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 Aspergillus 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, Chandigarh, 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°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
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 80°C 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_s) was recorded and the water content of the original digesta (V_o) was calculated

\[ V_o = V_s - 15 \]

A final volume (V) with a constant ratio of V_s 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) = \( \frac{Tx}{Tw} \)

**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) 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.

**Amylase activity** (Units/ml) =

\[ \frac{OD_{control} - OD_{test}}{X Volume of X Dilution factor} \]

OD of control supernatant

**Lipase activity** (Units/ml) =

\[ \frac{Titre value of test -Blank}{X Dilution factor} \]

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.5% FSM</th>
<th>1.0% FSM</th>
<th>1.5% FSM</th>
<th>Commercial enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Body weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Day old chick</td>
<td>45.50±1.10</td>
<td>45.47±1.07</td>
<td>45.45±0.95</td>
<td>45.48±0.95</td>
<td>45.43±0.97</td>
</tr>
<tr>
<td>1st week</td>
<td>123.42±4.45</td>
<td>124.08±4.71</td>
<td>130.67±3.56</td>
<td>121.50±4.22</td>
<td>130.17±4.07</td>
</tr>
<tr>
<td>2nd week</td>
<td>313.58±13.10</td>
<td>319.50±8.93</td>
<td>320.25±10.38</td>
<td>311.92±11.72</td>
<td>330.33±11.40</td>
</tr>
<tr>
<td>3rd week</td>
<td>631.92±23.96</td>
<td>646.42±14.34</td>
<td>632.83±22.05</td>
<td>620.50±21.30</td>
<td>643.00±21.26</td>
</tr>
<tr>
<td>4th week</td>
<td>1016.00±35.53</td>
<td>1065.42±20.49</td>
<td>1039.00±38.92</td>
<td>990.33±30.24</td>
<td>1050.75±30.17</td>
</tr>
<tr>
<td>5th week</td>
<td>1463.00±49.48</td>
<td>1568.33±37.95</td>
<td>1504.00±47.67</td>
<td>1416.17±41.20</td>
<td>1522.17±43.55</td>
</tr>
<tr>
<td>6th week</td>
<td>1783.75±51.75</td>
<td>1936.83±36.15</td>
<td>1840.92±48.88</td>
<td>1750.50±37.86</td>
<td>1818.92±45.64</td>
</tr>
<tr>
<td><strong>B. Feed consumption (g/bird)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st week</td>
<td>92.02±2.50</td>
<td>95.33±3.00</td>
<td>104.40±5.60</td>
<td>89.25±6.91</td>
<td>98.50±3.35</td>
</tr>
<tr>
<td>2nd week</td>
<td>272.08±3.75</td>
<td>275.00±5.67</td>
<td>278.33±5.00</td>
<td>268.72±7.09</td>
<td>250.00±5.00</td>
</tr>
<tr>
<td>3rd week</td>
<td>531.28±7.75</td>
<td>489.23±6.83</td>
<td>528.75±6.41</td>
<td>529.67±7.42</td>
<td>580.62±22.09</td>
</tr>
<tr>
<td>4th week</td>
<td>732.56±10.17</td>
<td>720.92±15.25</td>
<td>756.34±6.92</td>
<td>689.17±13.84</td>
<td>750.91±11.25</td>
</tr>
<tr>
<td>5th week</td>
<td>910.16±21.00</td>
<td>990.79±7.08</td>
<td>954.28±13.25</td>
<td>834.17±14.34</td>
<td>955.42±10.42</td>
</tr>
<tr>
<td>6th week</td>
<td>928.00±8.55</td>
<td>942.21±12.25</td>
<td>923.91±13.75</td>
<td>903.18±12.50</td>
<td>933.33±14.17</td>
</tr>
<tr>
<td><strong>C. Feed conversion ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st week</td>
<td>1.18±0.05</td>
<td>1.21±0.04</td>
<td>1.23±0.03</td>
<td>1.17±0.04</td>
<td>1.16±0.02</td>
</tr>
<tr>
<td>2nd week</td>
<td>1.43±0.02</td>
<td>1.41±0.02</td>
<td>1.47±0.02</td>
<td>1.41±0.02</td>
<td>1.25±0.03</td>
</tr>
<tr>
<td>3rd week</td>
<td>1.67±0.04</td>
<td>1.50±0.04</td>
<td>1.69±0.02</td>
<td>1.72±0.02</td>
<td>1.67±0.04</td>
</tr>
<tr>
<td>4th week</td>
<td>1.91±0.04</td>
<td>1.72±0.03</td>
<td>1.86±0.03</td>
<td>1.86±0.03</td>
<td>1.84±0.03</td>
</tr>
<tr>
<td>5th week</td>
<td>2.04±0.07</td>
<td>1.97±0.13</td>
<td>2.05±0.04</td>
<td>1.96±0.02</td>
<td>2.03±0.06</td>
</tr>
<tr>
<td>6th week</td>
<td>2.89±0.04</td>
<td>2.87±0.12</td>
<td>2.74±0.09</td>
<td>2.70±0.13</td>
<td>2.95±0.20</td>
</tr>
<tr>
<td>Overall FCR</td>
<td>1.94±0.03</td>
<td>1.82±0.04</td>
<td>1.93±0.01</td>
<td>1.88±0.03</td>
<td>1.91±0.04</td>
</tr>
</tbody>
</table>

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

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 than other groups.

However, during 3 and 4 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 not differed between treatments.
intestinal micro flora. This was evident with the report of improved, which may alter the composition of the digestibility of fermented or soaked feed may be 7 weeks (Samanta and Biswas, 1995). Secondly, the broilers through diet increased the body weight at 5 and supplementation separately and in combination to to 

Firstly, it is rich in lactic acid bacteria and is claimed that have beneficial effect especially against infective agents. Fermented feed has several characteristics that may with 

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 Lactobacillus or a mixture of 12 Lactobacillus strains. And finally, this feed may have 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) increased the levels of amylase in the small intestine in Arbor-Acres broilers. However, the proteolytic in the small intestine were not affected by addition of either Lactobacillus or a mixture of 12 Lactobacillus strains. And finally, this feed may have 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 .

Discussion

Feeding of fermented soybean meal 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
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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References


