colon[f]
W/O + 5	155	80	5.0	4.0
W/O + 20	451	260	10.2	8.7
W/O + 60	229	168	6.3	6.5
B + 5	203	115	5.8	4.1
B + 20	333	205	8.0	6.6
B + 60	241	148	6.4	5.4
A + 5	264	134	6.6	4.5
A + 20	426	204	10.1	7.7
A + 60	462	233	12.0	8.4
CV	28.6	24.3	24.2	27.9

[a](Marker intake, g/d)/(marker concentration in digesta sample/nutrient concentration in digesta sample).

[b]W/O = heat treatment without ethanol extraction; B = heat treatment before ethanol extraction; A = heat treatment after ethanol extraction; 5, 20 and 60 = minutes of autoclaving.

[c]Effect of heat treatment was quadratic ($P < .001$); B vs A ($P < .008$); heat treatment linear × B vs A ($P < .03$).

[d]Effect of heat treatment was linear ($P < .03$) and quadratic ($P < .001$); heat treatment linear × B vs A ($P < .02$).

[e]Effect of heat treatment was quadratic ($P < .002$); B vs A ($P < .005$); heat treatment linear × B vs A ($P < .02$).

[f]Effect of heat treatment was linear ($P < .04$) and quadratic ($P < .002$).

TABLE 5. EFFECTS OF ETHANOL EXTRACTION AND HEAT TREATMENT OF SOYBEAN FLAKES ON DIGESTIBILITY OF DRY MATTER AND NITROGEN

Treatment[a]	DM digestibility, %		N digestibility, %	
	Ileum[b]	Colon[c]	Ileum[d]	Colon[e]
W/O + 5	57.8	77.3	42.5	56.2
W/O + 20	63.2	78.8	67.9	72.7
W/O + 60	67.5	75.9	64.4	64.1
B + 5	69.9	83.0	67.4	76.6
B + 20	68.6	81.0	71.7	76.9
B + 60	68.7	80.7	68.1	73.3
A + 5	68.7	84.1	69.5	79.2
A + 20	64.5	83.0	68.2	75.8
A + 60	63.4	81.5	64.3	75.0
CV	12.3	3.9	9.2	7.0

[a]W/O = heat treatment without ethanol extraction; B = heat treatment before ethanol extraction; A = heat treatment after ethanol extraction; 5, 20 and 60 = minutes of autoclaving.

[b]No heat or extraction effect ($P > .18$).

[c]W/O vs B+A ($P < .002$).

[d]Effect of heat treatment was quadratic ($P < .006$); W/O vs B+A ($P < .001$); heat treatment linear × W/O vs B+A ($P < .001$); heat treatment quadratic × W/O vs B+A ($P < .02$).

[e]Effect of heat treatment was quadratic ($P < .05$); W/O vs B+A ($P < .001$); heat treatment linear × W/O vs B+A ($P < .02$).

tion (crypt depth and villus height/crypt depth). Because the SF treatments affected final weight, analysis of covariance was used to determine whether the morphological measurements should be adjusted for final BW. None of the measurements was affected by final BW ($P > .42$); thus, the data in Tables 6 and 7 are unadjusted means.

Villus height of duodenal samples responded in a quadratic manner to duration of heat treatment of the soybean flakes ($P < .02$). As with final pig weight and digestibility, there was a trend ($P < .08$) for the quadratic response to be most pronounced in pigs fed the W/O-OH soybean flakes; this trend was more subtle in pigs fed the B-OH and A-OH soybean flakes. Pigs fed the A-OH soybean flakes tended to have longer villi than pigs fed B-OH and W/O-OH treatments. Other measurements of villus size (area and perimeter length) responded in a manner similar to those of villus height.

Barratt et al. (1978) reported that digestive disturbances were severe in preruminant calves fed milk replacers containing soybean protein. Intestinal biopsies indicated that feeding soybean protein caused morphological disturbances in the intestinal villus and lamina propria; the villi had become shorter and broader with reduced surface area. Seegraber and Morrill (1979, 1982) found that calves fed milk replacers containing soybean protein had reduced absorptive capacity in the small intestine, as indicated by suppressed xylose absorption. Subjective evaluation of scanning electron micrographs indicated that exposure to soybean proteins caused villus atrophy, as characterized by shortening, blunting and obliteration of villi. Newby et al. (1984) contended that feeding intact casein protein, compared with feeding hydrolyzed casein, resulted in hyperplasia of mucosal epithelial cells (as indicated by increased crypt depth) in newly weaned pigs. The authors stated that mucosal cell hyperplasia is symptomatic of a gastrointestinal allergy. In the present experiment, the greater villus height and size in pigs fed ethanol-extracted soybean flakes correlates well with faster growth rate of pigs fed those soybean preparations and strengthens the argument that ethanol extraction of soybean proteins can improve the functional morphology of the intestinal mucosa. However, villus area/villus height (a measurement of villi shape) was not affected by treatment; indicators of epithelial cell hyperplasia (crypt depth and villus height/crypt depth) also were not affected by soybean flake treatment ($P > .08$). Thus, the greater height and area of villi from pigs fed soybean flakes extracted with ethanol

TABLE 6. EFFECTS OF ETHANOL EXTRACTION AND HEAT TREATMENT OF SOYBEAN FLAKES ON MORPHOLOGY OF THE PIG DUODENUM

Treatment[a]	Villus area, μm^{2}[b]	Villus height, μm[c]	Perimeter length, μm[d]	Crypt depth, μm[e]	Area/ height[e]	Height/ depth[e]
W/O + 5	5,006	370	824	445	13.5	.83
W/O + 20	7,746	493	1,060	508	15.7	.97
W/O + 60	6,821	461	1,026	520	14.8	.89
B + 5	6,608	437	1,002	540	15.1	.81
B + 20	6,720	474	1,055	511	14.2	.93
B + 60	5,310	379	831	556	14.0	.68
A + 5	7,219	454	1,034	528	15.9	.86
A + 20	8,232	539	1,168	530	15.3	1.02
A + 60	8,076	520	1,126	488	15.5	1.07
CV	20.4	14.2	13.0	18.1	10.6	28.4

[a]W/O = heat treatment without ethanol extraction; B = heat treatment before ethanol extraction; A = heat treatment after ethanol extraction; 5, 20 and 60 = minutes of autoclaving.

[b]B vs A ($P < .03$).

[c]Effect of heat treatment was quadratic ($P < .02$); B vs A ($P < .03$).

[d]Effect of heat treatment was quadratic ($P < .04$); B vs A ($P < .04$); heat treatment quadratic × W/O vs B+A ($P < .05$).

[e]No heat or extraction effect ($P > .08$).

was not accompanied by changes in shape and cell proliferative activity that would reflect reduced antigenicity. Probably some other factor(s), such as improved nutritional status of the mucosal enterocytes themselves, may contribute to the greater villus size for pigs fed ethanol-extracted soybean flakes.

Implications

The quality of soybean protein was affected by both duration of heat treatment and extraction with ethanol. Ethanol extraction improved the quality of soybean protein, especially when the soy protein was under- or over-processed. Results from this experiment do not support the hypothesis that ethanol extraction affects the utilization of soybean protein by altering the pH or flow rate of digesta. However, changes in intestinal morphology (i.e., longer and larger villi) for pigs fed ethanol-extracted soybean flakes may help explain why treatments improved performance of pigs.

TABLE 7. EFFECTS OF ETHANOL EXTRACTION AND HEAT TREATMENT OF SOYBEAN FLAKES ON MORPHOLOGY OF THE PIG ILEUM

Treatment[a]	Villus area, μm^{2}[b]	Villus height, μm[b]	Perimeter length, μm[c]	Crypt depth, μm[b]	Area/ height[b]	Height/ depth[b]
W/O + 5	3,841	301	664	294	12.8	1.02
W/O + 20	4,670	329	739	404	14.2	.81
W/O + 60	4,616	344	801	308	13.4	1.12
B + 5	5,335	368	850	345	14.5	1.07
B + 20	5,490	369	962	353	14.9	1.05
B + 60	4,149	284	644	352	14.6	.81
A + 5	5,588	408	988	362	13.7	1.13
A + 20	5,230	384	893	347	13.6	1.11
A + 60	5,275	353	767	345	14.9	1.02
CV	23.0	16.9	14.8	16.1	14.1	22.2

[a]W/O = heat treatment without ethanol extraction; B = heat treatment before ethanol extraction; A = heat treatment after ethanol extraction; 5, 20 and 60 = minutes of autoclaving.

[b]No heat or extraction effect ($P > .09$).

[c]W/O vs B+A ($P < .03$); heat treatment quadratic × B vs A ($P < .02$).

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