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Effect of dietary *Aspergillus* meal prebiotic on growth performance, carcass characteristics, nutrient digestibility, and serum lipid profile in broiler chick low-protein diets

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Abstract: The effects of 3 levels of commercial *Aspergillus* meal prebiotic (Fermacto®) in broiler diets with 2 levels of dietary crude protein (CP) on the growth performance, carcass characteristics, apparent ileal digestibility of CP and organic matter (OM), and serum lipid profile were evaluated using 240-day-old broiler chickens as a factorial arrangement in a completely randomized design. From the grower period, 3 groups received normal diets formulated to meet Ross-308 CP and other nutrient requirements with 3 levels of Fermacto®. The other 3 groups received experimental diets with 2% less CP, with the same levels of Fermacto®. None of the growth performance parameters were affected by the supplementation of Fermacto® from 11 to 49 days of age. Supplementation of diet with 1.5 g/kg Fermacto® significantly ($P < 0.05$) increased the apparent ileal digestibility of the OM and decreased the abdominal fat pad percentage and serum total cholesterol. It was concluded that the use of Fermacto® at a level of 1.5 g/kg improved the apparent OM digestibility and decreased serum total cholesterol and abdominal fat percentage; however, the growth performance parameters were not affected by the Fermacto® levels. There was no profit when Fermacto® was used in the low-protein diets.

Key words: Prebiotic, broiler, ileal digestibility, performance

Introduction

Antibiotic feed additives have been used in livestock production for about 50 years (1). A decrease in the therapeutic effectiveness of antibiotics in treating a wide array of bacterial infections in humans has prompted several European countries to ban the use of dietary antibiotics (2). Therefore, finding alternative ways to accelerate the gastrointestinal maturation of newly hatched birds may be necessary to replace antibiotic growth promoters. Some of the products that have been tested to try to achieve these goals include probiotics, prebiotics, organic

acids, and plant extracts (3). One of many such classes of alternatives is prebiotic, which includes fructooligosaccharides, galactooligosaccharides, transgalactooligosaccharides, and mannanoligosaccharides. Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of the bacteria in the intestine (4). Prebiotics have been shown to alter gastrointestinal microflora, alter the immune system, reduce invasion by pathogens such as *Salmonella enteritidis* and *E. coli*, and reduce serum cholesterol and odor compounds

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(5). Fermacto® (PetAg, Inc., Hampshire, IL, USA) is a feed additive containing *Aspergillus* meal (6) and is used as a prebiotic. *Aspergillus* meal is derived from an active fermentation of primary *Aspergillus* spp. and improves the digestive capacity of monogastric animals by providing nutrients and mycelia (fungal) fiber for the proliferation of intestinal bacteria (7). Mycelia fibers are compounds of mannanoligosaccharides, and they are thought to act by binding and removing pathogens from the intestinal tract (8). Several studies have investigated the effects of lactosucrose (9), fructooligosaccharide (10), and mannanoligosaccharide (11) as prebiotics on the growth performance of birds. In broiler diets, the price of protein is higher than other nutrients and the feedstuffs that are used as the source of protein in the diet are more expensive than others. Due to cost saving in the diet formula and environmental concerns for nitrogen excretion in feces, decreasing protein levels in diets with no significant difference in performance is of interest and importance for farmers. This point is very important for the poultry industry in Iran, because large amounts of protein sources are imported from abroad at high cost.

Therefore, the objective of the present study was to evaluate the effect of *Aspergillus* meal as a prebiotic feed additive on the growth performance, carcass characteristics, digestibility of organic matter (OM) and crude protein (CP), and serum lipid profile in broiler chick low-protein diets.

Materials and methods

Birds and diets

Mixed Ross-308 broiler chickens, 240 days of age, were randomly assigned to 6 treatments with 2 dietary protein levels and 3 *Aspergillus* meal additions (10 chickens per pen with 4 pens per treatment). Feed and water were provided ad libitum. The broilers were raised in floor pens (1 × 1 m) and maintained with 23 h of continuous light and 1 h of darkness. The ambient temperature started at 33 °C (from days 0 to 3) and was gradually reduced, according to usual brooding practices, to 22 °C on day 49. The study was carried out according to the guidelines of the animal care and use committee of the Animal Affairs Division of the Agriculture Organization of East Azerbaijan Province, Iran.

All of the chicks were fed a starter diet containing 23% CP from days 1 to 10 of age. From day 11, the chicks were fed the experimental diets; 3 chick group treatments received the normal diets, which were formulated to meet Ross nutrient requirements (with 21% CP in the grower diet from day 11 through 28 and 19% CP in the finisher diet from day 29 through 49) containing 3 *Aspergillus* meal prebiotic concentrations (0.0, 1.5, and 3.0 g/kg into the basal diets). The other 3 group treatments received diets with 2% less CP (19% in the grower diet from day 11 through 28 and 17% in the finisher diet from day 29 through 49) containing the same concentration of *Aspergillus* meal prebiotic (Table 1). *Aspergillus* meal was added to the basal diets as the last step in mixing.

Measurements

Body weight gain (after 8 h of fasting to remove any residual feed from the gastrointestinal tract) and feed intake were recorded weekly. The feed conversion ratio (FCR) was calculated as the unit of eaten feed per unit of body weight gain. The experimental diets were supplemented with 0.4% Cr₂O₃ as an indigestible marker 1 week before determining the ileal digestibility of the nutrients (day 28). On day 35, 3 birds per pen (3 chicks per pen with 4 pens per treatment) were killed by intracardial injection of ketamine. All of the ileal digesta between the yolk sac and the terminal ileum (4 cm above the ileal-cecal junction) were obtained immediately and carefully (12). The digesta from each of the 3 birds per pen were pooled as 1 sample into a plastic bag and immediately stored at -20 °C. Before analyses, the digesta samples were thawed and ground through a 1.00-mm mesh screen and then mixed thoroughly. Dry matter, OM, and CP contents were determined (13). The samples were also analyzed for chromic oxide (14). The apparent ileal digestibility values for dietary CP and OM were calculated as follows:

$$DD = 1 - [(ID \times AF) / (IF \times AD)],$$

where DD is the apparent digestibility of a nutrient in the diet, ID is the concentration of an indigestible marker in the diet, AF is the nutrient concentration in the ileal digesta, IF is the indigestible marker concentration in the ileal digesta, and AD is the nutrient concentration in the diet (15). A day prior to slaughter, blood samples were collected randomly from the wing vein of 5 male chicks of each treatment

Table 1. Ingredient composition and chemical analysis of the basal diets.

Ingredient (%)	Grower diets		Finisher diets	
	LP ³	NP	LP	NP
Corn	66.88	62.1	68.75	65.50
Soybean meal (CP 48%)	23.04	26.85	23.00	21.70
Vegetable oil	3.50	4.00	4.50	4.50
Fish meal (CP 64%)	3.00	3.50	-	3.70
DL-Methionine	0.10	0.10	0.10	0.10
L-Lysine	0.18	0.15	0.15	0.10
Oyster shell	1.00	1.00	1.10	1.10
Dicalcium phosphate	1.50	1.50	1.50	1.30
Vitamin premix ¹	0.25	0.25	0.25	0.25
Mineral premix ²	0.25	0.25	0.25	0.25
Wheat bran	-	-	-	1.20
Salt	0.30	0.30	0.40	0.30
Calculated chemical analysis				
ME (kcal/kg)	2962	2952	3019	3000
Crude protein (%)	19.09	21.04	16.93	18.81
Lysine (%)	1.13	1.24	0.94	1.06
Methionine (%)	0.43	0.46	0.37	0.43
Met + Cys (%)	0.73	0.79	0.66	0.73
Calcium (%)	0.91	0.94	0.84	0.89
Available phosphorus (%)	0.47	0.49	0.42	0.44

¹ Vitamin premix provided per kilogram of diet: vitamin A, 7.2 g; vitamin B₁, 0.72 g; vitamin B₂, 3.3 g; vitamin B₃, 4.0 g; vitamin B₆, 1.2 g; vitamin B₁₂, 0.6 g; vitamin D₃, 1.6 g; vitamin E, 14.4 g; vitamin K₃, 1.6 g; vitamin B₉, 0.5 g; vitamin B₅, 12 g; vitamin H₂, 2.0 g; and choline chloride, 400 g.

² Mineral premix provided per kilogram of diet: manganese oxide, 64 g; zinc oxide, 100 g; ferric sulfate, 44 g; copper sulfate, 16 g; calcium iodate, 0.64 g; and selenium premix, 8.0 g.

³ LP = low protein and NP = normal protein.

at 48 days of age. Serum samples were analyzed for total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels at the Imam Reza Hospital Biochemical Laboratory. At 49 days of age, 5 chickens from each treatment were randomly selected, weighed, and killed. The carcasses were opened and the gut, gizzard, and abdominal fat were removed and weighed. The thigh and breast muscles were separated and weighed separately. The relative weights of the organs, thighs, and breast muscles to live body weights were calculated.

Statistical analysis

The data were subjected to statistical analysis according to a completely randomized design as a factorial arrangement of 2 × 3 with 2 levels of dietary

CP and 3 levels of prebiotic, using the general linear model procedure of SAS (16). Means were compared using Tukey's tests at 5% probability.

Results

Performance

The effects of dietary CP and prebiotic levels on the growth performance of broiler chicks are shown in Table 2. In the grower period, chicks fed the low-protein diet had lower feed intake and body weight ($P < 0.05$). In the finisher period and during the entire experimental period, chicks fed the normal-protein diets had higher body weight and weight gain, and the FCR values were improved ($P < 0.05$). None of

Table 2. Effect of protein and prebiotic (*Aspergillus* meal) levels on growth performance parameters.

Treatment ¹	11–28 days			29–49 days			11–49 days					
	Feed intake (kg)	Body weight (kg)	Weight gain (kg)	FCR	Feed intake (kg)	Body weight (kg)	Weight gain (kg)	FCR	Body weight (kg)	Weight gain (kg)	FCR	
Protein levels												
NP	1.197 ^a	0.923 ^a	0.763	1.570	3.411	2.711 ^a	1.788 ^a	1.910 ^b	4.609	2.711 ^a	2.551 ^a	1.806 ^b
LP	1.147 ^b	0.895 ^b	0.737	1.563	3.350	2.588 ^b	1.693 ^b	1.982 ^a	4.497	2.588 ^b	2.430 ^b	1.851 ^a
SEM*	0.010	0.010	0.009	0.02	0.026	0.022	0.022	0.016	0.032	0.022	0.022	0.008
Prebiotic levels												
A	1.182	0.937	0.777	1.522	3.390	2.643	1.706	1.992	4.572	2.643	2.483	1.842
B	1.146	0.896	0.737	1.555	3.322	2.627	1.731	1.920	4.468	2.627	2.468	1.810
C	1.190	0.894	0.736	1.622	3.429	2.679	1.784	1.925	4.619	2.679	2.521	1.833
SEM	0.010	0.010	0.009	0.02	0.026	0.022	0.022	0.016	0.032	0.022	0.022	0.008
P-value												
Protein	0.012	0.051	NS	NS	NS	0.007	0.034	0.011	NS	0.007	0.008	0.007
Prebiotic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein × prebiotic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^{ab}Means within a column with different superscripts differ at $P < 0.05$.

*Standard error of the means (with 5 degrees of freedom for the model and 18 degrees of freedom for the error).

¹NP = normal protein; LP = 0.0 g prebiotics/kg diet; B = 1.5 g prebiotics/kg diet; and C = 3.0 g prebiotics/kg diet.

Means calculated with 40 birds on day 28 and with 28 birds on day 49 (each cage represents an experimental unit with 10 birds per cage on day 28 and 7 birds per cage on day 49 with 4 repeats).

the growth performance parameters were affected by supplementation of *Aspergillus* meal prebiotic from 11 to 49 days of age. However, the chicks fed diets supplemented with *Aspergillus* meal had a numerically lower FCR compared to the control; the difference was not statistically significant. There was no CP × Fermacto level significant interaction on growth performance parameters.

Carcass traits

The effects of dietary CP and prebiotic levels on the carcass characteristics and digestibility of CP and OM are presented in Table 3. Breast percent was affected by dietary CP levels and chicks fed the normal-protein diets had a higher breast meat yield ($P < 0.05$). Dressing percentage and thigh, gizzard, and gut percent were not affected significantly by dietary CP and *Aspergillus* meal prebiotic levels. There was

no CP × Fermacto level significant interaction on carcass characteristics.

Digestibility of CP and OM

The digestibility of CP and OM were not affected by dietary CP levels (Table 3). The CP digestibility of birds consuming the diet containing 1.5 g/kg *Aspergillus* meal prebiotic was significantly ($P < 0.05$) higher than that among birds consuming the diet containing 3.0 g/kg *Aspergillus* meal. There was no significant difference in CP digestibility between birds consuming the control diet and the diet containing 1.5 g/kg *Aspergillus* meal prebiotic. The apparent digestibility of OM was enhanced ($P < 0.05$) with supplementation of 1.5 g/kg *Aspergillus* meal compared to the control diet. There was no CP × Fermacto level significant interaction on digestibility of CP and OM of diets.

Table 3. Effect of protein and prebiotic (*Aspergillus* meal) levels on the carcass characteristics and apparent ileal digestibility of CP and OM.

Treatment ¹	Carcass characteristics (as percentage of live body weight)					Apparent digestibility of CP (%)	Apparent digestibility of OM (%)
	Dressing percentage	Breast	Thighs	Gut	Gizzard		
Protein levels							
NP	74.66	25.11 ^a	20.59	2.70	1.27	82	78
LP	73.99	24.12 ^b	20.20	2.60	1.32	81	79
SEM*	0.24	0.27	0.17	0.06	0.05	0.6	0.4
Prebiotic levels							
A	73.96	24.96	20.52	2.70	1.26	80 ^{ab}	77 ^b
B	74.50	24.20	20.49	2.64	1.35	83 ^a	79 ^a
C	74.61	24.68	20.17	2.67	1.28	79 ^b	78 ^{ab}
SEM	0.24	0.27	0.17	0.06	0.05	0.6	0.4
P-value							
Protein	NS	0.054	NS	NS	NS	NS	NS
Prebiotic	NS	NS	NS	NS	NS	0.043	0.050
Protein × prebiotic	NS	NS	NS	NS	NS	NS	NS

^{ab}Means within a column with different superscripts differ at $P < 0.05$.

*Standard error of the mean (with 5 degrees of freedom for the model and 24 degrees of freedom for the error for the carcass characteristic data, and 5 degrees of freedom for the model and 18 degrees of freedom for the error for the digestibility data).

¹NP = normal protein; LP = low protein; A = 0.0 g prebiotics/kg diet; B = 1.5 g prebiotics/kg diet; and C = 3.0 g prebiotics/kg diet.

Means for digestibility data calculated with one sample per pen. At 35 days of age, the digesta from each of the 3 birds per pen were pooled as 1 sample. Means for the carcass characteristics were calculated with 5 birds per treatment.

Serum lipid profile

The effects of dietary CP and prebiotic levels on serum lipid profile and abdominal fat pad are presented in Table 4. Neither the serum lipid profile nor the abdominal fat pad was affected by dietary CP levels. Serum total cholesterol was decreased ($P < 0.05$) with dietary supplementation of 1.5 g/kg *Aspergillus* meal compared to the control diet; however, HDL cholesterol and triglyceride levels were not affected significantly by *Aspergillus* meal levels. The abdominal fat pad as a percentage of live weight was significantly ($P < 0.05$) lower in the diet containing 1.5 g/kg *Aspergillus* meal prebiotic compared to control diet. There was no CP \times Fermacto level significant interaction on the serum lipid profile.

Discussion

Several studies have shown that supplementation of poultry diets with *Aspergillus* meal prebiotic enhanced body weight, weight gain, or the FCR (7,17–19). In contrast, some reports found no significant response in weight gain when the broilers were fed diets containing prebiotics (20,21). Stanczuk et al. (21) reported that supplementation with inulin and mannanoligosaccharide (1 and 4 g/kg) for 16 weeks had no effect on weight gain in turkeys. In another study, Yalçinkaya et al. (22) reported that the body weight gain, feed intake, and FCR of broilers were not significantly influenced ($P < 0.05$) by the addition of mannanoligosaccharide. Some experiments suggest that supplementation of *Aspergillus* meal may

Table 4. Effect of protein and prebiotic (*Aspergillus* meal) levels on the serum lipid profile and abdominal fat pad.

Treatment ¹	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	Triglycerides (mg/dL)	Abdominal fat** (%)
Protein levels				
NP	136.400	114.734	135.467	2.34
LP	139.600	114.467	143.667	2.41
SEM*	1.916	1.404	2.914	0.09
Prebiotic levels				
A	143.300 ^a	116.000	146.400	2.69 ^a
B	132.900 ^b	110.100	137.500	2.18 ^b
C	137.800 ^{ab}	117.700	134.800	2.25 ^{ab}
SEM	1.916	1.404	2.914	0.09
P-value				
Protein	NS	NS	NS	NS
Prebiotic	0.047	NS	NS	0.047
Protein \times prebiotic	0.021	NS	NS	NS

^{a,b}Means within a column with different superscripts differ at $P < 0.05$.

*Standard error of the means (with 5 degrees of freedom for the model and 24 degrees of freedom for the error).

¹NP = normal protein; LP = low protein; A = 0.0 g prebiotics/kg diet; B = 1.5 g prebiotics/kg diet; and C = 3.0 g prebiotics/kg diet.

**As percentage of live body weight.

Means for the serum lipid profile and abdominal fat pad calculated with 5 birds per treatment.

improve the performance of chicks fed suboptimal protein levels. Torres-Rodriguez et al. (17) observed that supplementation of *Aspergillus* meal improved body weight gain in broilers fed low-protein diets during the first 3 weeks of life. Ghiyasi et al. (23) showed that during the grower period (days 10–28), supplementation of *Aspergillus* meal in low-protein diets significantly reduced the FCR, but normal-protein diet supplementation of *Aspergillus* meal had no effect on the FCR. In the present study, adding *Aspergillus* meal to the basal diet numerically decreased the FCR, but this reduction was not statistically significant. Supplementation of *Aspergillus* meal did not improve the performance of birds fed the low-protein diet. These observations (17,23) seem to not be in agreement with the results of the present study, and it may be that the effects of the prebiotics were inadequate to offset the performance parameter deficits encountered in the low-protein diet. The results of the present study, combined with the earlier published studies, show that the effects of oligosaccharides on the growth performance of poultry are inconsistent. The lack of response with prebiotics in corn–soybean meal diets may be due to the effects of preliminary indigestible oligosaccharides in the diet (24) because the dietary soybean meal contains approximately 6% raffinose plus stachyose; thus, it has already provided a large amount of indigestible oligosaccharides.

The decreasing of breast meat percentage in chicks fed the low-protein diets was expected. Breast meat is the largest muscle in poultry and the reduction of breast percentage in birds fed the low-protein diets might be because of the decrease of essential amino acid intake, especially lysine.

Past studies have investigated the effects of oligosaccharides on the digestibility of dry matter, energy, calcium, CP, and phosphorus (15,25). However, these kinds of studies are scarce. The finding of improved digestibility of CP and OM is in agreement with the results of Huang et al. (15), who found that dietary supplementation of oligosaccharides improved nutrient digestibility in broiler chickens. They suggested that an increase in the digestion and absorption of nutrients is a major mechanism responsible for the enhanced growth performance of broilers in response to

dietary oligosaccharide supplementation. Dietary supplementation with prebiotics has been shown to improve the health status of the gastrointestinal tract; therefore, these substances are being actively investigated as indirect growth promoters (8). The nutrient digestibility enhancement in broilers supplied with oligosaccharide diets may be due to an improvement in gut health (26). According to a previous study (27), the enhanced ileal digestibility of nutrients in broilers fed a diet containing oligosaccharides might be explained by the following findings. First, oligosaccharide supplementation reduced the number of pathogenic bacteria (e.g., *Escherichia coli*, *Salmonella typhimurium*) and increased the number of beneficial bacteria (acid-producing bacteria, e.g., *Lactobacilli*) in the intestine. Second, oligosaccharides may stimulate the secretion of digestive enzymes from the stomach, pancreas, and intestinal mucosa. *Aspergillus* meal may provide nutrients to effectively stimulate the growth of beneficial microflora in the small and large intestine, and the result would be a better balance of the bacterial population. These new bacterial populations produce different digestive enzymes, which add to existing broiler endogenous enzymes (7).

The results of the present study are in agreement with previous observations about the positive effect of dietary supplementation with β -fructans (28) and mannanoligosaccharides (29) on reducing abdominal fat and serum total cholesterol.

Bacillus subtilis culture was shown to decrease the activity of acetyl-CoA carboxylase. Acetyl-CoA carboxylase has been widely suggested as the rate-limiting enzyme in fatty acid synthesis (29). In this study, the abdominal fat percentage reduction was probably due to increasing numbers of beneficial bacteria (e.g., *Bacillus subtilis*) as a result of supplementation with the *Aspergillus* meal. The mechanism for the reduction of serum cholesterol level is still not clear. Mannanoligosaccharide is considered as a substrate for lactic acid-producing bacteria. Increased levels of mannanoligosaccharides also increase the number of lactic acid-producing bacteria (27) and some lactic acid-producing bacteria (*Lactobacillus* and *Bifidobacterium*) are able to decrease the serum cholesterol by deconjugation of bile acids. Since the excretion of deconjugated

bile acids is enhanced, more cholesterol molecules are spent for the recovery of bile acids (30), and as a result, the level of serum cholesterol is reduced.

These experiments suggest that the available Fermacto®, as a prebiotic in the animal feed market of Iran, did not improve the growth performance

of broiler chicks; however, use of this feed additive decreased the abdominal fat pad and serum total cholesterol and improved the apparent ileal digestibility of OM. It should be mentioned that use of Fermacto® could not be improved in utilization of low-protein diets.

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