Effects of dietary supplementation of Aspergillus originated prebiotic (Fermacto) on performance and small intestinal morphology of broiler chickens fed diluted diets

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Abstract

Fermacto, that it is commercially a fermentation product of Aspergillus orizae, is proven to increase digestion efficiency of the gut. In this experiment, 450 one day old Cobb 500 broiler chicks (mixed-sex) were used in a completely randomized design with 3×2 factorial arrangement. There were three levels of Fermacto (0, 0.15 and 0.3% for 42 days) and two levels of diet dilution using rice hulls (0 and 40% at days 10-21 days of age). The treatments consisted of 3 replicates with 25 chicks per each.

Administration of Fermacto did not influence feed intake or feed conversion ratio, but the daily weight gain improved. Diluted diets significantly increased body weights and decreased feed intake; however, this manipulation didn’t affect feed conversion ratios. There was no interaction between probiotic supplementation and experimental diets dilution for performance traits. Diet dilution significantly decreased abdominal fat pad, villi height and increased epithelial thickness and goblet cell numbers. Dietary supplementation using Fermacto significantly decreased the abdominal fat pad and goblet cell numbers but increased the small intestinal villus heights. There were some conflicting observations for different segments of the small intestine. The 0.3% Fermacto inclusion in experimental diets caused more beneficial effects.

Contrary to previous reports on more efficiency of Fermacto on low protein diets, this trend is not seen for the whole diluted diets.

Introduction

Recently, the European Union and some other countries tended to ban antibiotics because of their side effects on both bird and human. The ban on antibiotic growth promoters has lead to a search for alternatives. A method that may be used to manipulate the gut ecosystem is the supplementation of the diet with small fragments of carbohydrates as prebiotics. The commercially available fermentation product of Aspergillus orizae, Fermacto (PET-AG, Ltd), has no live cells or spores, and is proven to enhance the digestive efficiency of the gut (Harms et al., 1988).

Fermacto has shown to enhance gut development and nutrient digestibility through an increase in gut beneficial microflora, short chain acids and duodenal and jejuna villi height (Gomez-Alcon et al., 1990; Beharka and Nagaraja, 1998; Hirayama et al., 2000). A diminishing effect on serum cholesterol is reported for Fermacto fed birds too (Ghiyasi et al., 2008). In some studies on broiler chickens, Fermacto resulted in better body weight gain, feed intake and feed conversion ratio (Piray et al., 2007; Khaksar et al., 2008; Piray and Kermanshahi, 2008). Khaksar et al. (2008) reported higher relative weights of breast and thigh in fed Fermacto broilers.

Reports indicate that Aspergillus meal may offer a protein sparing effect when used with low protein diets (Torres-Rodriguez et al., 2005). The results of the Ghiyasi et al. (2007) experiment showed that addition of Fermacto to broiler diets containing 10% less crude protein than NRC (1994) recommendation had a comparable performance to control group. Fermacto alters or affects intestinal microflora to alleviate nutrient deficiencies that would otherwise result in decreased performance (Grimes et al., 1997). This change may be accompanied by some intestinal alterations.

Because of reports on the use of Fermacto and whole diluted diets are lacking, the objective of the present study was to evaluate the effect of prebiotic (Fermacto) in strictly diluted diets on performance, carcass characteristics and the gut morphology of broiler chicks.

Materials and methods

In this experiment 450 one-day-old Cobb 500 broiler chicks (mixed-sex) were used in a completely randomized design with 3×2 factorial arrangement. There were three levels of Fermacto (0, 0.15 and 0.3% for 42 days) and two levels of diet dilution using rice hulls (0 and 40% at days 10-21 of age). The treatments consisted of 3 replicates with 25 chicks per each.

Experimental diets (Table 1) were formulated according to the Cobb 500 manual. Chicks had ad libitum access to feed during the entire experiment and were managed in accordance with the guidelines of the Cobb 500 manual. The photoperiod was 23L:1D, providing light from 02.00 to 23.00 h. The ambient temperature was 31°C at first week and decreased 2°C per each following week.

Feed intake and body weight gain were recorded for each pen at 42 days of age. At the end of experiment period, 5 male and 5 female birds per treatment were randomly sampled for carcass characteristics and morphometric analysis, and then killed. The weights of carcass, thigh, breast, liver and abdominal fat pad were measured.

The intestinal tract was removed immediately and severed from the gizzard and the pancreas was removed. The small intestinal weights and lengths were recorded. Three 1 centimeter tissue segments were taken from the proximal, middle and distal parts of duodenum, jejunum and ileum sections. Tissue samples were taken from the same area of each section of the tract, for each bird. Samples stored in 10% buffered neutral formalin for fixation, where they were gently shaken to
remove any adhering intestinal contents.

Cross sections (5 µm thick) of each intestinal segment were processed in low-melt paraffin and stained with hematoxylin and eosin. This procedure causes a longitudinal section of villi. Using a light microscope, 15 measurements per intestinal section were made for each parameter and averaged into one value per bird, having therefore each histological data obtained from the mean of 45 records (3 sections and 15 villi per section). Figure 1 illustrates samples of the small intestinal slides. Morphological indices were determined according to Xu et al., 2003, using image processing and analysis system (Leica Imaging Systems Ltd., Cambridge, UK).

Data on performance traits and histological parameters (Figure 1) were analyzed using the general linear model procedure and differences among treatments means were classified by Duncan's multiple range test (Version 6.12, SAS Institute, Inc.)

Results

No significant differences were found in the mortality rates of experimental treatments. Effects of dietary dilution and Fermacto supplementation on the performance traits of broiler chickens are showed in Table 2. The final body weight and daily weight gain of chicks were significantly increased (P<0.05) when they were fed with Fermacto at 0.3% level. Administration of Fermacto did not influence either the feed intake or the feed conversion ratio.

Diluted diets (with adding 40% rice hulls) significantly increased body weights, and decreased feed intake (P<0.05) compared with control; however, this manipulation didn’t affect feed conversion ratios (Table 2). There was no interaction between prebiotic supplementation and experimental diets dilution for performance traits.

The results presented in Table 3 show that the relative weights of liver, small intestine, breast, thigh and carcass were not affected by dietary dilution. However, the diluted diets caused a pronounced decrease in abdominal fat pad and a significant increase in small intestine length (P<0.05).

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Table 1. Composition and calculated nutrient content of Starter and Grower experimental diets supplemented with 0, 0.15 or 0.3% Fermacto and diluted with adding 40% rice bran.*

|                        | Original Starter (1-10 days of age) | Diluted Starter (10-21 days of age) | Grower (22-42 days of age) |
|------------------------|------------------------------------|-------------------------------------|---------------------------|
| Rice hulls, %          | -                                  | 40                                  | -                         |
| Corn, %                | 63.9                               | 36.34                               | 57.2                      |
| Soybean meal, %        | 23.49                              | 14.1                                | 15.97                     |
| Wheat, %               | -                                  | -                                  | 15                        |
| Fish meal, %           | 7                                  | 4.2                                | 3.5                       |
| Soybean oil, %         | 1.5                                | 0.9                                | 2.5                       |
| Oyster shell, %        | 1.1                                | 0.66                               | 1.01                      |
| Dicalcium phosphate, % | 1.02                               | 0.61                               | 1.01                      |
| Salt, %                | 0.3                                | 0.18                               | 0.22                      |
| HCl-Lysin, %           | 0.04                               | 0.02                               | 0.16                      |
| DL-Methionine, %       | 0.17                               | 0.1                                | 0.17                      |
| Vitamin/mineral supplement† | 1.49                           | 0.89                               | 0.76                      |
| Metabolisable energy, kca/kg | 2900                     | 1740                               | 3000                      |
| Crude protein, %       | 20.38                              | 12.23                               | 17                        |
| Calcium, %             | 0.99                               | 0.59                               | 0.87                      |
| Available phosphorous, % | 0.49                           | 0.29                               | 0.43                      |
| Sodium, %              | 0.15                               | 0.09                               | 0.15                      |
| Met+Cys, %             | 0.91                               | 0.55                               | 0.78                      |
| Lysin, %               | 0.97                               | 0.96                               |                           |

*At 10-21 days of age, for restricted fed birds, starter diet diluted with 40% rice hulls. †Provided per kilogram: vitamin A, 4,000,000 U; cholecalciferol 800,000 U; vitamin E, 14,000 U; vitamin K, 760 mg; vitamin B1, 2800 mg; vitamin B2, 1520 mg; vitamin B6, 7.6 mg; niacin, 18,000 mg; folic acid, 560 mg; pantothenic acid, 4400 mg; choline chloride, 19,000 mg; biotin, 45.5 mg; zinc, 16,000 mg; manganese, 25,000 mg; iron, 12,000 mg; copper, 1200 mg; selenium, 64 mg; iodine, 320 mg.

Table 2. Effect of prebiotic levels and diet dilution rate on performance traits of broiler chicks at 42 days of age.*

|                        | 0       | Fermacto % |                | Dilution rate % | SEM | Pr  | PDR | PFXDR |
|------------------------|---------|------------|---------------|-----------------|-----|-----|-----|-------|
| FBW, g                 | 2100b   | 2084a      | 2126a         | 2210a           | 2057b | 0.03 | 0.02 | 0.39  |
| DWG                    | 50.0a   | 49.5b      | 52.6a         | 52.6a           | 50.0b | 0.07 | 0.02 | 0.70  |
| DFI                    | 108.7   | 104.8      | 111.2         | 112.0a          | 104.6b | 3.2  | 0.25 | 0.03  | 0.66  |
| FCR                    | 2.18    | 2.11       | 2.10          | 2.13            | 2.14  | 0.03 | 0.39 | 0.26  | 0.39  |

*Calculations carried out using 3 observations per treatment. **means with different superscripts in each row differ significantly (P<0.05). FBW = Final body weight (42 days of age), DWG = Daily weight gain, DFI = Daily feed intake, FCR = Feed conversion ratio. SEM= Standard error mean.
pronounced increase in relative weight of thigh (P<0.05), but not of breast or total carcass as compared to control.

Table 4 summarizes the influence of dietary prebiotic and dilution on small intestinal morphology at 42 days of age. Villi of duodenum, jejunum and ileum sections were shortened significantly in birds fed with the diluted diets (P<0.05). Duodenum and jejunum villi height increased (P<0.05) in 0.3% prebiotic treated birds compared to the 0.15% prebiotic and control groups. At ileum, both prebiotic concentrations caused a taller villi compared to control (P<0.05). Interaction of diet dilution and prebiotic concentration on villi height was significant (P<0.05), showing that diet dilution reduced the positive effect of prebiotic on villi height (Figure 2).

Diet dilution increased the epithelium thickness of jejunum and ileum (P<0.05), but not of duodenum. Fermacto consumption caused a dose related decrease in epithelium thickness of duodenum (P<0.05). In jejunum and ileum, 0.3% Fermacto level decreased the epithelium thickness compared to 0.15% Fermacto and control group. There was a significant interaction among experimental factors on epithelial thickness (P<0.05), so that feed dilution aggravate the thinning effect of Fermacto on epithelium thickness of duodenum (Figure 2). Feed dilution decreased the crypt depth to villi height ratio (C/V) in duodenum and ileum (P<0.05), but didn’t affect this ratio in jejunum. Fermacto consumption did not affect the C/V ratio in duodenum and ileum, but increased it in jejunum (P<0.05).

Diet dilution caused a significant increase in goblet cell numbers at all the small intestinal segments (P<0.05). Goblet cell numbers of small intestinal epithelium decreased in duodenum (P<0.05). Goblet cell numbers of ileum (P<0.05), but didn’t affect this in goblet. Fermacto consumption did not affect the CV ratio in duodenum and ileum, but increased it in jejunum (P<0.05).

Table 3. Effect of prebiotic levels and diet dilution rate on small intestinal morphological parameters.

|                | Fermacto % | Dilution rate % | SEM  | P<DR | P<FXDR |
|----------------|------------|-----------------|------|------|--------|
|                | 0 0.15 0.3 | 0 40            |      |      |        |
| Carcass, %     | 70.4 72.9 70.9 | 70.7 69.5 | 0.18 | 0.66 | 0.61   |
| Thigh, %       | 27.1b 28.5a 28.9a | 28.5 28.0 | 0.3  | 0.04  | 0.43   |
| Breast, %      | 25.5 24.3 24.0 | 24.8 24.4 | 0.8  | 0.73  | 0.78   |
| Liver, %       | 2.28 2.28 2.01 | 2.17 2.22 | 0.07 | 0.24  | 0.82   |
| Abdominal fat pad, % | 2.57b 2.24a 1.91b | 2.35b 2.13b | 0.08 | 0.03  | 0.02   |
| Small intestine, % | 4.57 4.59 4.62 | 4.57 4.61 | 0.16 | 0.19  | 0.92   |
| Small intestine length, cm | 198.2 207.2 195.3 | 198.4 202.1b | 2.6 | 0.20  | 0.04   |

Calculations carried out using 10 observations per treatment. a-d: means with different superscripts in each row differ significantly (P<0.05). SEM= Standard error mean.

Table 4. Effect of prebiotic levels and diet dilution rate on small intestinal morphological parameters.

|                | Fermacto % | Dilution rate % | SEM  | P<DR | P<FXDR |
|----------------|------------|-----------------|------|------|--------|
|                | 0 0.15 0.3 | 0 40            |      |      |        |
| Duodenum       |            |                 |      |      |        |
| VH, μm         | 176b 177b 182a | 188a 175b 14.8 | 0.0001 | 0.0001 | 0.0001 |
| CD, μm         | 146.0b 145.8a 155.7a | 150.5a 147.7a 1.8 | 0.0001 | 0.0009 | 0.0001 |
| CV             | 0.083 0.082 0.082 | 0.082 0.084 0.083 | 0.0001 | 0.01  | 0.0019 |
| ET, μm         | 48.0b 46.1a 40.3c | 44.4 45.1 1.32 | 0.0001 | 0.33  | 0.0001 |
| GN             | 10.07b 9.77b 7.93b | 8.98b 9.53b 0.45 | 0.0001 | 0.03  | 0.0001 |
| Jejunum        |            |                 |      |      |        |
| VH, μm         | 842b 844a 872a | 863b 842b 4.52 | 0.0001 | 0.0001 | 0.0001 |
| CD, μm         | 126.2c 130.0b 138.8a | 133.7b 130.0b 2.18 | 0.0001 | 0.004  | 0.22   |
| CV             | 0.150b 0.154b 0.159a | 0.155 0.154 0.0027 | 0.0001 | 0.69  | 0.81   |
| ET, μm         | 39.9b 39.3a 37.6b | 39.3a 33.6b 1.11 | 0.0001 | 0.0001 | 0.0001 |
| GN             | 9.30b 9.43b 8.27b | 8.51b 9.49b 0.55 | 0.006  | 0.003  | 0.03   |
| Ileum          |            |                 |      |      |        |
| VH, μm         | 772c 788c 861a | 831a 783b 9.13 | 0.0001 | 0.0001 | 0.0001 |
| CD, μm         | 99.3b 101.6b 118.6a | 111.3b 101.7b 2.15 | 0.0001 | 0.0001 | 0.0001 |
| CV             | 0.128b 0.129b 0.129b | 0.134a 0.130b 0.0003 | 0.0001 | 0.04  | 0.012  |
| ET, μm         | 38.5a 37.4a 29.4b | 32.16b 33.6b 1.11 | 0.0001 | 0.0001 | 0.0001 |
| GN             | 10.23b 9.50b 7.90c | 8.73b 9.69a 0.47 | 0.0001 | 0.0006 | 0.0001 |

Calculations carried out using 45 observations per treatment. a-d: means with different superscripts in each row differ significantly (P<0.05). VH: Villus height; CD: Crypt depth; C/V: Crypt depth to Villus height ratio; ET: Epithelial thickness; GN: Goblet.

Discussion

Several studies have shown the beneficial effects of prebiotics on poultry performance (Spring et al., 2000; Xu et al., 2003; Pelicano et al., 2004), but the publications about Aspergillus originated prebiotics are almost limited.

In the present study, despite the positive effect of Fermacto (especially at 0.3% level) on body weight gain of birds, a severe dietary dilution by adding 40% rice hulls to diet, didn’t enhance the prebiotic effects as in the low protein diets, as reported by Ghiasi et al. (2007).

The body weight gain improvement in Fermacto fed birds is in agreement with some previous reports (Salamkhan et al., 2000; Midilli and Tuncer., 2001; Piray et al., 2007; Khaksar et al., 2008). In the present study, Fermacto prebiotic caused no differences in feed intake among treatments. Similar results were reported by Sims and Sefon (1999), Salamkhan et al. (2000) and Ghiasi et al. (2007). However, there are some positive reports, too (Piray et al., 2007). Fermacto failed to improve feed conversion ratio in this research, which seems to be in agreement with the finding of Ghiasi et al. (2007), but in disagreement with other authors (Salamkhan et al., 2000; Piray et al., 2007; Khaksar et al., 2008).

It is clear from the present study and published research that responses to prebiotic supplementation are inconsistent. A possible explanation for the differences among findings of different investigators may be related to the doses and type of prebiotic applied and diets composition.

Fermacto improves digestibility of the non-ruminant animals by providing nutrients and mycelial fibre for the proliferation of intestinal bacteria (Mamiek, 1993; Tangendjaja, 1993). Consequently, increased absorption of digest-
ed feed occurs, which enhances the performance traits and especially growth of broilers (Mamiek, 1993; Tangendjaja, 1993), as observed in the current study.

In previous reports, Fermacto enhanced the relative weights of thigh (Piray et al., 2007; Khaksar et al., 2008) or breast (Piray et al., 2007), but this is not observed in this study. Ghiyasi et al. (2007) and Salamkhan et al. (2000), couldn’t find a positive effect of Fermacto on thigh or carcass relative weights, in agreement with our findings. The effect of Fermacto on abdominal fat pad reduction is reported as well for other prebiotics (Yusrizal and Chen, 2003).

Overall gut surface area affects net utilization of dietary nutrients in chicken and is determined by gross morphological features such as length and cross-sectional area of the duodenal, jejunal and ileal segments, and by finer morphological features such as villus height and surface area of the epithelium in each of those segments (Jin et al., 1998). Studies with chickens and other species suggested that dietary restriction causes regression in mucosal development (Smith et al., 1991; Palo et al., 1995). Yamauchi et al. (1996) reported that villus height in the duodenum was significantly reduced in birds exposed to 24-h fasting. The regressed crypts and the delay in production of enterocytes can explain the retardation of growth in chicks following delayed access to feed (Pinchasov and Noy, 1993).

Prebiotic supplementation appeared to influence several intestinal parameters in

Figure 2. Significant interactions between dietary dilution and Fermacto on small intestinal morphometric parameters.
present study as well. We observed increases in villi heights and crypt depth, but a lower goblet cell number and thinner epithelial thickness in the Fermacto Prebiotic treated birds. It seems that diet dilution reduced the positive effect of Fermacto on villi height and epithelial thickness, but enhanced its favorable effect on goblet cell number reduction.

The increased duodenum villi height observed in our study has been previously reported in turkey poults (Noy et al., 2001) and broiler chickens (Solis et al., 2005) and may explain the enhanced efficiency of digestion and absorption of the duodenum due to an increase in population of beneficial bacteria supplying nutrients and stimulating vascularization and development of intestinal villi (Bedford, 2000; Gilmore and Ferretti, 2003).

In Solis et al. (2005) research, Fermacto fed birds had a thicker duodenum crypt, which is in agreement with our findings. Reduction in small intestinal epithelium thickness can facilitate absorption process, enhance nutrient absorption and reduce the metabolic demands of the gastrointestinal system (Visick, 1978). The reduction of gut epithelial thickness may be due to inhibition of microbial polysaccharides and volatile fatty acids production that causes an increase in electrolyte turn over and intestinal cell activity, so that the spared energy can be utilized for productive processes like meat production (Bedford, 2000).

The crypt can be regarded as the villus factory, and a large crypt indicates fast tissue turnover and a high demand for new tissue (Yason et al., 1987). The crypt:villus ratio is an indicator of the likely digestive capacity of the small intestine. A decrease in this ratio corresponds to an increase in digestion and absorption (Montagne et al., 2003). A fewer goblet cell number in intestinal tract epithelium following Fermacto administration can be attributed to a less stressful condition that leads to a reduced need for the protective mucus layer.

Conclusions

This study provides evidence that the administration of Aspergillus originated prebiotic (Fermacto) caused significant improvement on Broiler body weight gain and small intestinal morphological parameters. The 0.3% Fermacto inclusion in experimental diets caused more beneficial effects. Contrary to previous reports on more efficiency of Fermacto on low protein diets, this trend is not seen for the whole diluted diets.

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