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## Effects of Diet Supplementation of *Aspergillus* Meal Prebiotic (Fermacto®) on Efficiency, Serum Lipids and Immunity Responses of Broiler Chickens

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**Abstract:** This experiment was conducted to investigate the effects of *Aspergillus* meal prebiotic (Fermacto) on production performance, serum lipids and immune responses in broiler chickens. One hundred and forty four day-old Ross male broiler chicks were randomly assigned to three dietary treatments: a basal diet (control), a basal diet with 0.15% Fermacto and basal diet with 0.30% Fermacto. The production parameters, including Feed Intake (FI), Body Weight Gain (BWG) and Feed to Gain Ratio (FCR) were monitored weekly serum lipids, antibody productions and antibody titer against Infectious Bursal Virus Disease (IBVD) were measured at the end of a 6 week trial. At 28 days of age, four birds from each replicate of treatments were injected intrabreast with 0.2 mL of 5% Sheep Red Blood Cell (SRBC). The supplementation of 0.30% Fermacto in broiler diets improved the body weight gain from 21-28 and 35-42 days of age ( $p < 0.05$ ). The BWG was also significantly higher ( $p < 0.01$ ) in Fermacto-fed broilers than that of control treatment at 28-35 days of age, overall growing (21-42 day) and rearing (0-42 day) periods. FI was significantly ( $p < 0.05$ ) higher for 0.15% Fermacto-fed broilers than that of control broilers at 14-21 and 0-21 days of age. FI was significantly greater ( $p < 0.05$ ) in 0.30% Fermacto-fed broilers compared to control broilers at 35-42 days of age, during growing (21-42 day and rearing (0-42 day) periods. From 28-35 days of age, a lower FCR ( $p < 0.05$ ) was observed with 0.15% Fermacto when compared with control group. There were also no significant differences in total cholesterol, high density lipoprotein cholesterol (HDL-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol) and triglycerides among treatments. Dietary Fermacto did not affect antibody titer against IBVD. The serum IgG, IgA and IgM production significantly ( $p < 0.01$ ) decreased in Fermacto-fed broilers as compared to control broilers.

**Key words:** Prebiotics, fermacto, immunity response, performance, broilers

### INTRODUCTION

For many years antibiotics have been used in animal nutrition to improve growth, to reduce mortality, to improve reproductive performance and to protect animals from the pathogenic microorganisms. The continuous use of subtherapeutic levels of antibiotics in animal feeds may result in the presence of drug-resistant microorganisms in humans who consume these animal products. To avoid such problems, some alternatives have been suggested to antibiotics. Prebiotics have been defined as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). *Aspergillus* meal (Fermacto) is derived from a carefully controlled fermentation of *Aspergillus oryzae*, has no live cells or spores (Rodriguez *et al.*, 2005) and has been shown to increase gut development (Santose *et al.*, 2005). Several

studies have shown that addition of Fermacto to poultry diets improve performance (Potter and Shelton, 1984; Grimes *et al.*, 1997; Rodriguez *et al.*, 2005). The effect of growth promoters like Fermacto on blood parameters and immune responses are less studied. Therefore, the objective of this study was to evaluate Fermacto on performance, blood parameters and immune responses of broiler chickens.

### MATERIALS AND METHODS

This study was conducted at Poultry Research Station of Ferdowsi University of Mashhad, Iran in 2007. One hundred forty four day-old commercial Ross male broiler chickens were randomly allocated in a completely randomized design considering 3 treatments with 4 replicates of 12 chicks each. Three levels of Fermacto (0.0, 0.15 and 0.30%) were included into a typical corn-soybean meal based diets (Table 1) that met the broiler

**Table 1: Composition of experimental diets fed by broiler chickens**

| Experimental data           | Age (days) |         |         |         |         |         |
|-----------------------------|------------|---------|---------|---------|---------|---------|
|                             | 0-21       |         |         | 21-42   |         |         |
|                             | 0-7        | 7-14    | 14-21   | 21-28   | 28-35   | 35-42   |
| <b>Ingredients (%)</b>      |            |         |         |         |         |         |
| Corn                        | 57.55      | 57.55   | 57.55   | 66.34   | 66.34   | 66.34   |
| Soybean meal                | 36.62      | 36.62   | 36.62   | 29.43   | 29.43   | 29.43   |
| Wheat bran                  | 0.00       | 0.15    | 0.30    | 0.00    | 0.15    | 0.30    |
| Fermacto                    | 0.30       | 0.15    | 0.00    | 0.30    | 0.15    | 0.00    |
| Dicalcium phosphate         | 1.46       | 1.46    | 1.46    | 1.03    | 1.03    | 1.03    |
| Limestone                   | 1.15       | 1.15    | 1.15    | 1.27    | 1.27    | 1.27    |
| Vit. and Min. premix        | 0.50       | 0.50    | 0.50    | 0.50    | 0.50    | 0.50    |
| Salt                        | 0.42       | 0.42    | 0.42    | 0.31    | 0.31    | 0.31    |
| Veg. oil                    | 1.86       | 1.86    | 1.86    | 0.78    | 0.78    | 0.78    |
| DL-Methionine               | 0.14       | 0.14    | 0.14    | 0.04    | 0.04    | 0.04    |
| <b>Calculated analysis</b>  |            |         |         |         |         |         |
| ME (kcal kg <sup>-1</sup> ) | 2900.00    | 2900.00 | 2900.00 | 2950.00 | 2950.00 | 2950.00 |
| CP (%)                      | 20.84      | 20.84   | 20.84   | 18.40   | 18.40   | 18.40   |
| Ca (%)                      | 0.91       | 0.91    | 0.91    | 0.83    | 0.83    | 0.83    |
| Av. P. (%)                  | 0.41       | 0.41    | 0.41    | 0.32    | 0.32    | 0.32    |
| Na (%)                      | 0.18       | 0.18    | 0.18    | 0.14    | 0.14    | 0.14    |
| Arg. (%)                    | 1.38       | 1.38    | 1.38    | 1.18    | 1.18    | 1.18    |
| Lys. (%)                    | 1.14       | 1.14    | 1.14    | 0.97    | 0.97    | 0.97    |
| Met. + Cys. (%)             | 0.82       | 0.82    | 0.82    | 0.66    | 0.66    | 0.66    |

Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D<sub>3</sub>, 9790 IU; vitamin E, 121 IU; B<sub>12</sub>, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamine, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg

requirements (NRC, 1994). Feed and water were provided *ad libitum*. The broilers were reared on floor pens (1×1) and maintained on 24 h continuous lights. Feed Intake (FI) and body weight gain (BWG) were recorded weekly. Broiler chickens were immunized using killed infectious bursal disease virus (IBVD) at 11 and 20 days of age. At 28 days of age, one bird from each replicate of treatments were randomly selected and injected intrabreast muscle with 0.2 mL of 5% sheep red blood cell (SRBC). At 42 days of age, those birds were injected with SRBC, weighed and killed by cervical dislocation. The blood samples were collected in EDTA blood collection tubes, centrifuged and serum was separated and then stored at -20°C until assayed for measuring blood lipids using appropriate laboratory kits (Friedewald *et al.*, 1972). Serum samples were also analyzed for antibody responses against IBVD by ELISA technique using commercial kits and the plates were read at 405 nm on an ELISA reader. For measuring the concentration of blood immunoglobulins, IgA, IgG and IgM, the sera were determined by immunodiffusion assay. Data were analyzed based on a general linear model procedure of SAS (1993) and treatment means when significant ( $p < 0.05$ ), were compared using Duncan's multiple range test (Duncann, 1955).

## RESULTS AND DISCUSSION

There were significant effects of dietary treatments on the body weight gain, feed intake and feed conversion ratio. Fermacto-fed broilers gained more weight during first, second and third weeks and from 0-21 days of age,

although these improvements were not significant (Table 2). A significantly higher ( $p < 0.05$ ) body weight gain was observed in 0.30% Fermacto-fed as compared to control broilers from 21-28 and 35-42 days of age. No significant difference was observed between 0.15 and 0.30% Fermacto supplemented groups. BWG was significantly improved ( $p < 0.05$ ) when Fermacto added into the broiler diets from 28-35, 21-42 and 0-42 days of age as compared to control treatment. Rodriguez *et al.* (2005) observed that body weight significantly improved by including of 0.2% Fermacto into the low protein diets of broiler chickens. Potter (1972) observed improvements in body weight gain, feed intake and feed efficiency in medium white male and female turkeys fed diets containing 0.25% Fermacto up to 8 weeks of age. Waldroup *et al.* (2003) reported that supplementation of broilers diets with 0.1% mannan oligosaccharide (Bio-Mos) from 0-42 followed by 0.075% to 63 days of age did not influence body weight, feed conversion and mortality. FI was not affected by dietary treatments at 0-7 days of age. The same trend at 7-14, 21-28 and 28-35 days of age was observed. A significantly higher ( $p < 0.05$ ) feed intake was observed in 0.15% Fermacto as compared to 0.30% and control broilers from 14-21 days of age. A significantly greater ( $p < 0.05$ ) feed intake was observed in 0.15% Fermacto as compared to control broilers from 0-21 days of age. No significant difference was observed between 0.15 and 0.30% Fermacto supplemented groups. Feed intake was significantly greater ( $p < 0.05$ ) in 0.30% Fermacto-fed than that of control broilers from 35-42 days of age, during growing

**Table 2: Effects of experimental diets on body weight gain (g) of broiler chickens**

| Fermacto (%) | Age (days) |        |        |                      |                     |                      |        |                      |                      |
|--------------|------------|--------|--------|----------------------|---------------------|----------------------|--------|----------------------|----------------------|
|              | 0-7        | 7-14   | 14-21  | 21-28                | 28-35               | 35-42                | 0-21   | 21-42                | 0-42                 |
| 0.0          | 73.90      | 143.80 | 256.60 | 265.50 <sup>b</sup>  | 254.40 <sup>b</sup> | 270.90 <sup>b</sup>  | 474.40 | 790.90 <sup>b</sup>  | 1265.30 <sup>b</sup> |
| 0.15         | 80.70      | 148.60 | 272.90 | 315.40 <sup>ab</sup> | 342.40 <sup>a</sup> | 392.40 <sup>ab</sup> | 502.30 | 1050.30 <sup>a</sup> | 1552.70 <sup>a</sup> |
| 0.30         | 78.90      | 148.10 | 262.20 | 331.20 <sup>a</sup>  | 326.70 <sup>a</sup> | 425.90 <sup>a</sup>  | 489.20 | 1083.90 <sup>a</sup> | 1573.20 <sup>a</sup> |
| SEM±         | 3.30       | 7.10   | 10.17  | 14.50                | 15.09               | 38.11                | 18.86  | 42.79                | 51.20                |
| p-value      | 0.36       | 0.87   | 0.54   | 0.02                 | 0.006               | 0.04                 | 0.59   | 0.002                | 0.004                |

<sup>a,b,c</sup>: Means in each column with different superscripts are significantly different (p<0.05)

**Table 3: Effects of experimental diets on feed intake (g) of broiler chickens**

| Fermacto (%) | Age (days) |        |                     |        |        |                       |                      |                       |                       |
|--------------|------------|--------|---------------------|--------|--------|-----------------------|----------------------|-----------------------|-----------------------|
|              | 0-7        | 7-14   | 14-21               | 21-28  | 28-35  | 35-42                 | 0-21                 | 21-42                 | 0-42                  |
| 0.0          | 137.60     | 299.40 | 413.30 <sup>b</sup> | 647.40 | 704.30 | 917.70 <sup>b</sup>   | 850.40 <sup>b</sup>  | 2269.50 <sup>b</sup>  | 3119.90 <sup>b</sup>  |
| 0.15         | 142.50     | 312.10 | 484.20 <sup>a</sup> | 708.50 | 746.30 | 1089.30 <sup>ab</sup> | 938.80 <sup>a</sup>  | 2544.10 <sup>ab</sup> | 3483.00 <sup>ab</sup> |
| 0.30         | 139.30     | 322.90 | 421.10 <sup>b</sup> | 714.90 | 799.50 | 1229.00 <sup>a</sup>  | 883.40 <sup>ab</sup> | 2743.40 <sup>a</sup>  | 3626.80 <sup>a</sup>  |
| SEM±         | 3.78       | 14.28  | 12.57               | 32.64  | 30.84  | 55.90                 | 19.50                | 103.59                | 101.29                |
| p-value      | 0.66       | 0.53   | 0.006               | 0.31   | 0.14   | 0.01                  | 0.03                 | 0.03                  | 0.02                  |

<sup>a,b,c</sup>: Means in each column with different superscripts are significantly different (p<0.05)

**Table 4: Effects of experimental diets on feed conversion ratio (g g<sup>-1</sup>) of broiler chickens**

| Fermacto (%) | Age (days) |      |       |       |                    |       |      |       |      |
|--------------|------------|------|-------|-------|--------------------|-------|------|-------|------|
|              | 0-7        | 7-14 | 14-21 | 21-28 | 28-35              | 35-42 | 0-21 | 21-42 | 0-42 |
| 0.0          | 1.87       | 2.09 | 1.62  | 2.44  | 2.79 <sup>a</sup>  | 3.60  | 1.79 | 2.89  | 2.48 |
| 0.15         | 1.77       | 2.11 | 1.79  | 2.25  | 2.18 <sup>b</sup>  | 2.83  | 1.88 | 2.43  | 2.25 |
| 0.30         | 1.77       | 2.18 | 1.61  | 2.17  | 2.47 <sup>ab</sup> | 2.90  | 1.81 | 2.54  | 2.31 |
| SEM±         | 0.04       | 0.09 | 0.07  | 0.07  | 0.14               | 0.31  | 0.06 | 0.13  | 0.09 |
| p-value      | 0.17       | 0.74 | 0.18  | 0.07  | 0.03               | 0.20  | 0.50 | 0.07  | 0.22 |

<sup>a,b,c</sup>: Means in each column with different superscripts are significantly different (p<0.05)

(21-42 day) and rearing (0-42 day) periods. No significant difference was observed between 0.15 and 0.30% Fermacto supplemented groups (Table 3). FCR was improved significantly (p<0.05) by 0.15% Fermacto as compared to control at 28-35 days of age. No significant difference was observed between 0.15 and 0.30% Fermacto supplemented groups (Table 4). Grimes *et al.* (1997) reported that Fermacto increased the retention time of feed in gastrointestinal tract, improved feed utilization, which resulted in better feed conversion. Zhang *et al.* (2005) evaluated the effects of *Saccharomyces cerevisiae* (SC) cell components on broiler performance. The supplementation of SC cell wall (CW) and SC whole yeast (WY) in broiler diets improved the feed conversion ratio from 0 to 3 and 4 to 5 weeks of age, respectively. WY and CW-fed broilers gained more weight from 0-5 week of age. Kobayashi *et al.* (2002) reported that supplementation of broiler diets with chitosan from 14-35 days of age did not affect feed intake, body weight gain and feed efficiency in adequate or high ME diets. The serum total cholesterol, HDL-cholesterol, LDL cholesterol and triglyceride concentrations were not significantly different among treatments (Table 5). Pedersen *et al.* (1997) observed that addition of inulin to the low-fat diet of young women had not significant effects on serum total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride. Yusrizal and Chen (2003) found that inulin and oligofructose reduced

**Table 5: Effects of experimental diets on serum lipids (mg dL<sup>-1</sup>) of broiler chickens**

| Fermacto (%) | CH     | TG    | HDL    | LDL    | VLDL  |
|--------------|--------|-------|--------|--------|-------|
| 0.0          | 125.70 | 50.20 | 287.70 | 172.00 | 10.00 |
| 0.15         | 132.50 | 51.00 | 187.50 | 134.60 | 10.20 |
| 0.30         | 130.70 | 46.50 | 197.70 | 147.30 | 9.30  |
| SEM±         | 8.06   | 8.95  | 33.03  | 25.60  | 1.79  |
| p-value      | 0.83   | 0.93  | 0.11   | 0.59   | 0.93  |

Means in each column with no superscripts are not significantly different (p>0.05). CH: Cholesterol, TG: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein

**Table 6: Effect of experimental diets on immunoglobulin production (mg dL<sup>-1</sup>) and IBVD titer of broiler chickens**

| Fermacto (%) | IgG                 | IgM                 | IgA                | IBVD titer |
|--------------|---------------------|---------------------|--------------------|------------|
| 0.0          | 473.00 <sup>a</sup> | 175.25 <sup>a</sup> | 45.00 <sup>a</sup> | 6953.00    |
| 0.15         | 392.50 <sup>b</sup> | 135.00 <sup>b</sup> | 33.50 <sup>b</sup> | 4236.00    |
| 0.30         | 402.50 <sup>b</sup> | 139.25 <sup>b</sup> | 37.00 <sup>b</sup> | 3426.00    |
| SEM±         | 13.032              | 4.081               | 1.951              | 1957.47    |
| p-value      | 0.003               | 0.0001              | 0.007              | 0.44       |

<sup>a, b, c</sup>: Means in each column with different superscripts are significantly different (p<0.05)

serum cholesterol of broilers. Trautwein *et al.* (1998) found that supplementation of prebiotic (inulin) in Syrian hamsters cholesterol-enriched diet significantly decreased serum cholesterol and triglyceride. Antibody titer against IBVD was not significantly different among treatments. The serum IgG, IgA and IgM production significantly (p<0.01) decreased in Fermacto-fed broilers as compared to control broilers (Table 6). Shafey *et al.* (2001) observed

that mannan oligosaccharide (MOS) inclusion did not improve antibody titers against IBVD and Newcastle disease virus. MOS supplemented broiler breeders had higher antibody responses against IBVD (Shashidhara and Devegowda, 2003). Savage *et al.* (1996) reported that the turkeys fed MOS had higher plasma IgG and bile IgA concentrations. Cetin *et al.* (2005) reported that turkey poulters supplemented with mannan oligosaccharide had significantly higher serum IgG and IgM levels and significant decrease in the peripheral blood T-lymphocyte percentage compared with that of control turkey poulters.

### CONCLUSION

Under the conditions of this study, it was concluded that despite the fact that Fermacto acts as a growth promoter, it may suppress immune responses in broiler chickens.

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