The Effect of Soybean Galactooligosaccharides on Nutrient and Energy Digestibility and Digesta Transit Time in Weanling Piglets**

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ABSTRACT : Eight 12.4±0.6 kg initial body weight crossbred barrows were used to determine the effect of soybean galactooligosaccharides on nutrient and energy digestibility, and digesta transit time. Four dietary treatments were utilized in this trial. Treatment one was a corn-soybean meal based diet (SBM) containing raffinose and stachyose at the levels of 0.16% and 0.75%, respectively. Treatment two (control) was a corn-HP300 (soybean concentrate protein) diet. In treatments three and four, 1.1% and 2.2% commercial stachyose was added to the control diet to provide total dietary stachyose at the levels of 1% and 2%, respectively. The soybean galactooligosaccharides (raffinose + stachyose) level in treatment one was slightly lower compared to that in treatment three. Three collection periods were run with two pigs for each treatment/period. There was a 4 d adjustment period followed by a 3 d collection period. The results showed that the nitrogen retention (86.79%) of pigs fed treatment two diet was higher than that of pigs fed treatment one by 5.2% ($p<0.05$). The nitrogen retention of treatment three was intermediate 83.09%. The apparent fecal digestibility of all amino acids in treatment two was numerically highest, followed by treatments three and four. However, there were no significant difference among groups ($p>0.05$). The dry matter (DM), organic matter (OM), crude protein (CP), and crude fiber (CF) digestibility numerically decreased as the soybean galactooligosaccharides level increased, but were not significantly different ($p>0.05$). Chromium content in feces (from the inclusion of 0.3% chromic oxide in the diets) differed among treatments ($p<0.05$) at 15 h, 18 h, and 21 h after eating. This showed that the digesta transit time was differed significantly among treatments. Treatment four was the shortest, followed by treatment three, SBM and control. The results demonstrated that in the absence of antinutritional factors and soybean antigen protein, inclusion of 1% and 2% stachyose in corn-HP300 diet has no significant effect on the digestibility of DM, OM, CP, CF and amino acids. When the soybean galactooligosaccharide level in diet one and diet three were adjusted to be almost the same, antinutritional factors such as trypsin inhibitor and soybean antigen protein could decrease the nutrient digestibility and nitrogen retention rate of diet. High levels of soybean galactooligosaccharides shortened the digesta transit time in the intestinal tract. This trial suggested that the total level of soybean galactooligosaccharides (stachyose+raffinose) in the weanling piglet diet is better not to exceed 1% when common soybean meal is used as main protein source. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 11 : 1598-1604)*

Key Words : Soybean Galactooligosaccharide, Nutrient Digestibility, Weanling Piglet, Digesta Transit Time

INTRODUCTION

Soybeans contain about 35% carbohydrates, and defatted dehulled soy grits and flour contain about 17% soluble and insoluble carbohydrates (Smith and Cirek, 1972). The soluble carbohydrate fraction in soybean meal primarily consists of sucrose, raffinose, and stachyose. The use of soybeans has been limited in the human diet because of the flatulence produced by soybean galactooligosaccharide (raffinose and stachyose). These galactooligosaccharides cannot be metabolized in the small intestine by endogenous enzymes due to the absence of α-1,6-galactosidase in the intestinal mucosa (Gitzelmann and Auricchio, 1965).

Coon et al. (1988) suggested that the removal of oligosaccharides from soybean meal might result in greater fiber digestion because of a slower transit time and an enhanced cecal environment for the microbial hydrolyzation of polysaccharides.

The nutritional value of galactooligosaccharides in soybean meal has not been fully studied in swine. Risley and Lohrmann (1998) reported that apparent energy and dry matter digestibility decreased in early weanling piglets fed soybean meal with lower stachyose compared to pigs fed common soybean meal based diets, but the body weight gain was improved for pigs fed diets containing lower stachyose.

The objectives of this trial were: 1) to determine whether the inclusion of 1% and 2% stachyose in the diets affected available energy and nutrient digestibility; 2) to determine if feeding diets with 1% and 2% stachyose would change digesta transit time; 3) to determine if an interaction exists between soybean galactooligosaccharide and other anti-nutritional factors in common soybean meal in weanling piglets.

MATERIALS AND METHODS

Eight crossbred (Landrace×Large White×Duroc) barrows with initial BW 12.5±0.6 kg were used to determine nutrient

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and energy digestibility, and digesta transit time in the digestive tract. The barrows were fed one of four experimental diets (table 1). The four dietary treatments were: 1) corn-soybean meal based diet containing raffinose and stacchyose at the levels of 0.16% and 0.75%, respectively; 2) corn-HP300 (soybean concentrate protein) control diet; 3) and 4) control diet with 1.1%, 2.2% commercial stachyose added to provide total dietary stachyose levels of 1% and 2%, respectively. All diets for pigs between body weight 10 to 20 kg were formulated to meet or exceed the nutrient requirement (NRC, 1998). The nutrient, urease activity, soybean galactooligosaccharides and amino acids contents of soybean meal and HP300 are shown in tables 2 and 3. The commercial stachyose was purchased from China Food Fermentation Institute in Beijing and it contained 91.8% stachyose, 4.89% sucrose and 2.36% raffinose. HP300 was provided by Microvet Company, Beijing Office.

Three collection periods were run with two pigs for each treatment/period. Each period lasted a total of 7 days, consisting of a 4 d adjustment period to the diet followed by a 3 d collection period of feces and urine. After each collection period, pigs were allowed a 7 d recuperation period on a common corn-soybean meal based diet before starting the next collection period. Throughout the experiment, the barrows were individually housed in 0.5 m × 1.5 m cast iron metabolism crates equipped with a 0.25 m^3 round bottom feeder located at the front of the crate. The crates were located in an environmentally controlled barn with the temperature set at 24°C. The barrows were fed at 08:00 h and 16:00 h each day. Feed intake was maintained at a constant level for all pigs during each experimental period. The amount of feed offered to all pigs was the amount consumed with average daily intake during first 3 days of adjustment period. Water was added to the diets prior to feeding to form a moist, crumbly mixture, and the pigs typically consumed their ration within 30 minutes.

Feces and urine were collected in the morning and afternoon after feeding on days 4 to 7 of each period. The feces were weighed and mixed, and a subsample was obtained, freeze-dried to measure moisture content, allowed to come to air-dried weight, weighed and then ground. An aliquot amounting to 10% of urine was taken and frozen immediately at -20°C for later analysis. Samples of feed,

Table 1. Composition and nutrient levels of experimental diets

	SBM	HP300	HP300 +1% stachyose	HP300+2% stachyose
Ingredients (%)				
Corn	57.0	63.78	62.68	61.58
Dried whey	8.0	8.0	8.0	8.0
Soybean meal 45% CP	23.5			
HP300		18.7	18.7	18.7
Fishmeal	5.0	5.0	5.0	5.0
Dicalcium phosphate	1.4	1.4	1.4	1.4
Limestone	0.9	1.0	1.0	1.0
Premix*	1.0	1.0	1.0	1.0
Lysine·HCL	0.16	0.12	0.12	0.12
Commercial stachyose			1.1	2.2
Corn Oil	3.0	1.0	1.0	1.0
Total	100	100	100	100
Nutrient level**				
DE, Mcal/kg	3.34	3.36	3.36	3.36
CP, %	19.54	19.75	19.75	19.75
Calcium, %	1.02	0.97	0.97	0.97
Available phosphorus, %	0.49	0.50	0.50	0.50
Lys, %	1.09	1.09	1.09	1.09
Met, %	0.37	0.37	0.37	0.37
Met +Cys, %	0.73	0.75	0.75	0.75
Soybean galactooligosaccharides, %				
Raffinose, %	0.16	-	-	-
Stachyose, %	0.75	-	1.0	2.0

* Premix provided per kilogram of complete diet: vitamin A 7,500 IU, vitamin D_3 650 IU; vitamin E 70 mg; vitamin K_3 2.5 mg; thionine 1.5 mg; vitamin B_2 5.0 mg; pantothenic acid 20 mg; niacin 30 mg;vitamin B_6 4 mg; biotin 0.5 mg; vitamin B_{12} 0.05 mg; folic acid 2.0 mg; choline 500 mg; Mn 80 mg; Zn 110 mg; Fe 100 mg; Cu 10 mg; Se 0.3 mg; I, 0.35 mg.

** Nutrient levels were calculated values.

Table 2. Nutrient, urease activity and oligosaccharide contents in different soybean protein sources

Items	SBM	HP300
Nutrient level (fed basis)		
DM, %	88.56	92.03
CP, %	44.05	54.75
EE, %	1.09	2.52
CF, %	4.56	3.54
Ash, %	6.67	7.49
NFE, %	43.71	23.73
DE, Mcal/kg	4.05	3.38
Urease activity, △pH	0.26	0.12
Soybean oligosaccharides		
Raffinose, %	0.82	0.21
Stachyose, %	3.08	0.30
Sucrose, %	4.87	trace

Note: CP, crude protein; EE, extracted ether; CF, crude fiber; NFE, nitrogen free extraction.

Table 3. Amino acid contents in different soybean protein sources

Amino acids	SBM	HP300
Lys	3.29	3.41
Thr	1.58	2.20
Leu	3.08	4.29
Ile	1.90	2.64
Val	2.13	2.86
Trp	0.35	0.71
Phe	2.03	2.75
Tyr	1.71	2.15
Met	0.64	0.82
Cys	0.78	0.82
His	1.31	1.48
Arg	3.57	3.96
Glu	7.65	10.17
Gly	1.79	2.36
Pro	2.90	2.97

urine and feces were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), and nitrogen and amino acids concentrations. Dietary and fecal nitrogen were analyzed by the Kjeldahl method (AOAC, 1990), and crude protein was calculated as N×6.25. The amino acids were analyzed with Hitachi L-8800 automatic amino acid analyzer. The gross energy was determined with a PARR 1281 automatic energy analyzer. The diets and soybean protein sources were prepared for galactooligosaccharide analysis according to a modification of the procedures described by Delente and Ladeburg (1972), and Low and Sporns (1988). The soybean oligosaccharide content was determined on a HP 6890 gas chromatography.

The method used to estimate the digesta transit time was a modification of the procedure described by Mateos and Sell (1981). The marked experimental diets (0.3% chromic oxide) were made available for the first time feeding for each period, and then replaced by the unmarked experimental diets. Feces were collected at 3 h intervals for 12 h, beginning 15 h after eating the marked experimental diets. The marked experimental diets and feces samples were prepared for chromium analysis according to the procedures given by Williams et al. (1962). The chromium content was determined with a Hitachi Z-5000 atomic absorption spectrophotometer.

Statistical analysis

Data were analyzed as incomplete cross trial design using analysis of variance procedure of SPSS, using Duncan's multiple range test for post hoc multiple comparison after significant F from ANOVA.

RESULTS AND DISCUSSION

The growth performance of pigs is shown in table 4. Although there were no significant differences between treatments, average daily gain and feed/gain decreased as the stachyose level increased in the diet. Compared with the control diet, average daily gain in the 1% and 2% stachyose added treatments decreased by 18.18% and 33.33% respectively. Feed/gain in the control group was lowest (1.43), while the feed efficiency of the 1% and 2% stachyose added treatments worsened by 6.29% and 42.65% respectively. The feed/gain of the SBM diets was intermediate between the control and 1% stachyose treatments.

The energy digestibility and nitrogen retention in four treatments were shown in table 5. There were no significant effects of soybean galactooligosaccharides level in the diet on available energy intake and energy digestibility. The daily nitrogen intakes were very similar among treatments, with a range of 17.0-17.76 g/d. The daily nitrogen excretion from urine of the 1% stachyose added treatment was the same as that in control (0.06 g/d). However, the daily urinary

Table 4. The effect of soybean galactooligosaccharides on performance of weanling piglets

Items	SBM	HP300			SEM	P value
		Control	1% stachyose	2% stachyose		
ADG, kg/d	0.35	0.33	0.27	0.22	0.10	0.74
ADFI, kg/d	0.46	0.41	0.40	0.43	0.09	0.97
Feed/gain	1.47	1.43	1.52	2.04	0.27	0.35

Table 5. The energy digestibility and nitrogen retention in four treatments

Items	SBM	HP300			SEM	P value
		Control	1% stachyose	2% stachyose		
DE, Mcal/d	2.05	1.97	1.91	2.03	0.36	0.53
ME, Mcal/d	2.00	1.93	1.87	1.98	0.18	0.89
DE/GE intake, %	83.67	85.27	86.77	84.25	1.53	0.42
ME/GE intake, %	81.63	83.55	84.62	82.16	2.95	0.58
Nitrogen intake, g/d	17.0	17.41	17.76	17.70	1.37	0.81
Urinary nitrogen, g/d	0.19	0.06	0.06	0.13	0.03	0.29
Fecal nitrogen, g/d	2.94	2.30	2.96	2.99	0.12	0.86
Nitrogen retention rate, %	81.59[b]	86.79[a]	83.09	82.37	1.64	0.04

Note: Different superscript letters within a row indicate significant differences between means ($p<0.05$).

nitrogen excretion in the 2% stachyose added and SBM diets were 0.13 and 0.19 g/d, respectively. Therefore, nitrogen excretion increased as the stachyose level increased. The daily fecal nitrogen loss in the control diet was 2.30 g/d, but was substantially higher in the other three treatments (2.94-2.99 g/d). The nitrogen retention rate in control diet was 86.79%, which was 3.7% and 5.2% higher than those from the 1% and 2% stachyose added diets, respectively. The difference between SBM and control stachyose treatment was significant ($p<0.05$). The nitrogen retention rate was lowest in the SBM treatment (81.59%).

The apparent fecal digestibility of amino acids is shown in table 6. Apparent fecal digestibility of amino acids decreased as the soybean galactooligosaccharide level in the diet increased. Apparent amino acid digestibility for most amino acids was highest for the control diet, followed by the 1% added-stachyose, 2% added-stachyose, and SBM diets. However, there were no significant differences among treatments ($p>0.05$).

The digestibility of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF) is presented in table 7. Compared to the control diet, the digestibility of DM, OM, CP and CF decreased as the soybean galactooligosaccharide level increased in the diets, but there were no significant differences among treatments. The digestibility of CF was greatly affected by soybean galactooligosaccharide level. The CF digestibility in the 1% and 2% stachyose groups was 8.98% and 11.62%, respectively, and was lower than that of the control diet.

The content of chromium (Cr^{+6}) in feces at different time after eating the diets containing 0.3% Cr_2O_3 is shown in table 8. The content of Cr^{+6} in the feces differed among treatments ($p<0.05$) at 15 h, 18 h, and 21 h after intake, with Cr^{+6} passing through the pigs fed the 2% stachyose diets the quickest, followed by the 1% stachyose and SBM diets.

Risley and Lohrman (1998) conducted a study where piglets weaned at 18 d age (5.77 kg BW) were fed diets containing 30% soybean meal with lower stachyose. They observed that the apparent digestibility of energy (68.14% vs 71.62%) and DM (95.27% vs 95.95%) were lower for pigs fed low oligosaccharide diets than for pigs fed corn-SBM diets. The present experiment showed that while almost no soybean anti-nutritional factor and antigen protein existed in the diet, the DM digestibility of the diets declined as the addition level of soybean galactooligosaccharide increased (86.55% for control vs 84.19% and 83.98% for the 1% and 2% added-stachyose diets, respectively). This result was not consistent with the results reported by Risley and Lohrman (1998). This discrepancy may have resulted because the animals used in this trial differed in age and genetics, as well as differences in nutrition and experimental design. The pigs used in this experiment were weaned at 28 d of age with a body weight being about 12 kg, and Zhang et al. (2001) suggested that the ability of soybean galactooligosaccharide degradation by intestine bacteria was enhanced as the pigs got older.

Poultry research has shown that soybean galactooligosaccharides had a negative effect on DM and available energy digestibility. Leske et al. (1993) reported that in the adult rooster, the TMEn value of soybean concentrate protein was decreased ($p<0.05$) by adding raffinose and stachyose. There was no significant effect on GE among treatments.

Leske et al. (1993) also observed that galactooligosaccharides had a negative effect on protein digestibility for adult roosters. The protein digestibility of soybean meal without galactooligosaccharides was significantly higher than that of common soybean meal (73.1% vs 59.2%). Also, it was found that the effect of stachyose was greater than raffinose. When the stachyose level was equal to 40% stachyose in common soybean meal, the digestibility of CP was reduced by 1.5%, but when it was equal to 100%, the digestibility was reduced by 4%. Relative to stachyose, raffinose had slight effect on CP digestibility.

Studies on the effect of soybean galactooligosaccharides on amino acids are very few. Caugant et al. (1993), in the pre-ruminant calf, showed that the digestibility of amino

Table 6. The apparent fecal digestibility of amino acids of experimental diets (%)

Amino acids	SBM	HP300			SEM	P value
		Control	1% stachyose	2% stachyose		
Asp	84.60	88.41	88.20	86.39	1.81	0.44
Thr	81.80	85.35	84.52	82.55	2.24	0.66
Ser	84.99	87.96	87.21	86.88	2.15	0.78
Glu	87.61	90.87	90.39	88.35	1.39	0.33
Pro	85.11	90.68	89.04	87.78	2.09	0.33
Gly	81.11	84.73	83.80	82.12	2.03	0.60
Ala	76.24	82.36	81.74	77.82	2.88	0.40
Cys	79.04	78.80	80.21	81.40	3.00	0.91
Val	80.10	84.90	84.48	82.01	2.15	0.39
Met	75.91	80.88	81.01	79.08	2.95	0.58
Ile	83.14	87.82	87.12	84.74	1.79	0.28
Leu	85.65	88.92	87.94	85.42	1.73	0.44
Tyr	82.82	86.90	86.16	83.31	2.07	0.45
Phe	83.88	88.18	87.35	84.62	1.74	0.29
His	84.66	89.20	87.31	86.12	2.05	0.49
Lys	86.63	88.20	87.58	85.36	1.61	0.63
Arg	89.65	91.69	91.34	90.25	1.09	0.54
Average	83.11	86.81	86.20	84.36	2.05	0.50

Table 7. The percent digestibility of DM, OM, CF and CP in experimental diets

Items	SBM	HP300			SEM	P value
		Control	1% stachyose	2% stachyose		
DM, %	86.89	86.55	84.19	83.98	1.53	0.42
OM, %	87.87	87.79	86.50	85.57	1.42	0.60
CP, %	83.43	84.32	84.03	83.52	1.59	0.97
CF, %	62.56	61.85	52.87	50.23	6.73	0.14

Note OM; organic matter, CP; crude protein, CF; crude fiber.

Table 8. The content of Cr^{+6} in the digesta with different treatments, Unit %

Items	SBM	HP300			SEM	P value
		Control	1% stachyose	2% stachyose		
15 h*	0.16^{bc}	0.09^{a}	0.19^{cd}	0.22^{d}	0.01	0.003
18 h	0.28^{c}	0.11^{a}	0.29^{bc}	0.16^{a}	0.01	0.002
21 h	0.23^{c}	0.16^{a}	0.22^{bc}	0.16^{a}	0.08	0.005
24 h	0.16	0.20	0.20	0.14	0.04	0.77

^{a,b,c,d} Means within a row with unlike superscripts differ at the $p<0.05$ level.

* The time after eating the diets with inclusion 0.3% of chromic oxide.

acids increased after ethanol extraction. The results of our trial showed that the nitrogen retention of diets with 1% and 2% inclusion of stachyose decreased numerically as the soybean galactooli- gosaccharide level increased. Also, there was a significant difference between the 2% stachyose group and the control group ($p<0.05$). Compared to the control, the 2% stachyose diet decreased N retention by 5.2%. The CP digestibility had the similar tendency, which is in accordance with reports by Leske et al. (1993) and Caugant et al. (1993). However, Zuo et al. (1996) conducted an experiment with ileal-cannulated dogs to compare diets with stachyose soybean meal vs common soybean meal. The results showed no differences in CP and ileal amino acids digestibility.

The effect of raffinose and stachyose on TMEn of soybean products was associated with their levels in the diets. The negative effect was linearly related to the soybean galactooligosaccharides level. Leske et al. (1993) conducted another trial to determine the effect of different dietary levels of raffinose and stachyose on TMEn and DM digestibility. When the stachyose level was 1.36% (equivalent to 20% of stachyose content in common soybean meal), TMEn began to decrease significantly ($p<0.01$). As the level further increased, TMEn continued to decline. While the raffinose level exceeded 0.65% (equivalent to 60% of stachyose in common soybean meal) TMEn began to decrease. Our experimental results have also shown that nutrient and available energy digestibility decreased numerically as the soybean galactooligosaccharides level increased. Further research is needed to determine the effect of lower than 1% level soybean galactooligosaccharides on weanling piglets.

Coon et al. (1991), Leske et al. (1990; 1993) suggested that the alpha galactosidic linkage was the main cause in the reduction of TMEn, and was not related to sucrose. The results of our trial demonstrated that the transit time became shorter as the soybean galactooligosaccharide (raffinose and stachyose) level in the diet increases. Therefore, nutrient and energy digestibility decreased because the transit time was relatively shorter.

Although the galactooligosaccharide level (raffinose+ stachyose) in the soybean meal group was not over 1%, the nutrient digestibility and nitrogen retention rates were lower than that in 1% stachyose group. This showed that the nutrient digestibility and nitrogen retention rate were also possibly affected by other anti-nutritional factors such as trypsin inhibitor and antigen protein (glycinin and β-conglycinin). Many researchers have reported that trypsin could reduce the crude protein and amino acids digestibility (Miller et al., 1984; Li et al., 1990, 1991; Qiao et al., 1995, 1996; Chen et al., 1995).

Trypsin inhibitor can combine with trypsin in the small intestinal juice to produce a complex that reduces the digestibility of protein. Liener and Kadade (1980) found that rats fed a mixture of digested protein and trypsin inhibitor still exhibited growth repression, showing that the antinutritional effect of trypsin inhibitor was not only limiting the activity of trypsin but also limiting the others. Gallaher and Schneeman (1986) suggested that because the trypsin in intestine is combined with trypsin inhibitor and then is excreted, the lower amount of trypsin may induce the pig to secrete more into intestinal tract. Trypsin is rich in the sulfide amino acids so essential amino acids losses increased, resulting in growth repression.

CONCLUSION

Without antinutritional factors and soybean antigen protein, inclusion of 1% and 2% stachyose in corn- HP300 type diets has no significant effect on the digestibility of DM, OM, CP, CF and amino acids. The digestibility of CF was affected most. Comparing to the control treatment, the digestibility of CF in 1% and 2% stachyose added treatments decreased by 8.98% and 11.62%, respectively. The nitrogen retention rate was affected significantly by treatments. When the soybean galactooligosaccharides levels in the diets were adjusted to be almost same, antinutritional factors such as trypsin inhibitor and soybean antigen protein could decrease the nutrient digestibility and nitrogen retention rate in the diet further. High level soybean galactooligosaccharides shorten- ed the digesta transit time in intestinal tract. This trial indicated that the level of soybean galactooligosaccharide (stachyose and raffinose) in the weanling piglet diet should not exceed 1% when common soybean meal is used as main protein source. It is necessary to find some special soybean protein products to replace part of the SBM to avoid the negative effects on performance and nutrient digestibility from higher levels of soybean galactooligosaccharides.

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