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Effects of inulin and β-glucan supplementation in broiler diets on growth performance, serum cholesterol, intestinal length, and immune system

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Abstract: This study was designed to investigate the effects of a supplement of inulin and β-glucan into broiler rations on growth performance, serum cholesterol, intestinal length, and immune system. A total of 320 male 1-day-old Ross PM3 broiler chicks were divided into 1 control and 3 treatment groups, each comprising 80 birds. Each group was divided into 4 replicate groups of 20 birds each. The treatment groups were supplemented with 0.7% inulin, 0.014% β-glucan, and 0.7% inulin + 0.014% β-glucan, respectively. The experiment lasted for 6 weeks. Immunomodulatory roles were also investigated by measuring the effects on specific antibody titer, and, for this purpose, vaccination against the Newcastle disease (ND) virus was administered on days 10 and 26. Serum cholesterol and triglyceride levels were also investigated. At the end of the experiment, inulin supplementation to the diets significantly increased the intestine and cecum length. The supplementation of 0.7% inulin + 0.014% β-glucan to the diets not only had negative effects on performance parameters but also resulted in increased serum total cholesterol ($P < 0.001$) and triglyceride ($P < 0.01$) levels. The amount of abdominal fat of the carcasses also increased during this treatment in the animals in the inulin + β-glucan group by the end of the trial.

Key words: β-Glucan, blood parameters, immune system, inulin, performance

Introduction

Prebiotics are feed additives that can selectively stimulate and/or increase the specific activation of one or more kinds of bacteria. They also have positive effects on animal health and cannot be digested with digestive enzymes. Some lipids, along with some nondigestible carbohydrates, peptides, and proteins, work in the colon as potential prebiotics (1). Prebiotics decrease colonization of some bacteria such as *E. coli* and *Salmonella* and increase nonpathogenic microorganisms (2).

Feeding with inulin and oligofructose has positive effects on broiler performance and carcass and abdominal fat percentage (2,3), and it has also shown positive effects regarding the egg production of laying hens in some studies (4,5). Outcomes of the effect of oligofructose on the length, weight, and mucosa of the small intestine are contradictory (5,6). It has been observed that alimental oligosaccharides have an effect on decreasing lipids and blood cholesterol levels (7,8) and lessening plasma triglyceride concentrations (7).

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β-Glucan can be used as an enhancer and an alternative to traditional antimicrobials for poultry (9). Furthermore, chicks fed with β-glucan have much larger primary and secondary lymphoid organs, such as the bursa of Fabricius, the thymus, and the spleen. These findings show that β-glucan increases some basic immune reactions, leading to the idea that β-glucan can be used as a possible immune regulator (10). Lowry et al. (11) found that the addition of β-glucan into diets resulted in the decrease of *Salmonella enteritidis* levels in the liver/spleen from 76% to 7% compared to the control.

The poultry sector needs new products that can increase the productivity of rations and protect birds against the pathogenic effects of intestinal microorganisms. The aim of this study was to determine the effect of supplements added to broiler rations, such as inulin, β-glucan, and inulin + β-glucan, on some performance parameters [body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), yields of hot carcass and some organs (liver, spleen, heart, abdominal fat, bursa of Fabricius), some features of the intestine, total cholesterol and total triglyceride levels, and antibody level developed against Newcastle disease (ND)]. Therefore, our hypothesis for the present trial was that the inulin and β-glucan combination could provide increased productivity and health levels for broilers.

Materials and methods

A total of 320 male 1-day-old Ross PM3 broiler chicks were divided into 4 dietary groups of 80 birds each. Each main group was divided into 4 subgroups and each subgroup consisted of 20 chicks. The control group rations were not supplemented with inulin or β-glucan. The experimental groups received 0.7% inulin, 0.014% β-glucan, and 0.7% inulin + 0.014% β-glucan, respectively. The amount of wheat was decreased equal to the amount of inulin and β-glucan added to the rations.

The items used in this experiment, such as semipurified chicory inulin extract (RAFTIFEED® IPE, Orafti Animal Nutrition, Tienen, Belgium), included >70% inulin in its dry form. *Saccharomyces cerevisiae* (Leucogard®), which was extracted from bread yeast (1-3; 1-6 β-D-glucan), included 28% β-glucan (Fibona Health Products GmbH, Taunusstein, Germany).

The chicks were fed with starter diets (0-21 days) that contained 3200 kcal/kg metabolizable energy (ME) and 23.0% crude protein (CP), grower diets (21-42 days) that contained 3200 kcal/kg ME and 20% CP (Table 1), and water ad libitum during the 6-week feeding period.

The birds were housed in wire-bottomed pens fitted with electrical heaters during the 42-day experimental period. The temperature started at 34 °C the first week and was gradually reduced according to normal management practice (20 °C at week 5). The chicks were maintained on a 24-h constant light schedule until the end of the experiment. Feed and water were presented ad libitum to the animals.

Vaccination against the ND virus (Hitchner $B_{1,}$ Intervet, Boxmeer, the Netherlands) was administered on days 10 and 26. Coccidiostats (lasalocid sodium; 5 g/kg) were included in the diets on days 11, 15, and 21.

The study was approved by the Animal Ethics Committee of Ankara University.

Body weight (BW) and FI were recorded weekly. The FCR (feed to weight gain) was calculated by totaling the amount of feed consumed divided by the BWG of the birds.

At the end of the experiment, the birds were weighed individually to determine their preslaughter BW. Three birds from each single pen were then collected randomly for slaughter.

The liver, heart, spleen, abdominal fat, intestine, and bursa of Fabricius from each animal were weighed and divided by the BW to calculate the organ yields of the animals. Gut length was calculated by measuring the length of the small and large intestines.

In order to determine the maternal antibody level, on the first day, 20 reserved chicks were slaughtered. On day 26 and at the end of the experiment (before slaughtering), blood was taken from the subwing vein from 3 birds from each replication. Serum was kept at –20 °C. The animals were vaccinated against ND (Hitcher B1, Intervet) on days 10 and 26 of the experiment via the spraying method. Maternal antibody and specific antibody levels formed against ND were determined using a hemagglutination inhibition test (12).

Table 1. Basal composition and analyzed results of the experimental diets.

Ingredient	Starter (days 0-21)	Grower (days 21-42)
	% of diet	% of diet
Corn	42	51.85
Wheat	4.45	4.77
Soybean meal	26	18.83
Full-fat soybean	17	16.80
Fishmeal	3	2
Vegetable oil	4	2.3
Limestone	1.5	1.5
DCP	1.3	1.3
NaCl	0.25	0.25
Mineral*	0.10	0.10
Vitamin**	0.15	0.15
DL-methionine	0.25	0.15
Calculated value		
ME, kcal/kg	3200	3200
Crude protein,%	23	20

*Mineral premix supplies per kg diet: 150 mg Mn, 120 mg Fe, 150 mg Zn, 14 mg Cu, 0.4 mg Co, 3 mg I, and 0.3 mg Se.
**Vitamin premix supplies per kg diet: retinol acetate, 21,000 IU; cholecalciferol, 6000 IU; tocopherol acetate, 120 mg; menadione, 45 mg; thiamin, 4.5 mg; riboflavin, 12 mg; niacin, 60 mg; pantothenic acid, 18 mg; pyridoxine, 9 mg; cobalamin, 0.045 mg; folic acid, 3 mg; biotin, 0.225 mg; and ascorbic acid, 75 mg.
DCP: Dicalcium phosphate.

Blood samples were collected at the end of the experiment before slaughtering from the bronchial vein in the wing from 3 birds from each group, and the blood serum was separated by blood centrifugation. Serum cholesterol and triglyceride contents were analyzed. Total cholesterol in the blood was determined using kits (GD034000, GLOBE Diagnostics, Rome, Italy) and total triglyceride levels were determined spectrophotometrically (GD081500, GLOBE Diagnostics).

Statistical analyses of the data were performed using SPSS 10.0 for Windows. One-way ANOVA was used to determine the differences among the groups. When P-values were significant ($P < 0.05$), Duncan's multiple range test was performed. All of the data were expressed as means ± standard error (13).

Results

In this study, no significant differences were observed among the treatments regarding FI, BWG, FCR, and carcass yield (Tables 2 and 3). Organ weights and their ratio to 100 g BW also showed no significant differences among the groups ($P > 0.05$) (Table 4). The length of the duodenum was significantly different ($P < 0.05$) among the groups. The longest cecum and total intestine lengths were measured in the inulin group ($P < 0.001$) (Table 4). The ND-specific antibody titer determined (Table 5) in the first and second measurements were not different among the groups. It was found that addition of inulin + β-glucan into the diets resulted in a decrease of serum total cholesterol ($P < 0.001$) and total triglyceride ($P < 0.01$) levels (Table 6). Some nonsignificant differences ($P > 0.05$) were found among the groups in the abdominal

Table 2. Influence of inulin and β-glucan supplementation on the growth performance of the broilers.

	Control	Inulin	β-glucan	Inulin + β-glucan	P
0-3 weeks					
FI, g	1065.7 ± 23.9	1079.6 ± 23.7	1131.1 ± 7.4	1070.1 ± 42.9	0.349
BWG, g	671.1 ± 11.4	664.6 ± 16.8	687.7 ± 22.3	658.5 ± 18.2	0.683
FCR, kg/kg	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	0.825
3-6 weeks					
FI, g	3372.8 ± 55.1	3336.5 ± 119.2	3452.4 ± 80.4	3410.9 ± 62.2	0.783
BWG, g	1735.9 ± 18.4	1674.9 ± 27.5	1705.3 ± 24.6	1672.6 ± 18.2	0.210
FCR, kg/kg	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	0.565
0-6 weeks					
FI, g	4438.5 ± 58.7	4416.2 ± 128.5	4583.5 ± 87.5	4481.1 ± 83.9	0.606
BWG, g	2406.9 ± 11.9	2339.5 ± 37.1	2393.0 ± 44.2	2331.1 ± 29.2	0.310
FCR, kg/kg	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	0.511

Data are presented as mean ± standard error (n = 4).
FI = feed intake, BWG = body weight gain, FCR = feed conversion ratio.

fat content of the carcasses after removing the fatty tissues surrounding the gizzard and abdomen.

Discussion

At the end of the experiment, no significant differences were observed among the treatments for FI, BWG, and FCR. The performance of the broilers was not affected, even with levels of up to 0.7% inulin, 0.014% β-glucan, and 0.7% inulin + 0.014% β-glucan (Table 2).

Similarly, Chen et al. (5) observed no significant effects of inulin on growth performance in chickens, and Waldroup et al. (4) reported that supplementation of 0.375% oligofructose in the diet of broilers did not affect growth performance. However, Leeuwen et al. (14) observed that 1% and 2% inulin (Raftifeed®IPE) supplementation to broiler rations positively affected BWG, FI, and FCR ($P < 0.05$). Similar results were reported by Yusrizal and Chen (2) for male broilers fed with inulin or oligofructose. Ammerman et al. (3) reported that after supplementation with oligofructose, BWG was increased at day 47. Yusrizal and Chen (2) found that use of inulin or oligofructose improved the FCR in broilers.

There was no significant effect on the rate of BWG, FI, and FCR when 0.05% β-glucan was used (9). However, Chae et al. (15) indicated that adding more than 0.02% β-glucan to broiler rations positively affected BW.

Table 3. Average carcass rate and carcass yield of groups.

	Control	Inulin	β-glucan	Inulin + β-glucan	P
BW, g	2501.2 ± 45.1	2412.2 ± 46.9	2431.8 ± 52.8	2378.2 ± 48.8	0.341
HCW, g	1861.3 ± 40.1	1782.8 ± 38.9	1789.7 ± 40.2	1751.7 ± 38.2	0.259
HCP, %	74.3 ± 0.4	73.9 ± 0.4	73.6 ± 0.4	73.7 ± 1.0	0.806

BW = body weight, HCW = hot carcass weight, HCP = hot carcass percentage.
Data are presented as mean ± standard error (n = 12).

Table 4. Evaluated liver, spleen, heart, abdominal fat, bursa of Fabricius, and intestine weights and their rates to 100 g BWG.

	Control	Inulin	β-glucan	Inulin + β-glucan	P
Liver weight, g	50.6 ± 1.8	47.6 ± 1.6	49.3 ± 1.8	47.2 ± 1.3	0.425
Spleen weight, g	4.5 ± 0.2	4.1 ± 0.3	4.5 ± 0.3	4.0 ± 0.3	0.413
Heart weight, g	13.7 ± 0.5	13.1 ± 0.6	13.7 ± 0.6	14.2 ± 0.6	0.597
BF weight, g	5.1 ± 0.3	4.3 ± 0.3	4.4 ± 0.3	5.0 ± 0.3	0.172
Intestine weight, g	69.1 ± 2.3	65.3 ± 1.6	64.2 ± 2.1	60.9 ± 2.1	0.052
Abdominal fat weight, g	37.5 ± 4.5	30.7 ± 2.0	34.4 ± 2.9	41.7 ± 2.5	0.097
Liver ratio, g/100 g BW	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	0.846
Spleen ratio, g/100 g BW	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.626
Heart ratio, g/100 g BW	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.303
BF ratio, g/100 g BW	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.304
Intestine ratio, g/100 g BW	2.8 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	0.503
Abdominal fat ratio, g/100 g BW	1.5 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	1.8 ± 0.1	0.073
Small intestine, cm	150.0 ± 5.1	158.1 ± 2.8	155.3 ± 3.8	149.8 ± 4.8	0.426
Duodenum, cm	31.6 ± 1.5^{b}	30.6 ± 0.6^{b}	30.5 ± 0.7^{ab}	27.9 ± 0.7^{a}	0.043*
Cecum, cm	36.2 ± 1.7^{a}	48.3 ± 2.0^{b}	38.3 ± 1.6^{a}	38.4 ± 1.2^{a}	0.000***
Total intestine length, cm	217.8 ± 6.2^{a}	237.2 ± 3.1^{b}	224.1 ± 4.8^{ab}	216.1 ± 5.7^{a}	0.021*

BW = body weight, BF = bursa of Fabricius. Data are presented as mean ± standard error (n = 12).
^{abc}Means in a row with different superscripts differ significantly. $^{*}P < 0.05$, $^{***}P < 0.001$.

In this study, during the experimental period, the lowest BWG value was obtained with the inulin + β-glucan supplementation. Therefore, it can be concluded that there is a negative interaction between these 2 supplements.

There was no significant difference among the groups for average carcass weight and carcass yield values (P > 0.05, Table 3). This result was in agreement with the findings of Waldroup et al. (4). Despite this, Ammerman et al. (3) reported that supplementation of oligofructose in broiler diets at a level of 0.375% showed better carcass yield values. Yusrizal and Chen (2) stated that inulin or oligofructose enhanced carcass weight in broilers.

There were no statistically significant differences in the liver, spleen, heart, bursa of Fabricius, intestinal weights, and abdominal fat and their ratios to 100 g BW, but some numerical variations were found among the treatment groups (P > 0.05) (Table 4). Abdominal fat weight, which is considered to be a loss in carcass value and simultaneously means that additional energy was spent by the animal, was decreased by the supplementation of 0.7% inulin to the ration. This result is in agreement with the findings of Waldroup et al. (4). The length of the small intestine (jejunum + ileum) among the trial groups

Table 5. Antibody titers (log2) for ND after administration on days 10 and 26.

	Control	Inulin	β-glucan	Inulin + β-glucan	P
First, day 10	5.1 ± 0.8	4.5 ± 0.9	5.8 ± 0.9	5.3 ± 0.6	0.715
Second, day 26	7.2 ± 0.7	6.5 ± 0.7	6.7 ± 0.7	6.6 ± 0.8	0.908

Maternal = 5.90 ± 0.23. Data are presented as mean ± standard error (n = 12).

Table 6. Total serum cholesterol and triglyceride levels of the broilers.

	Control	Inulin	β-glucan	Inulin + β-glucan	P
TC, mg/dL	121.0 ± 4.83[a]	113.4 ± 2.2[a]	119.2 ± 2.7[a]	136.6 ± 3.6[b]	0.000***
TTG, mg/dL	32.5 ± 1.2[ab]	27.1 ± 1.0[a]	29.2 ± 1.0[ab]	34.0 ± 1.9[b]	0.002**

TC = total cholesterol, TTG = total triglyceride. Data are presented as mean ± standard error (n = 12).
[abc]Means in a row with different superscripts differ significantly. **P < 0.01 and ***P < 0.001.

was not statistically different (P > 0.05). The length of the duodenum showed statistically significant differences (P < 0.05) among the groups. The longest cecum length was measured in the inulin group (P < 0.001). This outcome was in agreement with the findings of Roberfroid (6).

Yusrizal and Chen (2) found that adding chicory-based fructans to rations increased the length of the intestine in female broilers. Chen et al. (5) stated that supplementation of 1% oligofructose and 1% inulin in white Leghorn chicken rations increased the length of small and large intestines.

In this study, the longest length value for total intestine (including jejunum + ileum + duodenum + cecum) (Table 4) was recorded for 0.7% inulin, followed by 0.014% β-glucan, the control group, and 0.7% inulin + 0.014% β-glucan. According to the values indicated above, statistically significant differences were found among the inulin, control, and inulin + β-glucan treatments (P < 0.05).

Some studies support the idea of using prebiotics for increasing the length of the intestinal villus, which also affects the length of the intestine, as well (16-19). The results show that the longer the intestine is, the better the nutrient absorption will be, and this causes an increase in BW (2). Even though the intestinal lengths increased in the β-glucan group, there were no parallel results for the BWGs.

In the present study, as a result of vaccination against the ND-specific antibody titer (Table 5) at the first measurement (day 26), the highest value was determined in the β-glucan group, followed by the inulin + β-glucan group, the control group, and the inulin group. At the second measurement (at the end of the experimental period), the highest value was determined in the control group, and there were no significant differences among the groups (Table 5). These results are in agreement with the findings of Cheng et al. (9). Based on this study, a low β-glucan supplement level (0.014%) may prevent ND.

Cheng et al. (9) conducted an experiment with broilers fed 0%, 0.012%, 0.025%, and 0.05% β-glucan for 6 weeks in which the antibody titer against ND viruses was evaluated. At the end of the study, no differences were observed in the antibody levels among the groups. Researchers (10,11,20) suggest that β-glucan can be effective in decreasing environmental stress and this effect may support the immunity.

Blood serum total cholesterol values were significantly different among the groups. When these values were taken into consideration, the lowest value was found in the inulin group (P < 0.001) (Table 6).

When total triglyceride values were taken into consideration, the lowest value was found in the inulin group (P < 0.01) (Table 6).

It was found that inulin and β-glucan decreased the blood serum total cholesterol and triglyceride levels significantly (P < 0.001 and P < 0.01, respectively). These results are in agreement with the findings of Yusrizal and Chen (2), who also observed that adding chicory-based fructans to feeds decreased the serum cholesterol level in broilers.

Adding 0.7% inulin to broiler rations increased cecum weight and intestinal length compared to the other groups, but decreased the abdominal fat weight. A possible reason for that is the unavailability of the stress condition (like disease) for producing the effects of inulin and β-glucan on the immune system. For this reason, it may be suggested that they be

used to increase the preventive effect and stimulate immunity.

During the experiment, 1 animal from each group died: in the first week, 1 from the inulin + β-glucan group; in the second week, 1 from the β-glucan group; and in the third week, 1 from the β-glucan group. These results did not statistically affect the results of the experiment.

It was concluded that combination of inulin and β-glucan is not advisable for broilers. Use of these products in combination resulted in a decrease in BWG and an increase in abdominal fat, although there were no statistical differences among the groups. Further studies should be conducted to support these results and to explain the mechanism of inulin and β-glucan on the effect of broiler performance.

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