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## **Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs**

### **Authors**

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**INTERRELATIONSHIP BETWEEN HYPERSENSITIVITY  
TO SOYBEAN PROTEINS AND GROWTH PERFORMANCE  
IN EARLY-WEANED PIGS**

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Summary

One hundred twenty-five pigs were orally infused with 6 g/d of either dried skim milk, soybean meal (48% CP), soy protein concentrate, extruded soy protein concentrate, or experimental soy protein concentrate from 7 to 11 d of age and then fed a diet containing the corresponding protein sources from weaning (d 21) to 35 d of age. All pigs were fed a corn-soybean meal diet containing 10% dried whey, 1.25% lysine, and 3% soybean oil for the remaining 21 d of the experiment. Skin-fold thickness following intradermal injection of protein extracts, xylose absorption, and anti-soy immunoglobulin G (IgG) titers were measured on d 6 postweaning. A total of 25 pigs (five pigs/treatment) was euthanized on d 7 postweaning. Villus height and crypt depth from duodenum samples were measured. These measurements were obtained to elucidate a relationship between the hypersensitivity responses to soybean products and growth performance of baby pigs. Pigs fed diets containing soybean meal had a lower rate of gain (ADG), lower villus height, higher serum anti-soy IgG titers, and increased skin-fold thickness following intradermal injection compared to those fed dried skim milk. Pigs fed other soy proteins also had lower ADG from d 0 to 14 postweaning; however, pigs fed moist-extruded soy protein concentrate tended to have higher ADG and improved feed utilization when compared to those pigs fed soybean meal. Skin-fold thickness and anti-soy IgG titers were negatively correlated with ADG at d 14 postweaning. Results indicate that a model including skin-fold thickness and anti-soy IgG titers provided a good estimate of nursery pig growth performance ( $R^2=.33$ ). Villus height was related to ADG at d 14 postweaning ( $R^2=.40$ ). A combination of skin-fold thickness, anti-soy IgG titers, xylose absorption, villus height, and crypt depth provided the best estimate of growth performance ( $R^2=.65$ ) for early-weaned pigs.

(Key Words: Starter, Piglet, SBM, Soybean, Process, Performance.)

**Introduction**

Research indicates that pigs fed diets containing commercially prepared soybean meal (SBM) have a transient hypersensitivity (allergy) response to soybean proteins. Sensitization and challenge by proteins present in SBM may lead to abnormalities in digestive processes,

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including disorders in digesta movement and an inflammatory response in the intestinal mucosa of the early-weaned pig.

Because it appears that the pig mounts an immune response to some of the detrimental antigens presented in conventionally processed SBM, this study was designed to determine if immune response criteria might be an effective indicator of soy protein quality. Therefore, the objective of this study was to establish a relationship between gut morphology, immunological responses to dietary soy protein, and growth performance in starter pigs fed different soy proteins.

### Procedures

One hundred twenty-five crossbred (Hampshire × Yorkshire × Duroc) pigs from 16 litters with an average birth weight of  $2.9 \pm .6$  lb were utilized. Sows were fed a corn-corn gluten meal (14% CP, .65% lysine) diet from d 109 of pregnancy throughout lactation in order to limit passive transfer of maternal anti-soybean protein antibodies to the baby pigs via colostrum. The pigs were allotted randomly to one of five treatment groups (25 pigs/group). In order to sensitize the pigs to dietary proteins, they were infused through a stomach tube from d 7 to d 11 with  $6 \text{ g.pig}^{-1}.\text{d}^{-1}$  of either dried skim milk (control), soybean meal (48% CP), soy-protein concentrate, extruded soy-protein concentrate, or experimental soy protein concentrate. Pigs were housed in an environmentally controlled nursery (5 pigs/pen; 5 replications/treatments) with pen dimensions of  $4 \times 5$  ft, with woven wire floors over a Y-flush gutter, and one nipple waterer and one, four-hole, self-feeder per pen. The room temperature was at 95°F for the first 7 d, and then was lowered 3°F each week thereafter. On d 6 postweaning, blood samples were taken from the jugular vein for determination of IgG titers to soybean proteins, glycinin, and  $\beta$ -conglycinin. All pigs were weaned on d 21 and until d 28 were reallocated by litter, sex, and weight to one of the five dietary treatments (Table 1) containing the same dietary protein sources that were infused during the preweaning period. Water and feed were available ad libitum. Postweaning diets were formulated to contain identical amounts of lysine, Ca, P, and ME. On d 7 postweaning, all pigs were fed the same corn-soybean meal diet (Table 2) containing 3% soybean oil and 1.25% lysine for the remaining 21 d of the experiment. Feed consumption and individual pig weights were measured at weekly intervals to determine average daily gain (ADG), average daily feed intake (ADFI), and feed/gain (F/G).

On d 7 postweaning, 25 pigs (5 pigs/treatment) were euthanized, and small intestines were immediately excised. Samples were collected, then sectioned at 6 mm thickness and stained with azur A and eosin. Intact villi were measured in four specimens for each pig within each group. Villus height was measured from the crypt mouth to the villus tip.

Cutaneous hypersensitivity to the corresponding protein to which the pigs were sensitized was tested on d 6 postweaning. Results were expressed as difference in skin-fold thickness (mm) obtained with protein compared to saline injections.

Small intestinal contents were obtained and mixed immediately after pigs were euthanized. Subsamples were prepared and plated in plate count agar (PCA) and violet red bile agar (VRB), followed by a standard plate count procedure.

Stepwise regression and simple correlations among villus height, crypt depth, anti-soy antibody titers, skin-fold thickness, and xylose absorption as related to ADG and F/G at d 14 postweaning were evaluated.

**Table 1. Diet Composition (D 0 to 14 Postweaning)<sup>a</sup>**

Ingredient, %	Diets				
	Milk protein	Soybean meal	Soy protein concentrate	Extruded soy protein concentrate	Experimental soy protein concentrate
Ground corn	9.82	7.50	7.50	7.50	7.50
Oat groats	29.13	11.69	27.08	27.08	27.08
Skim milk	35.00				
Dried whey	20.00				
Soybean meal (48%, CP)		38.13			
Soy protein concentrate			24.07		
Extruded soy protein concentrate				24.07	
Experimental soy protein concentrate					24.07
Dicalcium phosphate (18.5% P)	.24	2.26	2.29	2.29	2.29
Limestone	.26	.41	.39	.39	.39
Fat <sup>b</sup>	4.78	7.74	6.37	6.37	6.37
Trace mineral premix <sup>c</sup>	.12	.12	.12	.12	.12
Vitamin Premix <sup>d</sup>	.09	.09	.09	.09	.09
Lactose		31.50	31.50	31.50	31.50
L-lysine HCL (78%)	.10	.10	.13	.13	.13
CuSO <sub>4</sub> <sup>e</sup>	.09	.09	.09	.09	.09
Salt	.35	.35	.35	.35	.35
Ethoxyquin	.02	.02	.02	.02	.02

<sup>a</sup>Calculated analysis of the diet: ME, 1.59 Mcal/lb; lysine, 1.35%, .8%; P, .7%.

<sup>b</sup>Fat was 2% soybean oil and remainder was lard.

<sup>c</sup>Provided the following per lb of the complete diet (mg): Zn, 32; Fe, 23; Mn, 12; Cu, 2.3; Co, .23; I, .32; Se, .14.

<sup>d</sup>Provided the following per lb of complete diet: vitamin A, 2000 IU; vitamin D<sub>3</sub>, 200 IU; vitamin E, 6.7 IU; vitamin K, 1.3 mg; riboflavin, 2.0 mg; niacin, 12.6 mg; d-pantothenic acid, 8.0 mg; vitamin B<sub>12</sub>, 8.0 µg.

<sup>e</sup>Supplied complete diet with 240 ppm supplemental Cu.

**Table 2. Diet Composition (D 14 to 35 Postweaning)**

Ingredient	%	Ingredient	%
Corn	59.32	Vitamin premix <sup>a</sup>	.25
Soybean meal (48%)	23.75	Trace mineral premix <sup>b</sup>	.25
Dried whey	10.00	Se premix <sup>c</sup>	.05
Soy oil	3.00	L-Lysine.HCL (78%)	.35
Dicalcium phosphate	1.51	CuSO <sub>4</sub>	.10
Limestone	.82	Antibiotic <sup>d</sup>	.50
Salt	.25		

<sup>a</sup>Provided the following per lb of complete diet: vitamin A, 2000 IU; vitamin D<sub>3</sub>, 200 IU; vitamin E, 6.7 IU; vitamin K, 1.3 mg; riboflavin, 2.0 mg; niacin, 12.0 mg; d-pantothenic acid, 8.0 mg; vitamin B<sub>12</sub>, 8.0 µg.

<sup>b</sup>Provided the following per lb of the complete diet (mg): Zn, 32; Fe, 23; Mn, 12; Cu, 2.3; Co, .23; I, .32; Se, .14.

<sup>c</sup>Provided .3 ppm selenium.

<sup>d</sup>Provided the following per lb of complete diet: 50 mg chlortetracycline, 50 mg sulfamethazine and 25 mg penicillin.

### Results and Discussion

At d 14 postweaning, pigs fed soybean meal had lower ADG and ADFI and poorer F/G ( $P < .05$ ) than pigs fed the diet containing dried skim milk (Table 4). Pigs fed extruded soy protein concentrate tended to have higher ADG ( $P < .09$ ) than those fed soybean meal and improved F/G ( $P < .05$ ) compared with those fed other soybean products. There were no differences in ADG, ADFI, and F/G among the treatments from d 14 to 35 postweaning.

These results generally agree with previous reports. However, pigs fed milk gained the same as those fed soybean products from d 14 to 35 postweaning in the present study. Inclusion of soybean meal in starter pig diets may be responsible for shortened intestinal villus height and hypertrophy in the crypt. Moist-extruded soy protein concentrate improved F/G for starter pigs from d 0 to 14, when compared with other soybean products used in this experiment. With increasing villus height, ADG at d 14 postweaning increased ( $R = .63$ ;  $P < .05$ ), indicating a relationship between villus height in the small intestine and growth rate in starter pigs. This is not surprising, because the reduction of villus height could decrease total luminal villus absorption area and could result in inadequate digestive enzyme development and (or) transport of nutrients at the villus surface. Reduced enzyme content of cells of the intestinal mucosal barrier could also alter the capacity of the gastrointestinal tract to digest antigenic proteins.

Changes in skin-fold thickness following intradermal injection of the extracts of corresponding proteins are presented in Table 3. Pigs given dried skim milk had lower ( $P < .05$ ) skin-fold thickness than pigs fed any of the soybean products. Pigs fed soybean meal had greater ( $P < .05$ ) skin-fold thickness than pigs fed either milk protein or other soy proteins. Pigs orally infused with soybean meal preweaning and fed a diet containing soybean meal postweaning showed higher ( $P < .05$ ) cutaneous hypersensitivity, coinciding with lower ( $P < .05$ )

ADG during 14 d postweaning. Pigs fed soybean meal had higher serum ( $P < .01$ ) IgG titers than pigs fed diets containing dried skim milk, soy-protein concentrate, extruded soy protein concentrate, or experimental soy-protein concentrate (Table 3). This clearly indicates that antigenicity of dietary proteins may be critical in influencing overall pig performance. Thus, it may be advantageous to predict the suitability of soybean products by determining titers of antigenic proteins by ELISA before including them in the diets of baby pigs. Because skin-fold thickness is relatively easy to measure, it also may be used as an indicator of antigenicity of the soybean products for baby pigs. A relationship ( $P = -.54$ ;  $P < .05$ ) was found between skin-fold thickness and ADG at d 14 postweaning. Also, pigs fed a diet containing soybean meal had lower ( $P < .05$ ) xylose concentrations in plasma (Table 3) than pigs fed diets containing soy protein concentrate, experimental soy protein concentrate, or dried skim milk, suggesting a compromised absorptive ability of the small intestine. However, there were no differences ( $P > .20$ ) in plasma xylose concentration of pigs fed soy protein concentrate, extruded soy protein concentrate, or experimental soy-protein concentrate.

Plate count agar counts represent total bacterial numbers present in the small intestinal contents, and VRB counts represent total number of coliforms present in the small intestinal contents. The percentage of coliforms in total bacteria (Table 3) was lower ( $P < .05$ ) in pigs fed milk protein than in pigs fed soybean products. Among soybean products, pigs fed soybean meal had the highest percentage of coliforms, and those fed extruded soy protein concentrate and experimental soy protein concentrate were intermediate. This suggests that soybean meal protein may be unsuitable for baby pigs and may favor coliform proliferation.

Pigs fed soybean meal had shorter villus height, greater crypt depth ( $P < .01$ ), and higher anti-soy IgG titers ( $P < .05$ ) than pigs fed either milk protein or the other soybean products (Table 3). There were no differences in villus height among pigs fed soy-protein concentrate, extruded soy-protein concentrate, or the experimental soy-protein concentrate; however, villus heights of pigs on all soy treatments were lower than that of pigs fed milk protein.

Villus height was negatively correlated ( $R = .67$ ;  $P < .05$ ) with skin-fold thickness (Table 5). A stepwise model that included skin-fold thickness and anti-soy IgG titers provided an estimate of growth performance ( $R^2 = .33$ ;  $P < .05$ ). Villus height provided a good estimate of ADG for pigs at d 14 of age ( $R^2 = .40$ ;  $P < .01$ ). A combination of skin-fold thickness, anti-soy IgG titers, xylose absorption, villus height, and crypt depth provided the best estimate of growth performance ( $R^2 = .65$ ;  $P < .05$ ) for the early-weaned pig fed various soy protein products. The best prediction model used to estimate ADG is listed below.

$$Y = 161.9 + .33X_1 + 81.4X_2 - .51X_3 + 16.76X_4 - 11.33X_5$$

Where, Y = ADG, lb

X<sub>1</sub> = villus height, micrometer

X<sub>2</sub> = xylose absorption, mg/100 ml

X<sub>3</sub> = crypt depth, micrometer

X<sub>4</sub> = anti-soy antibody titers, log 2

X<sub>5</sub> = skin-fold thickness, mm

This model can be used to differentiate the quality of various soybean products for utilization in formulating diets for nursery pigs. However, a model that includes non-invasive criteria (skin-fold thickness and anti-soy IgG titers) may be more practical and cost effective in predicting soybean product quality.

**Table 3. Effect of Feeding Different Soybean Products on Gut Morphology, Skin-Fold Thickness, Xylose Absorption, Anti-Soy IgG Titers, and Intestinal Bacteria of Starter Pigs**

Criteria	Milk protein	Soybean meal	Soy protein concn.	Extruded soy prot. concn.	Exp. soy Prot. con.	CV
Residual antigens in products <sup>a</sup>						
Glycinin	ND*	2.4	.9	.8	.5	
Beta-conglycinin	ND*	3.6	1.5	1.3	1.2	
Anti-soy IgG titers (Log2) <sup>a</sup>						
D 7 preweaning	3.05	3.10	2.91	2.96	2.99	14.0
D 6 postweaning	3.12 <sup>c</sup>	5.16 <sup>b</sup>	2.94 <sup>c</sup>	3.14 <sup>c</sup>	3.26 <sup>c</sup>	17.0
Skin-fold thickness, mm	.82 <sup>d</sup>	3.33 <sup>b</sup>	2.65 <sup>c</sup>	2.50 <sup>c</sup>	2.59 <sup>c</sup>	37.0
Xylose absorption, mg/100 ml	.82 <sup>b</sup>	.42 <sup>b</sup>	.61 <sup>bc</sup>	.67 <sup>bc</sup>	.78 <sup>b</sup>	31.8
Bacteria						
Total bacteria (10 <sup>6</sup> )	52	3	14	5	6	9
Coliform (10 <sup>6</sup> )	.89	1.11	3.4	.2	1.14	10
Coliform/total bacteria, %	1.7	37.0	24.3	4.0	23.3	15
Villus height, $\mu$ m	364.2 <sup>b</sup>	234.0 <sup>d</sup>	309.0 <sup>c</sup>	319.0 <sup>c</sup>	280.0 <sup>c</sup>	18.0
Crypt depth, $\mu$ m	198.0 <sup>c</sup>	222.4 <sup>b</sup>	214.9 <sup>c</sup>	195.7 <sup>c</sup>	189.7 <sup>c</sup>	16.5

<sup>a</sup>ELISA titers (Log2)

<sup>bcd</sup>Means within a row with unlike superscripts differ (P<.01). Five replications per treatment.

\*ND=none detected.



**Table 4. Effect of Different Soybean Products on Starter Pigs Performance<sup>a</sup>**

Criteria	Treatment					CV
	Milk protein	Soybean meal	Soy protein concn.	Extruded soy prot. concn.	Exper. soy prot. concn.	
ADG, lb						
d 0 - 14	.72 <sup>b</sup>	.40 <sup>c</sup>	.46 <sup>c</sup>	.50 <sup>c</sup>	.46 <sup>c</sup>	15.5
d 14 - 35	.98	1.18	1.15	1.12	1.16	8.1
d 0 - 35	.87	.86	.87	.89	.87	8.1
ADFI, lb						
d 0 - 14	.66 <sup>b</sup>	.55 <sup>c</sup>	.51 <sup>c</sup>	.53 <sup>c</sup>	.55 <sup>c</sup>	13.1
d 14 - 35	1.72	1.91	1.86	1.74	1.84	11.0
d 0 - 35	1.29	1.33	1.50	1.31	1.29	10.2
F/G						
d 0 - 14	.99 <sup>b</sup>	1.38 <sup>c</sup>	1.14 <sup>c</sup>	1.07 <sup>b</sup>	1.18 <sup>c</sup>	18.8
d 14 - 35	1.77	1.65	1.63	1.58	1.65	8.2
d 0 - 35	1.50	1.57	1.72	1.47	1.52	5.6

<sup>a</sup>Five pigs per pen, five replications per treatment, avg initial age of 21 d.

<sup>b</sup><sup>c</sup>Means same row with different superscript differ (P<.05).

**Table 5. Simple Correlations among Growth Performance and Immunological Criteria**

Item	Correlation coefficient					
	ADG	F/G	Skin-fold thickness	Villus height	Xylose absorption	Anti-soy IgG titers
Skin-fold thickness	-.54	.30	—	-.67	-.11	.22
Villus height	.63	-.46	-.67	—	.43	-.56
Xylose absorption	.54	-.49	-.11	.43	—	-.53
Anti-soy titers	-.30	.40	.22	-.56	-.53	—