Evaluation of HP300 Soybean Protein in Starter pig Diets

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ABSTRACT : One growth trial and one digestibility trial were conducted to evaluate the nutritional value of HP300, a commercially processed soybean meal product for weanling pigs. Dried whey, fish meal and/or full fat extruded soybeans (FFES) as well as portions of soybean meal (SBM) were replaced with HP300 in weanling pig diets. The objectives were to investigate the effects of HP300 on growth performance, digestibility, ileal amino acid digestibility and blood amino acid concentrataion in weanling pigs. One hundred and twenty crossbred (Duroc × Beijing Black × Landrace) pigs weaned at 28 days of age were used in the growth trial. The pigs were randomly allocated to five treatments, with three pens per treatment and eight pigs per pen. The trial duration was 28 days. The control (CTRL) diet contained no HP300; in treatments 2, 3 and 4, dried whey and fish meal were replaced by 3.0%, 7.5% and 10.5% HP300; in treatment 5, full fat extruded soybeans were replaced by 10.5% HP300 plus soybean oil to attain the same metabolic energy content as FFES. Five T-cannulated barrows were used in a digestibility trial with a 5 × 5 Latin square design to determine nitrogen retention and amino acid ileal digestibility of HP300 used alone or mixed with other ingredients. The results indicated that replacement of dried whey, fish meal, full fat extruded soybeans and a part of the soybean meal with HP300 in piglet diets improved average daily gain and feed conversion ratio ($p < 0.05$). There was a trend toward improved DM, crude protein and amino acid ileal digestibilities and improved protein net availability with the use of HP300 in swine diets.

(**Key Words**: HP300, Piglet, Digestibility, Amino Acid Ileal Digestibility, Free Amino Acids in Blood)

INTRODUCTION

The shortage of protein resources for livestock feeding is increasingly becoming more critical. Methods to improve bioavailability of protein resources have been developed. (Hancock et al., 1990; Li et al., 1993). It has been reported that antigenic materials in soybeans can reduce the performance of piglets. (Li, 1990, 1991a, 1991b) HP300 (Hamlet Protein, Denmark) is a soybean product manufactured by purifying and defatting the soybean via a proprietary microbial process which decreases levels of trypsin inhibitors and other antinutritional factors. This project was designed to investigate the effects of HP300 on performance, digestibility and amino acid ileal digestibility.

MATERIALS AND METHODS

Growth trial

HP300 soy protein product was provided by the HAMLET PROTEIN A/S (HP) Company of Denmark. Its nutrient composition is shown in table 1.

This trial was conducted for 4 weeks at the YuanDa Pig Farm in a suburb of Beijing. One hundred and twenty crossbred pigs (Duroc × Beijing black × Landrace) were weaned at 28 days of age, with initial weights of 9.5 kg ± 1.0 kg. All pigs were randomly allocated to 5 groups, according to body weight, sex and parentage. There were 3 pens per treatment with 8 pigs per pen with identical feeding, penning and environment. All pigs were housed on slotted wire floors and fed *ad libitum* throughout the trial period. Body weight and feed consumption were measured at the end of week 2 and week 4.

Data were collected for calculation of average daily gain (ADG), average daily feed intake (ADFI), Feed conversion ratio (F/G), blood urea nitrogen (BUN) and free amino acid concentration in serum.

Measurement of BUN and free amino acid concentration in serum: On day 14, blood samples were

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Table 1. Nutrient composition of HP300[a]

Item	Percentage (%)	
Moisture	8.00	
Protein	55.00	
Fat	2.50	
Fiber	3.50	
Ash	7.30	
Calcium	0.25	
Phosphorus	0.80	
Carbohydrate	23.70	
Other Characteristics:	Value	Units
Trypsin Inibitor	1	mg/g
Glycinin	< 100	ppm
b-conglycinin	< 100	ppm
Lectins	< 1	ppm
Oligosaccharides	1	%
pH	6.1	
Particle Size	80% in 75-200 mm range	
Water binding	1 : 5	
Density	0.45	g/ml
Amino Acids	Grams/100 g Protein	
Lysine	6.2	
Methionine	1.5	
Cystine	1.5	
Threonine	4.0	
Tryptophan	1.3	
Leucine	7.8	
Isoleucine	4.8	
Phenylalanine	5.0	
Valine	5.2	

[a] Nutrients are on an as fed basis.

taken before the morning feeding via the jugular vein, using a vacuum container. Blood samples were centrifuged for 15 minutes at 3,000 rpm to separate the serum. The serum was divided into two parts-one part was stored at −20℃ for BUN analysis using diacetyl monoxime; the other was deproteinated and centrifuged at 10,000 RPM at 4℃ for determination of free amino acid concentration by HPLC. Analyses of BUN and amino acids were made according to the standard methods developed at MAFIC.

Dietary nitrogen was analyzed by the Kjeldahl method (AOAC, 1984) and crude protein was calculated by N × 6.25. Chromic oxide (Cr_2O_3) was added to the diets as a marker. Chromic oxide concentration in the diet and the digesta was determined by spectrophotometric analysis for calculation of amino acid ileal digestibilities.

Statistical methods: The primary data were collected on the basis of replicates. ADG, ADFI, feed/gain ratio were analyzed by the GLM procedure of SAS (1988).

Diet formulation: The control (CTRL) diet was a corn-soy based diet plus dried whey, fish meal and full fat extruded soybeans (FFES). In treatment 2, 3 and 4, dried whey and fish meal were replaced by increasing proportion of HP300 at the same nitrogen level;. in treatment 5, full fat extruded soybean was also replaced by HP300 plus soybean oil (SO) to provide a dietary metabolic energy content similar to the diets containing FFES. The crude protein and lysine content were formulated to meet NRC nutrient requirements (1988) (table 2).

Digestibility trial

The trial duration was 50 days and was conducted in the Monogastric Animal Nutrition Lab., College of Animal Science and Technology, China Agricultural University.

Five ileal T-cannulated barrows of similar age, parentage and body weight were randomly allotted into 5 treatments with one pig per treatment. The procedure for ileal T-cannulation was as follows.

Nylon T-cannulae with a threaded 1.2 cm O. D. tube and curved T-flange 6 cm long were prepared at the BAU Machine Shop from nylon rod stock purchased locally in Beijing. A 4 cm diameter washer and a 3 cm knurled round nut was fitted to each cannula.

Piglets were anaesthetized with 0.1 ml/kg of "Fufang Liuantong" (ketamine-complex injection) containing 150 mg ketamine hydrochloride + 150 mg xylazine hydrochloride injected intramuscularly in the cervical musculature, and supplemented with inhalation of diethyl ether as necessary to maintain anaesthesia. Pigs were placed in right lateral recumbancy and the right side of the pig from the 8th rib to the rear leg was shaved, scrubbed and prepped with 5% tincture of iodine. A vertical incision 10 cm in length was made in the skin 8 cm caudal to the xiphoid, and extended through the abdominal musculature by blunt dissection to the peritoneum. The peritoneum was picked up with thumb forceps and carefully incised parallel with the skin incision. The ileum was located using the cecum and ileal mesenteric lymph node chain as landmarks. The ileum was exteriorized and a 2 cm incision made in the antimesenteric aspect of the ileum 12 to 15 cm proximal to the ileocecal junction. The T-flange of the cannula was carefully introduced into the incision, and the ileal incision was closed proximal to the cannula with silk sutures in a continuous Lembert pattern. The ileal mucosa surrounding the cannula was carefully inverted and held in place by a silk purse string suture. A notch was made in the end of the cannula to serve as a

Table 2. Diet compositions for growth trial[1]

Ingredient	CTRL	SBM+FFES+ 3.5% HP300	SBM+FFES+ 7.0% HP300	SBM+FFES+ 10.5% HP300	SBM+SO+ 10.5% HP300
Corn	56.63	58.45	59.29	60.33	60.16
FFES	4.00	4.00	4.00	4.00	–
Dried whey	3.00	–	–	–	–
SBM	27.00	27.30	22.80	18.30	21.50
Fish meal	3.00	–	–	–	–
Soybean oil	1.00	1.00	1.00	1.00	2.00
HP300	–	3.50	7.00	10.50	10.50
Organic acid	1.50	1.50	1.50	1.50	1.50
Flavor	–	0.03	0.03	0.03	0.03
Premix[2]	1.00	1.00	1.00	1.00	1.00
Salt	0.34	0.34	0.34	0.34	0.34
Bone meal	2.20	2.50	2.70	2.70	2.70
Lysine	0.20	0.23	0.21	0.18	0.19
DL-methionine	0.13	0.15	0.13	0.12	0.08
Total	100.00	100.00	100.00	100.00	100.00
Nutrient (as fed basis, %)					
Crude Protein	21.20	21.30	21.60	21.60	21.70
Ca	1.00	1.00	1.00	1.00	1.00
P	0.78	0.75	0.76	0.78	0.74
Lysine	1.30	1.29	1.29	1.29	1.30
Met+Cys	0.82	0.82	0.81	0.80	0.80

Notes: [1] CTRL = control; SBM = soybean meal; FFES = full fat extruded soybean; SO = soybean oil.
[2] Provided per kilogram feed: furazolidone, 110 mg; VA, 5512 IU; VD_3, 551 IU; VE, 66.1 IU; riboflavin, 5.5 mg; pantothenic acid, 13.8 mg; nicotinic acid, 30.0 mg; choline, 551 mg; VB12, 2.6 mg; Mn, 90 mg; Fe, 100 mg; Zn, 100 mg; Cu, 250 mg; I, 0.3 mg; Co, 1 mg; Se, 0.3 mg.

marker for proper rotational orientation of the cannula. A 20 cm length of heavy silk suture was tied around the end of the cannula, and the cannula was exteriorized by drawing it through a stab incision in the left paralumbar fossa 4 cm caudal to the incision, taking care not to exteriorize the peritoneum and to orient the cannula in the proper rotational position to permit normal flow of the ileal digesta. The suture was removed from the cannula and the cannula snugly fastened to the abdominal wall with the washer and nut.

Each pig was numbered, weighed and housed individually in a metabolic cage. The trial used a 5 × 5 Latin-square design. Ileal digesta was collected continuously (150 ml each day). Ileal digesta was frozen at −20℃ immediately after measuring its pH. At the end of the trial, digesta for every period was mixed according to the individual time periods, and was frozen and dried. The dried samples were ground to pass through a 1 mm sieve.

Diet formulation: Diets were formulated to be of similar metabolic energy and protein content and to meet NRC (1988) requirements. Treatment 1 consisted of a corn-soybean meal-based diet as a control, containing 3.0% dried whey and 1.0% fishmeal. Treatment 2 was a purified diet containing HP300 and corn starch. In treatment 3, 4 and 5, dried whey, full fat extruded soybeans and equivalent portions of the soybean meal were replaced by 7.0%, 10.5% and 10.5% HP300 plus soybean oil, respectively. All diets were formulated to have the same metabolic energy and lysine level. Diet compositions are shown in table 3.

The digestibility trial included 5 feeding periods. A initial period of 8 days was taken, of which 3 days were used for adjustment to metabolic cages, and 5 days used for feed intake determination. All feces and urine were collected in the following collection period. During the 5 day collection period, all Pigs were fed two times per day with equal allowances in the morning and evening. Water was provided *ad libitum*. Urine was filtered through gauze into a collection bottle containing 10 ml HCl (1 : 1).

Table 3. Diet compositions for digestibility trial

Ingredient	CTRL	32.8% HP300	SBM+FFES+ 7.0% HP300	SBM+FFES+ 10.5% HP300	SBM+SO+ 10.5% HP300
Corn	62.72	–	67.40	68.61	67.85
FFES	4.00	–	4.00	4.00	–
Dried whey	3.00	–	–	–	–
SBM	23.30	–	14.70	10.00	13.50
Fish meal	1.00	–	1.00	1.00	1.00
Soybean oil	1.00	2.00	1.00	1.00	2.00
HP300	–	32.80	7.00	10.50	10.50
Organic acid	1.00	1.00	1.00	1.00	1.00
Limestone	0.68	1.44	0.74	0.85	0.86
Dicalcium phosphate	1.80	1.30	1.67	1.55	1.54
Premix[1]	1.00	1.00	1.00	1.00	1.00
Salt	0.34	0.34	0.34	0.34	0.34
Lysine	0.15	–	0.12	0.11	0.11
DL-methionine	0.01	–	0.03	0.04	0.05
Cr_2O_3	0.25	0.25	0.25	0.25	0.25
Corn Starch	–	56.87	–	–	–
Fiber	–	3.00	–	–	–
Total	100.00	100.00	100.00	100.00	100.00
Nutrient (as fed basis, %)					
Crude Protein	19.00	19.10	19.40	19.00	19.00
Ca	0.90	0.90	0.90	0.90	0.90
P	0.60	0.60	0.60	0.60	0.60
Lysine	1.05	1.03	1.05	1.04	1.04
Met+Cys	0.63	0.58	0.64	0.64	0.65

Notes: [1] Provided per kilogram feed: furazolidone, 110 mg: VA, 5512 IU; VD_3 551 IU; VE, 66.1 IU; riboflavin, 5.5 mg; pantothenic acid, 13.8 mg; nicotinic acid, 30.3 mg; choline, 551 mg; VB_{12} 2.6 μg; Mn, 90 mg; Fe, 100 mg; Zn, 100 mg; Cu, 250 mg; I, 0.3 mg; Co, 1 mg; Se, 0.3 mg.

CTRL = control; SBM = soybean meal; FFES = full extruded soybean; SO = soybean oil.

An aliquot amounting to 10% of the urine was taken and frozen immediately at −20℃ for storage untill analysis. Feces and digesta were dried at 55℃ for 48 hours to measure moisture, allowed to come to air-dry weight, weighed, and ground. Samples of feed, urine and feces were analyzed for nitrogen.

Criteria measured: Apparent digestibility of dry matter, Apparent digestibility of Protein, Amino acid-digestibility, Protein net availability, and protein biological value (BV).

RESULTS AND DISCUSSION

Growth trial

The resulting data of ADG, ADFI and Feed Conversion Ratio during weeks 0-2, weeks 3-4 and overall, and the BUN at the end of week 2 are shown in table 4.

No significant differences in ADG and ADFI (table 4) among treatments were detected during weeks 0-2 ($p > 0.05$). However, pigs which consumed diets containing 7.0% and 10.5% HP300 had improved ($p < 0.05$) feed per unit gain as compared with those fed other diets. During weeks 3-4, ADG of treatments 5 differed ($p < 0.05$) from the other treatments.

FCR of treatment 1 differed ($p < 0.05$) from other treatments except 3; treatment 2 differed ($p < 0.05$) from treatment 3. During the overall period, pigs fed diets containing HP300 had improved ($p < 0.05$) feed over gain ratio as compared with the control group. Pigs fed diets containing HP300 had higher ($p < 0.05$) ADG than that of the control. When dried whey, fish meal, full fat extruded soybeans and portion of the soybean meal were replaced with HP300, no significant differences in BUN concentration were detected (table 4).

Table 4. Effects of HP300 on growth performance and BUN of piglets

Phase Item	CTRL	SBM+FFES+ 3.5% HP300	SBM+FFES+ 7.0% HP300	SBM+FFES+ 10.5% HP300	SBM+SO+ 10.5% HP300	SE
0–2 wks						
ADG	196.58[a]	193.93[a]	211.04[a]	212.41[a]	216.47[a]	16.5
ADFI	380.71[a]	371.03[a]	383.73[a]	374.94[a]	378.49[a]	18.1
FCR	1.96[a]	1.94[a]	1.79[b]	1.81[b]	1.79[b]	0.24
3–4 wks						
ADG	378.06[a]	427.76[a]	428.57[a]	450.78[a]	463.57[ab]	22.0
ADFI	678.91[a]	603.25[b]	655.28[a]	652.02[a]	642.14[a]	31.0
FCR	2.10[a]	1.41[b]	1.53[b]	1.45[b]	1.40[b]	0.26
0–4 wks						
ADG	287.32[a]	310.84[b]	319.81[b]	331.60[b]	340.02[b]	28.0
ADFI	529.81[a]	487.14[b]	519.51[a]	513.48[a]	510.32[a]	24.0
FCR	1.84[a]	1.57[b]	1.62[b]	1.55[b]	1.50[b]	0.30
BUN at week 2, (mg/100 ml)	14.42[a]	16.15[a]	12.35[a]	14.27[a]	13.47[a]	1.2

[a,b] Same superscripts within a row mean no significant differences ($p > 0.05$).
CTRL = control; SBM = soybean meal; FFES = full fat extruded soybean; SO = soybean oil; wks = weeks; BUN = blood urea nitrogen.

Digestibility trial

The apparent digestibility of dry matter and protein, protein net availability and protein biological value (BV) are shown in table 5.

Dry matter digestibilities were increased ($p < 0.05$) when pigs were fed HP300 as the only protein source. This indicated that HP300 was more easily digested than the other protein sources fed.

Apparent digestibility of protein in the control diet was 87.29%, while that of treatments 2, 5, 3 and 4 were 94.37%, 88.75% 88.51% and 88.45%, respectively. Protein digestibility was higher ($p < 0.05$) than the control when HP300 was the only protein source. Digestibilities of DM and protein were improved with increasing inclusion rates of HP300 ($p < 0.05$). This result is likely due to the high digestibility of HP300 and the lower antinutritional factor content.

Protein net availability and biological value were improved ($p < 0.05$) when HP300 was added to the diets. This perhaps can be attributed to the process of manufacturing HP300, which lowers antinutritional factors and improves protein availability.

Table 5. Effect of HP300 on digestibility and protein utilization (%)[a]

Item	CTRL	32.8% HP300	SBM + FFES + 7.0% HP300	SBM + FFES + 10.5% HP300	SBM + SO + 10.5% HP300	SE
Apparent digestibility of DM[b]	85.94	94.17	85.97	86.65	86.68	5.4
Apparent digestibility of Protein[c]	87.29	94.37	88.51	88.45	88.75	4.8
Protein net availability[d]	43.59	51.08	44.90	47.82	47.95	6.2
Protein BV[e]	43.95	54.13	49.39	55.00	49.43	5.6

CTRL = control; SBM = soybean meal; FFES = full fat extruded soybean; SO = soybean oil; DM = dry matter; BV = biological value.
[a] Mean values represent five observations.
[b] 32.8% HP300 > SBM + FFES + 7% HP300, SBM + FFES + 10.5 HP300, SBM + SO + 10.5% HP300 ($p < 0.05$).
[c] 32.8% HP300 > SBM + FFES + 7% HP300, SBM + FFES + 10.5 HP300, SBM + SO + 10.5% HP300 ($p < 0.05$).
[d] 32.8% HP300 > CTRL ($p < 0.04$).
[e] 32.8% HP300, SBM + FFES + 10.5% HP300 > CTRL ($p < 0.02$).

Amino acid ileal digestibilites are shown in table 6. There was a trend toward improved digestibilities of amino acids as HP300 supplementation increased. No significant differences were observed when full fat extruded soybeans were replaced by HP300 + SO and soybean meal on an equal metabolic energy and protein content basis. This indicates that HP300 has a amino acid profile and amino acid availability comparable to full fat extruded soybeans.

The efficiency of use of amino acids in diets can be obtained from the concentration of free amino acids in serum. There was a trend toward lower free amino acids concentration (table 7) in serum in those groups fed HP300 as compared with the control, particularly in treatments four and 5, in which free lysine and methionine concentration in serum were significantly lower ($p < 0.05$) than those of the control. This is indicative of a better balance of available amino acids than the control diet. Theoretically, free amino acids decrease with tissue synthesis and amino acid degradation after the digesting and absorption of nutrients. Free amino acids in serum have been effectively used to determine

Table 6 Effect of HP300 on amino acid ileal digestibilities for piglets (%)

Amino Acid	CTRL	32.8% HP300	SBM + FFES + 7.0% HP300	SBM + FFES + 10.5% HP300	SBM + SO + 10.5% HP300	SE
Asp	73.49	83.58	81.33	81.72	80.49	3.6
Ser	75.15	82.95	80.73	83.50	79.41	2.2
Glu	73.58	86.70	80.19	81.74	80.22	4.8
Thr	75.61	92.98	80.08	82.07	78.01	4.2
Gly	66.18	75.96	71.01	67.78	70.61	3.2
Arg	84.02	93.53	89.88	88.46	88.69	1.6
Ala	70.73	83.00	78.22	76.40	74.60	3.8
Tyr	75.91	87.87	92.14	95.16	86.77	4.3
Pro	66.37	86.24	75.26	72.98	69.13	10.2
Val	97.45	85.24	80.50	76.77	75.56	12.4
Phe	76.07	100.00	75.91	78.80	77.95	15.2
Leu	79.41	86.11	82.61	79.16	80.71	1.1
Ile	75.72	90.00	84.43	81.72	81.09	6.4
Lys	75.53	83.15	81.25	80.82	78.68	2.1

Notes: CTRL = control; SBM = soybean meal; FFES = full fat extruded soybean; SO = soybean oil.

Table 7 Effect of HP300 inclusion level on free amino acid concentrations in serum of piglets (mg/dl)

Amino Acid	CTRL	SBM + FFES + 3.5% HP300	SBM + FFES + 7.0% HP300	SBM + FFES + 10.5% HP300	SBM + SO + 10.5% HP300	SE
Asp	4.80^{a}	2.58^{ab}	1.69^{ab}	1.76^{b}	2.34^{ab}	1.8
Ser	3.20^{a}	2.10^{a}	2.98^{a}	2.36^{a}	2.12^{a}	0.9
Glu	6.08^{a}	3.85^{a}	4.28^{a}	4.07^{a}	3.77^{a}	1.2
Thr	2.99^{a}	2.35^{a}	2.54^{a}	2.07^{a}	1.84^{a}	0.6
Gly	5.03^{a}	5.24^{a}	5.22^{a}	5.42^{a}	3.40^{a}	0.8
Arg	5.98^{a}	3.64^{a}	5.27^{a}	3.98^{a}	3.40^{a}	1.1
Ala	5.21^{a}	4.91^{a}	4.63^{a}	4.21^{a}	4.21^{a}	1.2
Tyr	3.37^{a}	3.90^{a}	3.76^{ab}	3.32^{a}	3.81^{a}	0.5
Pro	3.46^{a}	3.29^{a}	3.60^{a}	2.85^{a}	2.83^{a}	1.1
Val	3.09^{a}	2.73^{a}	2.06^{a}	2.08^{a}	1.91^{a}	0.7
Phe	3.06^{a}	1.99^{ab}	2.03^{ab}	1.66^{b}	1.52^{ab}	1.6
Leu	2.28^{a}	1.34^{a}	1.34^{a}	2.29^{a}	2.29^{a}	1.0
Ile	3.33^{a}	1.98^{a}	1.89^{a}	1.35^{a}	1.43^{a}	1.2
His	3.43^{a}	3.22^{a}	3.08^{a}	2.75^{a}	2.52^{b}	0.6
Lys	3.58^{a}	3.69^{a}	2.09^{ab}	1.52^{b}	1.81^{b}	1.3
Met	0.47^{a}	0.52^{a}	0.32^{a}	0.26^{b}	0.30^{b}	0.2

Notes: ^{a,b}: Same superscripts within a row mean no significant differences ($p > 0.05$).
CTRL = control; SBM = soybean meal; FFES = full fat extruded soybean; SO = soybean oil.

the adequacy of amino acid supply in animals (Lewis, 1992). A previous study by Eggum (1977) demonstrated a negative relationship between concentration of free amino acids in plasma and dietary amino acid balance. This relationship can be used to determine amino acid requirements in pigs (Brown and Cline, 1974).

Free amino acids in plasma or serum can reflect the status of protein metabolism and amino acid supply in the animal's body. The blood pool is the most important source of amino acids for metabolism. A significant positive relationship exists between free amino acids and available amino acids in the diet. An improvement in the balance of available amino acids is accompanied by a decrease in free amino acid concentrations. The results obtained from the feeding trial and digestibility trial confirm these concepts.

CONCLUSIONS

In conclusion, based on the results from the growth and digestibility trials, the replacement of dried whey, fish meal, full fat extruded soybeans and a protion of the soybean meal with HP300 can improve growth performance and efficiency of protein utilization in diets for baby pigs. There was a trend toward improved digestibility of dry matter, protein and amino acids, with improved amino acid balance with increasing inclusion rates of HP300 in the diet. There was a tendency toward reduction in cost per unit of gain when HP300 was supplemented in starter pig diets.

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