

TRANSIENT HYPERSENSITIVITY TO SOYBEAN MEAL IN THE EARLY-WEANED PIG¹

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ABSTRACT

An experiment was conducted to determine whether baby pigs develop hypersensitivity to dietary soybean proteins. Thirty-two pigs were orally infused with either dried skim milk (5 g/d; control) or soybean meal (48% CP; 5 g/d) from d 7 to 14 after birth. Sows were fed a corn-corn gluten meal-based diet supplemented with lysine and tryptophan to avoid exposure of pigs to soybean proteins. Pigs were weaned at 21 d of age and fed diets containing either soybean meal or milk proteins until d 56. One half of the pigs were killed at 28 d of age and the rest at 56 d of age. Segments of small intestine were collected, and intraepithelial lymphocytes were isolated. At 28 d of age, pigs fed diets containing soybean meal had lower ($P < .05$) villus height (221 vs 298 μm) and rate of gain (86 vs 204 g/d) than control pigs did. Pigs fed a diet containing soybean meal had higher ($P < .05$) immunoglobulin G (IgG) titers to soybean protein than did pigs fed a milk protein-based diet. Blood and intestinal lymphocytes collected on d 28 and 56 did not exhibit any proliferative response when cultured with purified soy proteins (2.5 or 5 $\mu\text{g}/\text{ml}$). Phytohemagglutinin- and pokeweed mitogen-induced lymphocyte proliferations were higher ($P < .05$) at d 56 than at d 28, but there were no differences attributable to protein source. There were no differences ($P > .05$) in skin-fold thickness measurements following intradermal injection with soy or milk proteins. Decreased villus height and increased serum IgG titers to soybean proteins coinciding with inferior performance of early weaned pigs fed diets containing soybean meal indicate that conventionally processed, commercial soybean meal may retain some antigens that can cause transient hypersensitivity in piglets. (Key Words: Piglets, Soybean Meal, Intestines, Hypersensitivity.)

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Introduction

The trend of the swine industry is to wean pigs at younger ages in order to increase sow productivity. Research has focused on minimizing the severity of postweaning lags in pig performance. The allergenicity of soybean

proteins was first noted in humans by Duke (1934). Feeding a new dietary antigen may transiently increase crypt cell production rate, resulting in malabsorption and villus atrophy (Stokes et al., 1986). Soybean proteins, glycinin and beta-conglycinin, may be responsible for the hypersensitivity response of pigs (Stokes et al., 1984), calves (Kilshaw and Sissons, 1979; Dawson et al., 1988) and mice (Mowat and Ferguson, 1981). Miller et al. (1984a) found that abrupt changes in diet imposed on pigs at weaning can trigger an aberrant immune response. Enteropathic changes may occur even in the complete absence of microbial involvement, and because proliferation of *E. coli* is commonly observed subsequent to such changes, it has been suggested that *E. coli* is an opportunist rather

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than a primary pathogen (Miller et al., 1984b). This response may have a cascading effect within the small intestine and affect pig performance in the first few weeks following weaning.

Villus atrophy and malabsorption can be produced experimentally as a hypersensitivity response to dietary antigens by the intestine of the pig (Stokes et al., 1984). If the inferior performance of baby pigs fed soybean protein can be related to a hypersensitivity response, it may be necessary to consider immunological criteria in the quality control of soybean processing. Therefore, the objective of this study was to determine the effect of transient hypersensitivity to conventionally processed soybean meal on villus height, crypt depth, proliferative responses of blood and intestinal lymphocytes to mitogens and purified soy protein, and serum IgG titers specific to soy protein in early-weaned pigs.

Experimental Procedures

Animals and Experimental Design. Thirty-two crossbred (Hampshire × Yorkshire × Duroc) pigs from four litters with an average birth weight of 1.3 kg were utilized. Pigs in each litter were allotted randomly to one of the two treatment groups. In order to sensitize the pigs to the dietary proteins, pigs in treatment group 1 were infused orally every day with 5 g soybean meal (48% CP) through a stomach tube from d 7 to 14 of age; the remaining 16 pigs (treatment group 2) received dried skim milk. All the pigs were weaned at d 21 of age and were allowed ad libitum access to either a control (milk protein diet; treatment group 2) or an experimental diet (treatment group 1) containing soybean meal (Table 1) until d 56. Eight pigs selected randomly (two pigs/litter) from each treatment were killed at 28 d of age, and the remaining 16 were killed at 56 d of age. Samples of the duodenum were taken to isolate lymphocytes and for scanning electron and light microscope determination of villus height and crypt depth. One day before pigs were killed, blood samples were taken from the jugular vein to measure immunoglobulin G (IgG) titers to soybean protein and to isolate

lymphocytes. The pigs were kept in a temperature-controlled nursery with eight pigs/pen and weighed at d 1, 21, 28 and 56 of age.

Sows were fed a corn-corn gluten meal (14% CP) diet from d 109 of pregnancy through the lactation period in order to limit passive transfer of maternal anti-soybean protein antibodies to baby pigs through colostrum.

Lymphocyte Blastogenesis Assay. Lymphocytes from both blood and small intestines were tested for blastogenic response to mitogens and purified soybean proteins.

Peripheral Blood Mononuclear Cells. Heparinized whole blood, obtained by jugular venipuncture, was centrifuged (10 min at 700 \times g) to allow collection of the white blood cell layer. The buffy coat was diluted with an equal volume of RPMI medium⁵ supplemented with 25 mM HEPES⁶, 100 U/ml penicillin and 100 μ g/ml streptomycin⁵, then layered onto 4 ml of a density gradient (Histopaque-1077)⁶ and centrifuged for 40 min at 950 \times g. Cells at the interface were collected, mixed with RPMI, again layered over a density gradient and centrifuged for 30 min at 700 \times g. The cells then were collected from the interface and washed three times in medium, with the final wash in medium plus 10% (v/v) fetal bovine serum⁶ (FBS). Triplicate assays, with phytohemagglutinin⁵ (PHA; 100 μ l/ml), pokeweed mitogen⁵ (PWM; 100 μ l/ml) and medium controls, were performed in U-bottom microtiter plates⁷, using 2×10^5 cells per well in a 200- μ l final volume of medium + 10% FBS. Purified soybean proteins (1:1, wt/wt mixture of glycinin and β -conglycinin) were used at concentrations of 2.5 and 5 μ g/ml. Glycinin and β -conglycinin were purified in our laboratory by the method of Lei et al. (1983). Cultures were incubated for 48 h in a 93% air, 7% CO₂ humidified atmosphere at 37°C, the pulse labeled with 1 μ Ci/well of [³H]thymidine⁸ and incubated for an additional 18 h before being harvested onto glass-fiber discs. After liquid scintillation counting, results were reported as net cpm (cpm of mitogen-stimulated cultures minus cpm of nonstimulated cultures).

Intraepithelial Lymphocytes. The small intestine was excised immediately after slaughter. A 10-cm segment from the middle jejunum was removed and rinsed with saline then put into cold calcium- and magnesium-free Hanks Balanced Salt Solution (CMF-HBSS), with 2 μ g/ml fungizone⁵, 20 mM HEPES, 100 U/ml

⁵Gibco Laboratories, Grand Island, NY.

⁶Sigma Chemical Co., St. Louis, MO.

⁷Flow Laboratories, Inc, McLean, VA.

penicillin, and 100 µg/ml streptomycin for lymphocyte isolation. The samples were transported to the laboratory immediately.

The intestinal segments were washed five times with medium 1 (CMF-HBSS), and the serous layer was removed and placed in sterile petri dishes. Samples then were cut into 2- to 3-mm pieces, transferred into Windsor Flasks⁹, stirred in medium 2 (CMF-HBSS containing .145 mg/ml dithiothreitol⁵ (DTT), 5% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 µg/ml fungizone) for 15 min and then stirred in medium 3 (CMF-HBSS containing .37 mg/ml EDTA⁶, 5% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 µg/ml fungizone) for 45 min. The tissues were washed five times with medium 1 and two times with medium 4 (RPMI 1640 containing 20% FBS, .20 mg/ml collagenase⁵ (chromatographically purified), 100 U/ml penicillin, 100 µg/ml streptomycin, 2 µg/ml fungizone). Samples then were placed in 25 ml of medium 4 and incubated in loosely capped Windsor flasks in a 7% CO₂, 93% air, humidified atmosphere at 37°C with constant gentle stirring using a magnetic four-place stirrer¹⁰. After 6 h of digestion, the samples were centrifuged at 400 × g for 10 min at 25°C, supernatant fluids were discarded, and the pellet was washed with 10 ml of medium 5 (RPMI 1640, 5% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 µg/ml fungizone) and diluted with medium 5 (about 10 ml). The cell suspension then was layered over a density gradient and centrifuged at 400 × g for 30 min. Cells at the interface were harvested and washed three times with medium 5 before use in the blastogenesis assay as described above for blood lymphocytes.

In Vivo Hypersensitivity. Soybean protein-induced cutaneous hypersensitivity was tested on d 27 and 57. Whey, purified soybean protein (1 mg/ml) and physiologic saline were injected intradermally (.5 ml) in the skin fold of the flank. Skin-fold thickness was measured 24 h later using a constant pressure dial micrometer (Blecha et al., 1983). Results were expressed as difference in skin-fold thickness (mm) after protein vs saline injections.

TABLE 1. DIET COMPOSITION

Ingredient, %	Dietary treatments	
	Soybean	Milk
Soybean meal (48% CP)	36.9	
Dried skim milk		35.0
Lactose	30.0	
Dried whey		20.0
Corn	12.6	20.0
Oat groats	11.4	19.0
Choice white grease	3.6	3.0
Monocalcium phosphate (21% P)	2.2	.3
Soybean oil	2.0	2.0
Calcium carbonate	.4	.1
Salt	.3	
Swine trace mineral premix ^a	.3	.3
Vitamin premix ^b	.1	.1
Copper sulfate ^c	.1	.1
L-lysine-HCl (78% lysine)	.1	.1
Calculated composition		
ME, Kcal/kg	3,409	3,503
CP, %	20.6	18.7
Lysine, %	1.3	1.3
Ca, %	0.8	0.8
P, %	0.7	0.7

^aProvided the following per kg diet: 220 mg Fe; 150 mg Zn; 10 mg Mn; .1 mg I; 10 mg Cu and .3 mg Se.

^bProvided the following per kg diet: 4,400 IU Vitamin A; 330 IU Vitamin D₃; 22 IU Vitamin E; 5 mg riboflavin; 1.7 mg menadione, 13 mg pantothenic acid, 27.5 mg niacin, 500 mg choline chloride and 24.2 µg vitamin B-12.

^cProvided 240 mg Cu/kg diet.

Measurement of Serum IgG Titers to Soy Protein. Serum IgG titers specific to purified soy protein were measured at d 27 and 57. Ninety-six-well microtiter plates were coated (.1 ml/well) with purified soy protein (50 µg/ml diluted in carbonate buffer, .05 M, pH 9.6) for 1 h. The plates were washed twice with phosphate-buffered saline (PBS) + .1% Tween 20, and the uncoated sites were blocked by adding PBS with 1.0% ovalbumin (.2 ml/well) for 30 min. Serial doubling dilutions (diluted in PBS + .1% Tween 20) of test serum samples then were applied (.1 ml/well) to the plates (three wells/dilution). After a 1-h incubation, plates were washed three times with PBS + .1% Tween 20, and horseradish peroxidase-labeled anti-porcine IgG (dilution 1:3000; .1 ml/well) was added, followed by addition of peroxidase substrate¹¹ (.1 ml/well). After incubation for 20 min at room temperature, the color reaction was determined in a Bio-tek EIA reader¹². The antibody titers were expressed as reciprocals of highest dilutions showing optical density of at least .1 above background.

⁸ICN Radiochemicals, Inc., Irvine, CA.

⁹100 ml with a stir bar, Belco Glass Inc., Vineland, NJ.

¹⁰ETL Testing Laboratories Inc., Cortland, NY.

Light and Scanning Electron Microscopy. Cross-sections of intestinal samples from formalin-preserved segments were prepared using standard paraffin embedding techniques. Samples were sectioned at 6 μm thickness and stained with azur A and eosin. Villus height was measured on the stained sections at 40 \times magnification using a light microscope equipped with an ocular micrometer. A minimum of 10 well-oriented, intact villi were measured in triplicate specimens for each pig within each group. Height was measured from the crypt mouth to the villus tip in 10- μm increments.

Scanning electron microscopy was performed on the 2-mm specimens. Samples were dehydrated in an ethanol-freon series as described by Liepens and DeHaven (1978). Following platinum coating, specimens were viewed with an ISI scanning electron microscope (ETEC UI Model 30) and photographed.

Trace Mineral Analysis. Concentrations of Fe, Cu, Zn, and Se in serum were analyzed according to AOAC procedures (1980).

Statistical Analysis. Data obtained from these experiments were subjected to analysis of variance and means were separated by Student's *t*-test and analysis of variance as described by Ott (1988). Differences between the two treatment means for weight gains, villus height, blood and intestinal lymphocyte proliferative response, serum antibody response to soybean protein, serum trace minerals were compared at both d 28 and 56. Differences between the means for skin-fold thickness were compared on d 28.

Results

The body weight of pigs on d 1, 21, 28 and 56 were 1.28, 5.50, 6.93 and 16.23 kg for the milk protein treatment versus 1.32, 6.02, 6.62 and 15.64 kg for the soybean protein treatment, respectively. The daily average feed intakes were 230 and 218 g from d 21 to 28 and 438 and 464 g from d 28 to 56, respectively, for the milk protein and soybean meal treatments.

Pigs sensitized to soybean protein and then fed diets containing soybean meal had lower

TABLE 2. EFFECT OF INTRADERMAL INJECTION OF SOYBEAN AND WHEY PROTEIN ON SKIN-FOLD THICKNESS (mm) ON DAY 28

Diet	Injections	
	Soybean	Milk
Soy protein	.185 ^a \pm .042	.230 ^b \pm .053
Milk protein	.156 ^a \pm .036	.470 ^b \pm .065

^aSkin-fold thickness after soybean protein injection minus thickness after physiologic saline injection given on d 27.

^bSkin-fold thickness after milk protein injection minus thickness after physiologic saline injection given on d 27.

($P < .01$) ADG at 1 wk after weaning than pigs fed diets based on milk protein (86 vs 204 g/d). However, they showed a compensatory increase in weight gains in the later weeks, so that from weaning to 5 wk of age, there were no differences ($P > .05$) due to diet in BW gains (331 vs 322 g/d).

On d 27 and 55 of age, no differences ($P > .05$) were found between the treatment groups of pigs in skin-fold thickness following intradermal injection of either milk or soy proteins (Table 2), though reaction to milk protein was greater than to soybean protein. However, pigs fed soybean meal had greater ($P < .01$) IgG titers to soy protein than did pigs fed milk protein at both 4 and 8 wk of age (Figure 1).

At 28 d of age, pigs fed the diet containing soybean meal had shorter ($P < .01$) villus height (Figure 2) than pigs fed milk proteins; however, at 8 wk of age, the pigs fed the diet containing soybean meal had villus heights comparable to those of pigs fed the milk protein-based diet. Feeding soybean meal diet resulted in slightly increased ($P < .10$) crypt depth (228 vs 213 μg at 4 wk and 241 vs 234 μm at 8 wk) compared with feeding milk protein.

Scanning electron microscopy showed that pigs fed soybean meal had shorter and broader villi, whereas the pigs fed milk proteins had long, round, tapering villi. However, there were no apparent differences in villi appearance among treatments at 56 d of age (Figure 3).

Proliferation of intraepithelial lymphocytes from mitogenic stimulation by PHA and PWM was greater at d 56 than at d 28 (Table 3). The proliferative responses of peripheral blood lymphocytes to mitogens PHA and PWM (Table 4) were higher than those of intraepithelial lymphocytes (Table 3); however,

¹¹Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD.

¹²Model EL 309, Bio-Tek Instruments, Highland Park, Winooski, VT.

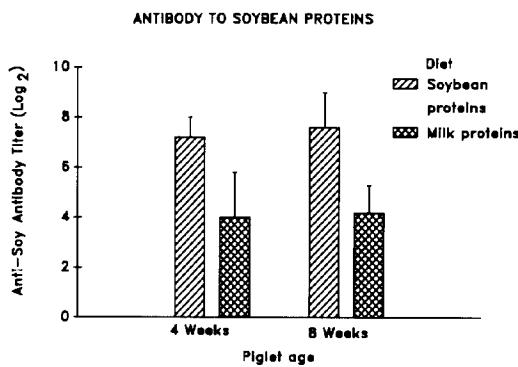


Figure 1. Effect of feeding baby pigs diets containing commercial soybean meal or milk proteins on their serum anti-soy antibody immunoglobulin G response. Soybean protein > milk protein ($P < .01$) at both d 28 and 56.

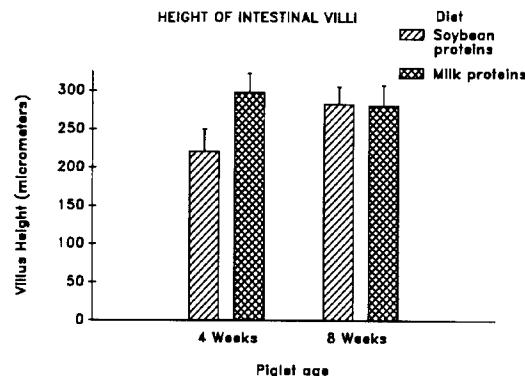


Figure 2. Effect of feeding baby pigs diets containing commercial soybean meal or milk proteins on villus height. Soybean protein < milk protein ($P < .01$) at 4 wk.

no effect of diet or age was detected. With purified soybean protein, both blood and intraepithelial lymphocytes showed very low proliferative responses (Tables 3 and 4).

Trace mineral concentrations in serum are presented in Table 5. There were no differences in Zn, Fe, Se and Cu concentrations between protein source treatments ($P > .20$) at either 4 or 8 wk of age.

Discussion

Pigs fed the diet containing soybean meal had depressed weight gain from 3 wk to 4 wk of age. This inferior growth coincided with abnormal villus morphology and increased serum IgG titers specific to soybean protein. Formation of complexes between residual antigens present in the soybean meal and systemic IgG antibodies plus activation of complement may have been responsible for the damage to villi. Increased serum IgG titers to soy protein is interpreted to indicate that macromolecules of soybean proteins crossed the intestinal mucosal barrier. The mechanism by which this occurs is obscure. Perhaps transfer of macromolecules is, in part, related to the maturity of the intestine. Pigs showed depressed performance and abnormal villus morphology at 4 wk, but not at 8 wk. It also is possible that pigs at this young age are not fully able to digest soybean protein; undigested protein reaching the small intestine might support more bacterial growth, resulting in

damage to villi (Miller et al., 1984a). Whether this age effect is due to induced tolerance to soybean protein or increased ability to digest soybean meal is not clear.

Immediately after weaning, the number of enteropathogenic bacteria increases (Miller et al., 1984a), perhaps as a result of the change in diet. Low preweaning feed intake, which commonly occurs when weaning at 3 wk or less, results in a transient hypersensitivity to dietary antigens (Miller et al., 1984b), which may, in turn, lead to an increase in mitotic rate in enterocytes (Stokes et al., 1984). The effect of this increase is an elongation of the crypts. As the rate of migration of the enterocytes up the villi increases, the enterocytes are shed from the top of the villi at a greater rate; therefore, the number of mature enterocytes decreases. Because these cells have absorptive ability and high sucrose activities in their brush borders (Miller et al., 1983), a reduction in the number of mature cells will result in lower enzyme activity and absorptive capacity. These changes are associated with increased enterocyte turnover and malabsorption, which increases the susceptibility of the intestine to pathogenic bacteria such as *E. coli*. In this experiment, pigs infused daily with 5 g of either soybean meal or milk proteins for 7 d before weaning did not have any noticeable diarrhea. This finding is contrary to the findings by Miller et al. (1984a) that a preweaning intake of at least 400 g of feed was necessary to prevent postweaning diarrhea.

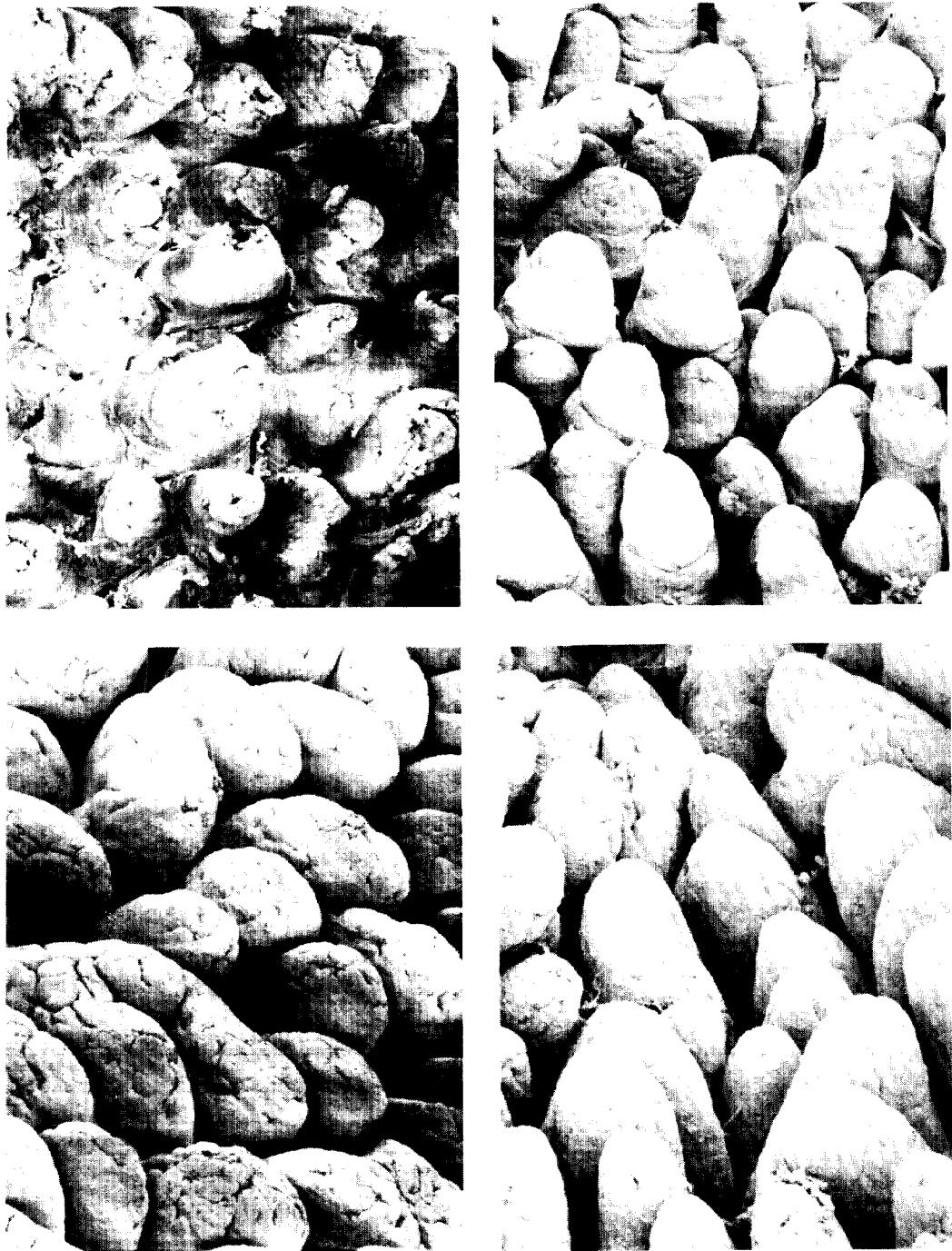


Figure 3. Scanning electron micrographs of villi in intestine of early-weaned pigs fed soy proteins and milk proteins. Width of photomicrograph field is 730 μm . Left above: Soy protein at 2 wk. Right above: Milk proteins at 4 wk. Left below: Soy protein at 8 wk. Right below: Milk protein at 8 wk.

TABLE 3. INTRAEPIHELIAL LYMPHOCYTE BLASTOGENESIS IN STARTER PIGS
FED MILK PROTEIN OR SOYBEAN PROTEIN (NET CPM $\times 10^3 \pm SE$)^a

Mitogen	Diet			
	Soybean protein		Milk protein	
	4 wk	8 wk	4 wk	8 wk
PHA ^b	2.0 ± 2.0	21.0 ± 9 ^f	2.0 ± 3.0	20.0 ± 10 ^f
PWM ^c	1.0 ± .1	3.0 ± 4 ^f	.4 ± .9	15.0 ± 8 ^f
Purified soybean protein ^d				
2.5 µg/ml	.5 ± .6	.2 ± .1	.7 ± .8	.2 ± .1
5.0 µg/ml	1.5 ± 1.2	.4 ± .2	.6 ± .9	.5 ± .6
Control ^e	1,511.4	1,201.3	1,201.3	1,485.3

^aNet cpm = cpm of stimulated culture - cpm of control culture. Values are least square means.

^bPhytohemagglutinin.

^cPokeweed mitogen.

^dPurified soybean proteins were glycinin and beta-conglycinin, 1:1 ratio, wt/wt.

^eControl was cultures without mitogenic stimulation.

^fNet cpm at 8 wk > at 4 wk ($P < .01$).

This dose of protein appeared to be effective in inducing transient hypersensitivity, because the performance of pigs sensitized with soybean meal before weaning was reduced. This is in agreement with an experiment using 20-d-old pigs (Barratt et al., 1978) in which perfusion with soybean protein solutions inhibited flow of digesta through the intestine. The effect was only observed after previous sensitization with soybean protein. Preruminant calves fed milk replacer containing heated soybean flour had delayed abomasal emptying followed by rapid movement of digesta through the small intestine and decreased N absorption (Sissons and Smith,

1976). This effect was observed only for calves sensitized with soybean protein. Calves may suffer from gastrointestinal hypersensitivity responses to certain soybean products because major proteases (pepsin and trypsin) of the digestive tract fail to denature soluble antigenic constituents of soybean protein (Sissons and Thurston, 1984). Whether this is true also for baby pigs has not been determined.

Following prolonged feeding with ovalbumin, the ability of rats to develop delayed hypersensitivity had completely abated within 3 wk after treatments were applied (Chase, 1946). These observations are similar to the results of earlier studies by Arnaud-Battandier

TABLE 4. PERIPHERAL BLOOD LYMPHOCYTE BLASTOGENESIS IN STARTER PIGS
FED MILK PROTEIN OR SOYBEAN PROTEIN (NET CPM $\times 10^3 \pm SE$)^a

Mitogens	Diet			
	Soybean protein		Milk protein	
	4 wk	8 wk	4 wk	8 wk
PHAb	158 ± 90	196 ± 97	243 ± 81	198 ± 93
PWM ^c	41 ± 23	46 ± 21	57 ± 30	67 ± 23
Purified soybean protein ^d				
2.5 µg/ml	1 ± 1.1	4 ± 6	2 ± 2.2	1 ± .8
5.0 µg/ml	2 ± 6.0	1 ± .8	2 ± 2.0	1 ± 1
Control ^e	1,338	2,009	2,662	1,985

^aNet cpm = cpm of stimulated cultures - cpm of control cultures. Values are least squares means.

^bPhytohemagglutinin.

^cPokeweed mitogen.

^dPurified soybean proteins glycinin and beta-conglycinin, 1:1 ratio (wt/wt).

^eControl was cultures without mitogenic stimulation.

TABLE 5. CONCENTRATION OF TRACE MINERALS IN SERUM OF PIGS FED SOYBEAN PROTEIN OR MILK PROTEIN ($\mu\text{g}/\text{ml}$)

Diet	Zn ^a		Fe ^a		Cu ^a		Se ^a	
	4 wk	8 wk						
Soybean protein	61	120	191	232	217	250	3.6	11.6
Milk protein	64	114	188	222	219	314	5.6	11.4
SE	27.0	62.5	97.8	42.3	72.8	85.1	.9	1.3

^aNo differences ($P > .05$) between treatment means at 4 and 8 wk of age.

and Nelson (1982) in which contact sensitivity in guinea pigs was suppressed by a short series of feedings with a hapten. However, species vary considerably in their response to protein antigens administered orally. In rats and mice, immunological tolerance develops quickly with little or no antibody production (Mowat and Ferguson, 1981). On the other hand, calves mount a sustained antibody response that has been implicated in the development of gastrointestinal disturbances (Kilshaw and Sissons, 1979; Kilshaw, 1981).

The results of the present study support the hypothesis that antigens administered orally evoke a local immune response that result in inferior performance of pigs at 28 d of age. This also is in agreement with the findings by Barratt et al. (1978) that previously sensitized calves responded to reintroduction of soybean meal with marked increases in antibody levels. In a study involving chickens, Klasing et al. (1988) found a similar trend that prior challenge with isolated soy protein decreased gain and feed conversion and increased immunoglobulin titers to soy protein. The fact that neither blood nor intestinal lymphocytes showed any proliferative responses to purified soy protein suggests that antigenic proteins of soybeans do not evoke cell-mediated immune responses. Also, there were no cutaneous hypersensitive responses to intradermal injections of purified soy protein.

In our study, serum trace mineral concentrations were not affected by protein sources. This tends to conflict with the findings of Hill (1985) that preruminant lambs fed a milk substitute containing isolated soybean protein absorbed and retained less Fe and Zn from the diet than those fed a milk substitute containing casein as the only source of protein. In chicks, decreased concentrations of Zn in serum resulted from sensitization by isolated soybean proteins (Klasing et al., 1988). Reduction in the availability of trace minerals from diets

containing soybean products is thought to be due to the formation of insoluble complexes with the phytate present in those products. These complexes are indigestible in the digestive tract of monogastric species (Welch and Van Campen, 1975; Halberg, 1981). Perhaps various soybean products differ in phytase contents and, thus, may have different effects on the availability of trace minerals. The reduction in the availability of trace minerals also might depend on the amount of soybean meal included in the diet and the duration of feeding such diets.

Reduced villus height and slightly greater crypt depth were found at 4 wk for pigs sensitized with soybean meal before weaning compared with pigs primed with milk protein. This is in agreement with previous findings that villus atrophy and malabsorption may be produced experimentally by hypersensitivity to soy proteins in mice (Mowat and Ferguson, 1981), pigs (Stokes et al., 1982; Newby et al., 1984) and calves (Seegarber and Morrill, 1986). Pigs would be susceptible to such responses at weaning because the conventional starter diets contain potent oral antigens from soybean meal (Sissons and Thurston, 1984). Delayed-type hypersensitivity responses to dietary antigen increased mitotic rate in enterocytes; the effect of this increase was an elongation of the crypts. However, there were no differences ($P > .05$) in villus height and crypt depth for pigs fed either soybean meal or milk proteins at 56 d of age in this study. One explanation might be that the allergic response to dietary antigen decreased as age increased. Heppell and Kilshaw (1982) confirmed that the age of pigs could be an important factor in responsiveness to new dietary antigens.

There were no treatment differences ($P > .05$) in the skin-fold thickness test on d 27 between pigs given soybean protein or milk protein. This is in agreement with Giesting et al. (1986), who reported that skinfold thickness

of pigs was not affected by soybean protein treatment in the preweaning or postweaning periods. Intraepithelial lymphocytes showed higher proliferative responses to PHA and PWM at 8 wk than at 4 wk in pigs fed the diet containing soybean meal. This might be the result of stress associated with early weaning and its adverse effect on immune response. Blecha et al. (1983) reported a temporary depression in the peripheral blood lymphocyte blastogenic response to PHA in pigs weaned at 3 wk, but not in pigs weaned at 5 wk of age. The PHA- and PWM-induced intraepithelial lymphocyte proliferation was higher ($P < .05$) at d 56 than at d 28 for all pigs. This finding is in agreement with that of Wilson et al. (1986) that intraepithelial lymphocytes do not show any response to concanavalin A until 10 wk of age.

The scanning electron microscopy data are supported by the results of Seegraber and Morrill (1986), who found that calves fed soybean protein had greater variation in size and conformation of villi than calves fed milk proteins. Intestinal damage may play a role in the poor postweaning performance of early-weaned pigs (Risley et al., 1988).

In summary, piglets sensitized to soybean protein by dosing and then fed a diet containing soybean meal processed by conventional methods showed transient hypersensitivity, as indicated by lowered villus height and increased serum anti-soybean immunoglobulin titers. These observations coincided with depressed growth from 3 to 4 wk. Our results suggest that it may be necessary to apply immunological criteria in evaluating the suitability of various soybean protein products for piglets.

Implications

Nursery pigs sensitized to soybean protein by dosing from d 7 to 14 and subsequently fed diets containing soybean meal exhibited a transient hypersensitivity. This hypersensitivity response may be partly responsible for poorer performance of pigs fed soybean protein than of pigs fed only milk protein.

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