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Feeding of Fermented Soybean Meal on Broiler Performance

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Abstract: Soybean meal was fermented with *Aspergillus niger* for 48 h, dried and supplemented in the broiler diet at 0.5, 1.0 and 1.5 percent to study its effect on production performances and intestinal characteristics by using 200 day-old Vencob broilers for 6 weeks period and compared with control diet and commercial enzyme preparation. The result revealed that there was no difference in body weight between treatments up to 4th weeks of age. However, body weight of 0.5 percent FSM fed group was significantly (P<0.05) higher than control at 5th and 6th weeks of age. The cumulative feed consumption was significantly (P<0.05) lesser in 1.5 percent FSM group at 5th and 6th weeks of age. During 3rd and 4th weeks, 0.5 percent FSM fed group recorded better (P<0.05) FCR than other groups. The livability was 100 percent in all the treatment groups. The percentage of intestine, pancreas, ready-to-cook weight to live weight, intestine length and viscosity of intestinal content were not significantly differed between the treatments. However, the pH of intestinal content was significantly (P<0.05) lower in 0.5, 1.0 percent FSM and commercial enzyme supplemented groups as compared to control. The ileum villi length and width was significantly (P<0.05) higher in 0.5, 1.0 FSM and commercial enzyme supplemented group compared to control. The activities of digestive enzymes did not differ significantly between treatments except lipase activity where, the lipase activity was significantly (P<0.05) higher in 0.5 percent and 1.0 percent FSM than other groups. It was concluded that fermented soybean meal may be supplemented in broiler diet at 0.5 percent level as microbial enzyme supplement to improve the production performance of broilers.

Key words: Fermented soybean meal, *A. niger*, microbial enzyme, production performance

Introduction

The broilers are mostly fed with maize, soybean meal-based diet since the high calorie and protein diet is the pre-requisite for the better growth rate and feed efficiency. These ingredients contain considerable amounts of non-starch polysaccharides, which have a tendency to create a viscous environment within the intestinal lumen (Choct and Annison, 1992; Choct *et al*., 1996) and thereby reduced the absorption of nutrients. However, supplementation of fungal enzymes improved the nutrient utilization (Fengler *et al*., 1988).

A. niger is one of the potent fungus having capacity to produce enzymes viz. hemicellulases, hydrolases, pectinolytic and lipolytic enzymes. The lipase enzymes produced by *A. niger* are most stable under acidic conditions that approximate the proventriculus compared to the stability of animal lipases and lipases from *Rhizopus arrhizus* (Kermanshahi *et al*., 1998).

The growth medium for this fungus should have adequate level of protein, carbohydrate as well as minerals. Among the commonly used feed ingredients, soybean meal is having balanced proportion of nutrients with approximately 38 percent protein, 31 percent carbohydrate, 8 percent water and high level of some minerals and vitamins (Lotong, 1998) which is conducive for the growth of fungus. Feeding broilers with diets containing soybean cultured with Aspergilli significantly improved growth and feed utilization in broilers by increasing the availability of nutrients (Chah *et al*., 1975). Hence, an attempt was made to study the effect of direct-feeding of *A. niger* fermented soybean meal (FSM) on broiler performance.

Materials and Methods

Fermented feed preparation: The pure culture of *A. niger* was purchased from the Institute of Microbial Technology, Chandigrah, India. The lyophilized culture was reconstituted and subcultures were made with the help of potato dextrose agar. The inoculated subcultures were kept at room temperature until the spore formation begins and thereafter the cultures were preserved in refrigerated temperature (approximately 4^{o}C) till the organisms were used for solid medium inoculation.

The fermented soybean meal (FSM) was prepared as per the procedure described by Zamora and Veum (1979). Briefly, the soybean meal was purchased from commercial feed plant and soaked in water at the ratio of 1:3 (1 part soybean meal : 3 parts water). The soaked

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soybean meal was autoclaved at 121°C for 30 minutes. The autoclaved soybean meal was spread on sterilized stainless steel pans to a depth of 2-3 cm and cooled to 37°C. The cooled fermented medium was inoculated with *A. niger* and covered with muslin cloth. These cultures were incubated at 37°C for 48 hours. After incubation, the samples were dried in a hot air oven at 80ºC for three days. The dried samples were ground in a grinding mill and stored until mixed with the diets.

Birds housing and feeding: Two hundred commercial, straight run day-old broiler chicks belonging to single hatch were purchased from local hatchery, wing banded, weighed and randomly allotted into five treatment groups with four replicates of ten chicks each. The chicks were reared in broiler cages in a gable roofed, open sided house. All the chicks were provided with uniform floor, feeder and waterer space and were reared under standard management conditions throughout the experimental period of six weeks. The diets were formulated having isocaloric and isonitrogenous content as per B.I.S (1992) and FSM was included with basal diet (T1) at 0.5 (T2), 1.0 (T3) and 1.5% (T4) percent and compared with commercial enzymes (T5). Feed and water were provided for *ad-libitum*. Data on body weight, feed consumption were recorded every week and mortality was recorded at occurrence. From the above data feed efficiency and livability were calculated.

Slaughter and sampling of birds: At 42nd day of age, six birds from each treatment were humane slaughtered. Ready to-cook weight, pancreas weight, intestinal weight and length were recorded. Intestinal segment of ileum portion was taken to study the villi and crypt structure of intestine. Individual intestinal content from small intestine were collected in a graduated tube and divided in to two parts. One part was used to measure the pH and viscosity immediately and another part was stored at –20°C to analyze the digestive enzymes.

Analysis and measurements

pH: The pH of the intestinal content was recorded by using digital pH meter immediately after slaughter.

Viscosity: The relative viscosity of the digesta was calculated by the method of Choct and Annison (1992) using Ostwald U-tube viscometer. Immediately after slaughter, the ileal contents were squeezed out, collected in 15 ml of triple glass distilled water and centrifuged at 10,000 g for 15 min. The volume of the supernatant (V_1) was recorded and the water content of the original digesta (V_0) was calculated

$V_0 = V_1$-15

A final volume (V) with a constant ratio of V_0 to V was obtained by the addition of water. The time (Tx) for an aliquot digesta supernatant and time (Tw) for distilled water to flow through the viscometer was recorded. The relative viscosity of the sample was obtained from the following relationship

Relative viscosity (cP) = Tx / Tw

Intestinal enzymes: Digesta supernatant was taken and diluted to 1:1000 with 0.9 percent saline. Estimation of amylase activity of the digesta was assessed as per the method of Coles (1986). Briefly, One ml of 0.02 percent buffered (pH 7.0) starch substrate (prepared by addition of 13.3 g of anhydrous disodium phosphate and 4.3 g of benzoic acid to 250 ml of triple glass distilled water and boiled. A soluble starch 200 mg was prepared separately in 5 ml of cold distilled water and mixed to the boiling mixture. The beaker was rinsed with the additional cold water, boiled, centrifuged for one min, cooled to room temperature and diluted to 500 ml triple glass distilled water) was incubated at 37°C exactly for 8.5 min along with 0.1 ml of diluted supernatant solution and one ml of working iodine solution (25 g of potassium fluoride with 50 ml of 0.1N iodine solution. The 0.1N iodine solution was prepared by dissolving 3.567 gm of potassium iodate and 45.0 gm of potassium iodide in approximately 800 ml of distilled water and slowly adding 9 ml of concentrated hydrochloric acid with constant mixing. The mixture was diluted to one liter with distilled water). The mixture was thoroughly mixed and the volume was made up to 10 ml with triple distilled water. The optical density (OD) was read at 660 nm in a digital photoelectric colorimeter using distilled water as blank.

$$\text{Amylase activity (Units/ml)} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times \text{Volume of supernatant} \times \text{Dilution factor}$$

The lipase activity of the digesta was determined as per the method of Boutwell (1962). The substrate buffer mixture was prepared by stirring five volume of 0.067 M phosphate buffer (pH 7.0) along with one volume of olive oil emulsion. Twelve milliliters of the substrate buffer mixture was equally transferred into two tubes and warmed in a water bath at 37°C. To one tube 5 ml of the intestine homogenate was added and mixed thoroughly by gentle inversion. The second tube served as a blank. Both the tubes were incubated at 37°C for 24 h. At the end of incubation, 5 ml of homogenate was added to the blank. The mixture was titrated along with 4-6 drops of phenolphthalein against 0.05 N sodium hydroxide to get a distinct pink colour. The lipase unit was the quantity of enzyme required to release acid equivalent to one ml of 0.05 N NaOH from an olive oil substrate in 24 h.

$$\text{Lipase activity (Units/ml)} = \frac{\text{Titre value of test - Blank}}{\text{Weight of the intestine content(g)}} \times \text{Dilution factor}$$

Table 1: Mean body weight, feed consumption and feed conversion ratio (± S.E.) of broilers from1 to 6 weeks of age as influenced by dietary inclusion of fermented soybean meal

A. Body weight (g)	Control	0.5% FSM	1.0%FSM	1.5% FSM	Commercial enzymes
Day old chick	45.50±1.10	45.47±1.07	45.45±0.95	45.48±0.95	45.43±0.97
1st week	123.42±4.45	124.08±4.71	130.67±3.56	121.50±4.22	130.17±4.07
2nd week	313.58±13.10	319.50±8.93	320.25±10.38	311.92±11.72	330.33±11.40
3rd week	631.92±23.96	646.42±14.34	632.83±22.05	620.50±21.30	643.00±21.26
4th week	1016.00±35.53	1065.42±20.49	1039.00±38.92	990.33±30.24	1050.75±30.17
5th week	1463.00^{a}±49.48	1568.33^{b}±37.95	1504.00^{ab}±47.67	1416.17^{a}±41.20	1522.17^{ab}±43.55
6th week	1783.75^{a}±51.75	1936.83^{b}±36.15	1840.92^{ab}±48.88	1750.50^{a}±37.86	1818.92^{ab}±45.64
B. Feed consumption (g/bird)					
1st week	92.02±2.50	95.33±3.00	104.40±5.60	89.25±6.91	98.50±3.35
2nd week	272.08±3.75	275.00±5.67	278.33±5.00	268.72±7.09	250.00±5.00
3rd week	531.28±7.75	489.23±6.83	528.75±6.41	529.67±7.42	580.62±22.09
4th week	732.56±10.17	720.92±15.25	756.34±6.92	689.17±13.84	750.91±11.25
5th week	910.16^{b}±21.00	990.75^{b}±7.08	954.28^{b}±13.25	834.17^{a}±14.34	955.42^{b}±10.42
6th week	928.00^{b}±8.55	942.21^{b}±12.25	923.91^{b}±13.75	903.18^{a}±12.50	933.33^{b}±14.17
Total feed	3466.02^{b}±41.39	3513.44^{b}±21.25	3546.01^{b}±31.46	3314.15^{a}±14.09	3509.24^{b}±35.80
C. Feed conversion ratio					
1st week	1.18±0.05	1.21±0.04	1.23±0.03	1.17±0.04	1.16±0.02
2nd week	1.43^{b}±0.02	1.41^{b}±0.02	1.47^{b}±0.02	1.41^{b}±0.02	1.25^{a}±0.03
3rd week	1.67^{b}±0.04	1.50^{a}±0.04	1.69^{b}±0.02	1.72^{b}±0.02	1.67^{b}±0.04
4th week	1.91^{b}±0.04	1.72^{a}±0.03	1.86^{b}±0.03	1.86^{b}±0.03	1.84^{b}±0.03
5th week	2.04±0.07	1.97±0.13	2.05±0.04	1.96±0.02	2.03±0.06
6th week	2.89±0.04	2.87±0.12	2.74±0.09	2.70±0.13	2.95±0.20
Overall FCR	1.94±0.03	1.82±0.04	1.93±0.01	1.88±0.03	1.91±0.04

a,,b Means within a row with no common superscript differ significantly (P<0.05).

The tryptic activity of the digesta was assessed by the method of Hawk *et al.* (1947). The digesta was homogenized, centrifuged and the supernatant was collected. It was serially diluted at the rate of 1, 1/2., 1/4, 1/8 1/16 and 1/32. One drop of phenolphthalein solution was added to each tube followed by addition of 2 percent sodium bicarbonate solution drop by drop until the development of light pink color. To all the tubes, 0.5 ml of casein solution (0.4 g of casein in 40 ml of 0.1 N NaOH, 130 ml of triple glass distilled water and 30 ml of 0.1 N hydrochloric acid) was added and incubated at 40° C for 5 h. The undigested casein was precipitated by drop wise addition of precipitating solution (glacial acetic acid 1 ml; alcohol (95%) 50 ml and distilled water 50 ml). Development of a clear solution indicated complete enzymatic digestion. The boiled supernatants and distilled water were taken in separate tubes with casein solution followed by the test reagents served as control, which showed turbidity on addition of the precipitating solution. The tryptic values were expressed in terms of dilution.

Histomorphology: Ileum portion of the small intestine were collected during slaughter and fixed in 10 percent buffered formal saline. The paraffin embedded tissues were sectioned to 3 to 4 µ thickness and stained with hematoxylin and eosin for histomorphological studies.

Statistical analyses: All the data were subjected to completely randomized design as per the procedure of Snedecor and Cochran (1989) and significance were compared at the probability of 0.05 and 0.1 percent levels.

Results

Production parameters: Analysis of data revealed no difference in body weight between treatments up to 4th week of age. However, body weight of 0.5 percent FSM group was significantly (P<0.05) higher than control and 1.5 percent FSM fed group at 5th and 6th week of age, while there was no significant difference between 0.5 and 1.0 percent FSM and commercial enzyme added group. Similarly, no significant difference was observed between 1.0 and 1.5 percent FSM and commercial enzyme added group.

Statistical analysis of feed consumption revealed no difference up to 4th week of age. However, during 5th, 6th and cumulative feed consumption were significantly (P<0.05) lesser in 1.5 percent FSM as compared to other groups. No significant difference was observed between other treatment groups.

The feed conversion ratio (FCR) was better (P<0.05) in commercial enzyme fed group at 2nd week of age. However, during 3rd and 4th week, 0.5 percent FSM fed group recorded better (P<0.05) FCR than other groups. The cumulative FCR was not showed any significant difference between treatment groups though 0.5 percent FSM group recorded a non-significant better FCR. The livability was 100 percent in all the treatment groups.

Carcass and intestinal characteristics: The percentages of intestine, pancreas, ready-to-cook weight to live weight were not significantly different between treatments. Similarly, the intestine length and viscosity of intestinal content were not differed between treatment groups. However, the pH of intestinal content was

significantly (P<0.05) lower in 0.5, 1.0 percent FSM and commercial enzyme supplemented groups as compared to control and 1.5 percent FSM.

The lieum villi length was significantly (P<0.05) higher in 0.5, 1.0 FSM and commercial enzyme supplemented group as compared to control and 1.5 percent FSM, which were not differed significantly between them. Similarly, the ileum villi width was significantly (P<0.05) higher in 0.5 and 1.0 FSM, which were not differed significantly with control and 1.5 percent FSM. While commercial enzyme fed group recorded lower width, which were not differed significantly with control and 1.5 percent FSM fed groups. The crept length was significantly (P<0.05) higher in FSM supplemented groups than control group with no significant difference as compared to commercial enzymes. The ileum crept width was not differed between treatment groups.

The activities of digestive enzymes did not differ significantly between treatments except lipase activity where, the lipase activity was significantly (P<0.05) higher in 0.5 percent and 1.0 percent FSM than other groups. However the difference did not exist between 1.0 percent FSM and commercial enzyme supplemented groups.

Discussion

Production parameters: The body weight of 0.5 percent FSM fed group was significantly (P<0.05) higher than control and 1.5 percent FSM fed group at 6th week of age. This finding is in agreement with earlier reports of Chah *et al.* (1975) who reported that chicks received diets containing soybeans cultured with desirable Aspergilli significantly improved the growth rate. This may be due to higher levels and digestibility of threonine, lysine, leucine and methionine as a result of fermentation (Zamora and Veum, 1979). The increased digestibility is may be due to positive influence of fermented diet on gastrointestinal health by lowered gastric pH, increased level of short chain fatty acids, reduced pathogenic microbial activity and improved mucosal architecture (Scholten *et al.*, 1999). The similar observations of reduced intestinal pH, improved mucosal structure of ileum villi were also observed in this study. Similarly, Nagra *et al.* (1998) recorded better growth rate (P<0.05) by feeding fermented guar meal with *A. niger* to broilers. However, no significant differences were observed between 1.0 and 1.5 percent FSM, commercial enzyme and control groups.

Fermented feed has several characteristics that may have beneficial effect especially against infective agents. Firstly, it is rich in lactic acid bacteria and is claimed that feeding of *L. acidophilus* and *L. bulgaricus* supplementation separately and in combination to broilers through diet increased the body weight at 5 and 7 weeks (Samanta and Biswas, 1995). Secondly, the digestibility of fermented or soaked feed may be improved, which may alter the composition of the intestinal micro flora. This was evident with the report of Jin *et al.* (2000) who observed that feeding of single strain of *L. acidophilus* or a mixture of 12 *Lactobacillus* strains at 0.1 percent in diet for 40 days significantly (P<0.05) increased the levels of amylase in the small intestine in Arbor-Acres broilers. However, the proteolytic and lipolytic activities in the small intestine were not affected by addition of either *L.acidophilus* or a mixture of 12 *Lactobacillus* strains. And finally, this feed may has high concentration of lactic and other organic acids, which improved the performance of broilers (Chitra, 2000). However, no significant difference was observed in body weight between 0.5 and 1.0 percent FSM and commercial enzyme group.

Cumulative feed consumption was significantly (P<0.05) lesser in 1.5 percent FSM consumed group than other groups. However, there was no significant difference in cumulative FCR between treatment groups. This observation is contrary to the findings of Chah *et al.* (1975) who reported better feed utilization in Aspergilli fermented soybean fed chickens due to more efficiency in utilization of nitrogen than unfermented soybean in broilers .

Carcass and intestinal characteristics: The intestine, pancreas, ready-to-cook weight to live weight percentage was not significantly different between all the treatments. Similarly, the intestine length and viscosity of intestinal content were not differed between treatment groups.

The pH of the intestinal content was significantly (P<0.05) lower in 0.5, 1.0 percent FSM and commercial enzyme supplemented groups than control and 1.5 percent FSM and this was attributed to higher level of volatile fatty acids - lactic acid and acetic acid production during fermentation (Scholten *et al.*, 2002), which decreased the viable counts of pathogenic bacteria in the caeca of broiler chickens.

Feeding of fermented diet significantly (P<0.05) increased the villus height, width and crypt length, which might helped for better cumulative feed conversion ratio in 0.5 percent FSM than control though not differed significantly at 3rd and 4th week of age. This findings is in agreement with report of Scholten *et al.* (2002) who observed that villus height, villus shape and villus:crypt ratio was increased in swine by feeding of fermented diet, which may due to higher lactic acid and total short chain fatty acids level present in fermented feed.

The activities of digestive enzymes did not differ significantly between treatments except lipase activity where, the lipase activity was significantly (P<0.05) higher in 0.5 percent and 1.0 percent FSM groups, which may be due to better subtract nature of soybean meal for microbial lipase production (Mathivanan *et al.*, 2006), and hence improved the fat utilization in broilers.

From the above study, it was concluded that feeding of broilers with fermented soybean supplementation at 0.5 percent level from 0-6 weeks age increased the body weight, feed consumption and improved the feed conversion ratio. These effects may be attributed to

Table 2: Mean carcass and intestinal characteristics (± S.E.) of broilers at 6 weeks of age as influenced by dietary inclusion of fermented soybean meal

	Control	0.5% FSM	1.0%FSM	1.5% FSM	Commercial enzymes
A. Organ weight % to live weight					
Intestine weight	4.60±0.30	4.10±0.16	4.62±0.08	4.58±0.35	4.61±0.62
Pancreas weight	0.29±0.03	0.22±0.03	0.28±0.01	0.32±0.02	0.24±0.03
Ready –to –cook weight	72.33±3.56	75.80±5.99	73.03±6.72	73.86±4.09	77.81±6.81
B. Intestine length (cm)	181.00±10.50	160.00±21.94	168.67±11.26	189.33±9.82	183.00±6.11
C. Viscosity of intestinal Content (cP)	3.89±0.04	3.14±0.06	3.61±0.30	4.01±0.15	3.58±0.06
D. pH of intestinal content	6.83^{b}±0.03	6.30^{a}±0.06	6.37^{a}±0.09	6.73^{b}±0.09	6.43^{a}±0.03
E. Ileum villi length (µm)	1532.28^{a}±47.62	1665.48^{b}±33.41	1598.46^{b}±25.34	1499.25^{a}±40.25	1652.54^{b}±25.37
F. Ileum villi width (µm)	163.60^{ab}±8.39	166.73^{b}±12.08	171.01^{b}±7.92	163.00^{ab}±9.87	159.49^{a}±9.50
G. Ileum crypt length (µm)	149.12^{a}±8.31	160.43^{b}±12.57	162.01^{b}±12.75	165.68^{b}±7.00	158.29^{ab}±9.33
H. Ileum crypt width (µm)	66.95±1.98	64.97±2.40	95.43±3.05	66.08±1.28	66.59±2.27
I. Digestive enzymes activity (U/ ml of intestinal content)					
Amylase	72.54±11.93	70.58±3.40	72.54±11.93	77.25±12.65	84.31±11.96
Lipase	7.33^{C}±1.17	16.25^{A}±2.63	13.08^{AB}±1.44	8.08^{C}±1.36	10.58^{BC}±1.08
Trypsin	10.66±2.13	12.80±2.33	10.66±4.26	13.30±2.50	14.00±1.30

^{a,b}Means within a row with no common superscript differ significantly (P<0.05)

lowered pH of intestine by feeding of fermented feed, which in turn helped for better utilization of nutrients by improved intestinal environment.

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