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RESEARCH ARTICLE

Effects of Chitosan Oligosaccharide and/or Beta-Glucan Supplementation to Diets Containing Organic Zinc on Performance and Some Blood Indices in Broilers

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ABSTRACT

This study was carried out to investigate effects of chitosan oligosaccharides and/or beta-glucan addition into diets containing organic zinc on performance and biochemical profiles in broilers. One-day old broiler chicks (n=540) were assigned to six groups for six replicates (15 chicks for each). Chicks in control group were fed basal diet containing soybean meal and corn, and experimental groups were fed diets containing 1% organic zinc (Or.Zn) or 0.025% chitosan oligosaccharides (COS) or 0.050% beta-glucan (BG) or 1% Or.Zn plus 0.025% COS or 1% Or.Zn plus 0.050% BG during 42 days. There were no significant differences between groups for performance (body weight, daily body weight gain, daily feed intake, and feed conversion ratio) during experimental period. Although, there were no differences between all groups for serum total cholesterol, HDL-cholesterol, VLDL-cholesterol, urea, insulin and glucose levels, statistical significances were determined between Or.Zn plus COS and Or.Zn plus BG groups for total protein, and Or.Zn plus COS and BG groups for GPT on d 21 ($P<0.05$). GOT levels were lower only in control group on d 42 ($P<0.05$). Groups fed diet with COS had lower serum LDL-cholesterol levels than control group at the end of the experiment. As a result of this study, there were no significant effects of organic zinc, beta-glucan and chitosan oligosaccharide supplementations into diets on performance. However, the use of chitosan oligosaccharides in diets decreased LDL-cholesterol levels without any alteration in HDL-cholesterol levels in broilers.

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INTRODUCTION

Nowadays, several feed additives are widely used in poultry nutrition. Especially, intensified food production requires the use of various additives to feed which promote animal growth process. Zinc, chitosan oligosaccharides (COS) and beta-glucan are considered as antioxidant and immune-stimulant feed additives. Zinc, a co-factor of over 200 enzymes including alkaline phosphatase, alcohol dehydrogenase, carbonic dehydrogenase, is an essential nutrient for growth, protein metabolism, energy metabolism, gene regulation, cell membranes and bone structure (Powell, 2000). It was reported that the bioavailability of organic zinc sources such as zinc-methionin or zinc-propionate was more than inorganic zinc sources such as zinc-oxide or zinc-sulphate in farm animals (Hahn and Baker, 1993). Hess *et al.* (2001) reported that the addition of 40 mg/kg zinc-amino acid complex into broiler diet improved feed efficiency on days 0-42.

Chitosan is derived from chitin, a polysaccharide formed by N-acetyl-D glucosamine units found in insects, marine diatoms, algae, fungi and crustacea like crab, by deacetylation (Synowiecki and Al-Khateeb, 2005), demineralization, deproteinisation, decoloration (Bilgin and Fidanbaş, 2011) and it was determined that chitosan content of freshwater crabs was 4.65% (Bolat *et al.,* 2010). Unlike chitosan, COS are readily soluble in water due to their shorter chain lengths and free amino groups in D-glucosamine units. The low viscosity and greater solubility of COS at neutral pH have attracted the interest of many researchers to utilize chitosan in its oligo-saccharide form (Jeon *et al.,* 2000). It was reported that there was no effect of chitosan on body weight gain, feed consumption and feed conversion ratio, however,

abdominal fat and lipase activity in small intestines were lower without any alteration in total lipase activity in broilers (Kobayashi *et al.,* 2002). Shi *et al.*(2005) reported that optimum growth performance, feed efficiency and protein utilization were determined in broilers fed diets contained 0.5 and 1.0 g/kg chitosan. Huang *et al.* (2005) reported that the best results for performance and ileal digestibility of nutrients in broilers were obtained by the addition of 100 and 150 mg/kg COS rather than those of groups fed diets with 0 and 50 mg/kg COS.

Glucans with β-1,3 and 1,6 glycosidic linkages (β-glucan) are major structural components of yeast and fungal cell walls (Jorgensen and Robertsen, 1995). In an experiment conducted to evaluate the efficacy of β-glucan on broilers, it was reported that the addition of 0.04 % β-glucan to diet had beneficial effect on growth performance and immunity (Chae *et al.,* 2006).

MATERIALS AND METHODS

Animal Care and Experimental Diets: A total of 540 day-old male Ross broiler chicks were used in this study. Birds were assigned to six dietary treatment groups (15 birds/pen and 6 pens/treatment) according to body weight to equalize the mean body weight in each group. Room temperature was maintained at 33ºC for first 3 d, which was gradually reduced by 3ºC a week until reaching 24ºC. During the entire experiment, 24-h constant light and ventilation were maintained. A corn-soybean meal basal diet was formulated and used adequate in all nutrients (NRC, 1994). Basal diet composition for starter (d 1-21) and grower (d 22-42) phase are listed in Table 1. All diets were fed in mesh form. Control group were fed basal diet and other 5 experimental groups were fed basal diet supplemented with 1% zinc propionate as organic zinc [(Or.Zn), purchased from Alltech Bioteknoloji Ltd., Izmir, Turkey] or 0.025% chitosan oligosaccharide [(COS), purchased from GlycoBio Company, Dalian, China] or 0.05% beta-glucan [(BG), purchased from Centurk Organik Urunler San. Tic. Ltd., Derince, Kocaeli, Turkey] or 1% Or.Zn plus 0.025% COS or 1% Or.Zn plus 0.05% BG. Chicks were provided *ad libitum* access to feed and water. Experimental design is presented in Table 2.

Sample Collection and Analytical Profiles: Chicks were weighed individually at the beginning of study for initial body weights. On d 21 and 42, broilers were fasted for 12 h and then weighed. Amounts of offered feeds and refused feeds were recorded daily and feed consumption was determined at the end of experiment. Data were recorded for statistical performance analysis for body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). Diets for each group were prepared for starter (d 1-21) and grower (d 22-42) phase, and samples of each diet were collected and chemical analyses was carried out according to the standard procedures of Association of Official Analytical Chemists (AOAC, 1990) being presented in Table 1.

Blood samples were collected from 12 chicks selected randomly from each group on d 21 and 42. 144 samples were taken from brachial vein by using vacuum tubes before morning feeding. Serum samples were separated by centrifugation and stored at -20ºC for analyses.

Table 1: Composition of basal diets and nutrient levels

	Starter (d 0-21)	Grower (d 22-42)
Ingredients (%)		
Corn	57.40	65.40
Corn starch	1.00	1.00
Soybean meal	35.50	27.30
Sunflower meal	1.30	1.50
Salt	0.25	0.25
Dicalcium phosphate	2.10	2.10
Limestone	1.20	1.20
Methionine	0.08	0.08
Vitamin-mineral premix	1.17	1.17
Nutritional content (calculated)		
ME, MJ/kg	12.34	12.74
CP, %	21.00	18.20
Ca, %	0.99	0.99
P, %	0.44	0.44
Na, %	0.20	0.20

* Provided per kg of diet: vitamin A, 1204 µg; cholecalciferol, 25 µg; vitamin E, 4.5 mg; riboflavin, 2.25 mg; niacin, 15.0 mg; d-pantothenic acid, 4.0 mg; folic acid, 0.25 mg; vitamin B12, 5 µg; choline chloride, 200 mg; thiamine, 0.5 mg; biotin, 25 µg; ethoxyquin, 12.5 mg; menadione sodium bisulfite, 1.25 mg; pyridoxine, 0.5 mg; manganese, 24.9 mg; zinc, 22 mg; iodine, 0.2 µg; iron, 13.6 mg; copper, 1.6 mg.

Table 2: Experimental design

Groups	n	Replicates	Diets
Control	15	6	Basal diet
Or.Zn	15	6	Basal diet + 1% Or.Zn
COS	15	6	Basal diet + 0.025% COS
BG	15	6	Basal diet + 0.05% BG
Or.Zn + COS	15	6	Basal diet + 1% Or.Zn + 0.025% COS
Or.Zn + BG	15	6	Basal diet + 1% Or.Zn + 0.05% BG

Serum triglyceride, free fatty acids, total cholesterol, HDL-cholesterol, LDL-cholesterol, total protein, glucose, urea, GOT and GPT levels were detected with a commercial kit (Accurex Biomedical Pvt. Ltd., Boisar, Thane, India) by automatic analyser (Hitachi-704). Insulin levels were detected with DRG® commercial ELISA kit (DRG Instruments GmbH, Germany Division of DRG International Inc. Frauenbergstraße 18, D-35039 Marburg, Germany) by ELISA.

Statistical Analysis: Statistical analyses were performed by using software package program (SPSS for windows, Standard version 10.0, 1999; SPSS Inc., Headquarters, Chicago, IL, USA) package software. One way analysis of variance (ANOVA) was used for each experiment and mean differences were determined by Duncan's multiple range tests.

RESULTS

BWs (initial, on d 21 and 42), ADGs, ADFIs and FCRs (d 0-21, 22-42 and 0-42) are given in Table 3. There were no significant differences between groups for BW, ADG, ADFI and FCR during the experiment (P>0.05).

The levels of total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, free fatty acids (FFA), total protein, urea, insulin, glucose, GOT and GPT determined in serum samples obtained from broilers on d 21 and 42 are presented in Table 4.

There were no significant differences between groups for serum total protein, HDL-cholesterol, VLDL-cholesterol, urea, insulin and glucose levels in both period (P>0.05). Although there were no differences between groups for triglyceride levels on d 21, group Or.Zn plus COS had lower serum triglyceride levels than those of

Table 3: Performance values of broilers (n=90)

Parameter/days	Control	Or.Zn	COS	BG	Or.Zn+COS	Or.Zn+BG
BW (g)						
Initial	40.36±0.39	41.13±0.49	40.40±0.40	41.43±0.45	41.26±0.43	40.93±0.36
d 21	663.14±11.52	709.14±16.97	677.43±21.82	666.94±19.00	678.61±10.45	686.57±12.25
d 42	2061.05±31.47	2096.15±51.53	2178.09±57.46	2101.52±45.77	2060.12±47.07	2134.28±49.20
ADG (g/day)						
d 0-21	29.65±0.19	31.81±0.22	30.33±0.25	29.78±0.18	30.35±0.20	30.74±0.23
d 22-42	66.56±0.40	66.05±0.42	71.46±0.46	68.31±0.41	65.78±0.48	68.93±0.51
d 0-42	48.11±0.45	48.92±0.39	50.89±0.42	49.05±0.43	48.07±0.37	49.84±0.40
ADFI (g/day)						
d 0-21	50.74±0.46	54.26±0.41	53.13±0.45	50.65±0.39	48.65±0.43	50.95±0.44
d 22-42	124.14±1.51	120.92±1.78	129.63±2.00	125.23±1.65	125.12±1.60	124.25±1.55
d 0-42	87.44±1.41	87.59±1.38	91.38±1.36	87.94±1.39	86.88±1.42	87.60±1.35
FCR						
d 0-21	1.71±0.008	1.71±0.021	1.75±0.014	1.70±0.010	1.60±0.013	1.66±0.009
d 22-42	1.87±0.011	1.83±0.017	1.81±0.020	1.83±0.015	1.90±0.008	1.80±0.011
d 0-42	1.82±0.007	1.79±0.013	1.80±0.009	1.79±0.008	1.81±0.011	1.76±0.015

BW: Average body weight, ADG: Average daily gain, ADFI: Average daily feed intake, FCR: Feed conversion ratio

Table 4: Some blood parameters according to the groups (n=12)

Parameter/days	Control	Or.Zn	COS	BG	Or.Zn+COS	Or.Zn+BG
Total cholesterol (mg/dl)						
d 21	129.50±9.33	145.40±7.92	140.53±10.59	151.88±6.92	129.91±7.19	137.18±8.00
d 42	147.44±4.41	146.43±2.88	137.27±5.20	136.80±4.25	139.80±3.69	144.90±3.22
LDL-cholesterol (mg/dl)						
d 21	25.91±2.39^{ab}	24.11±1.11^{ab}	23.51±2.43^{ab}	26.71±1.00^{ab}	21.18±2.40^{b}	28.31±1.88^{a}
d 42	39.97±3.98^{a}	33.29±4.16^{ab}	28.90±0.91^{b}	33.60±3.13^{ab}	25.31±0.82^{b}	34.92±3.73^{ab}
HDL-cholesterol (mg/dl)						
d 21	93.00±5.93	103.33±5.61	98.16±6.79	105.83±4.11	94.00±5.02	90.33±5.59
d 42	92.91±1.91	95.83±1.74	94.83±3.10	91.83±2.18	93.25±1.47	91.66±2.46
VLDL-cholesterol (mg/dl)						
d 21	14.70±1.13	17.95±2.41	18.85±1.83	19.33±2.24	14.73±1.54	18.53±1.60
d 42	18.41±2.20	17.30±2.44	15.44±1.61	15.50±1.05	21.24±1.41	19.95±2.28
Triglyceride (mg/dl)						
d 21	60.16±5.92	68.33±5.82	71.83±7.40	74.50±13.09	64.33±9.94	58.83±5.36
d 42	68.75±3.21^{bc}	72.50±2.83^{abc}	75.41±3.49^{ab}	75.66±2.74^{ab}	62.66±2.60^{c}	80.50±5.25^{a}
Free fatty acids (mg/dl)						
d 21	5.53±0.77	6.61±0.72	6.35±0.56	6.46±0.98	5.41±0.89	4.81±0.42
d 42	5.65±0.15^{b}	6.19±0.18^{ab}	6.01±0.17^{ab}	6.05±0.17^{ab}	5.63±0.11^{b}	6.57±0.27^{a}
Total protein (g/dl)						
d 21	2.64±0.09^{ab}	2.74±0.03^{ab}	2.73±0.05^{ab}	2.60±0.06^{ab}	2.52±0.10^{b}	2.78±0.09^{a}
d 42	3.44±0.15	3.81±0.16	3.79±0.17	3.81±0.11	3.76±0.11	3.72±0.13
Urea (mg/dl)						
d 21	21.65±1.26	24.40±1.15	20.85±1.09	23.01±0.96	19.98±0.74	23.23±1.30
d 42	20.84±0.82	22.10±0.30	23.02±0.61	24.24±0.32	22.45±0.84	24.35±0.72
Insulin (µIU/ml)						
d 21	3.54±0.30	3.85±0.25	4.15±0.28	3.72±0.24	4.09±0.28	3.92±0.29
d 42	3.51±0.22	4.00±0.39	3.88±0.26	3.72±0.24	4.32±0.21	3.60±0.26
Glucose (mg/dl)						
d 21	271.66±7.92	263.21±4.92	269.28±3.33	257.45±3.86	259.98±9.04	279.96±7.48
d 42	261.55±8.46	264.13±7.15	250.15±3.60	245.58±4.82	251.09±5.18	263.26±5.70
GOT (IU/L)						
d 21	257.66±6.56	239.00±3.54	241.66±4.82	234.50±10.00	231.00±14.45	249.83±8.51
d 42	242.58±9.13^{c}	268.83±4.31^{b}	292.75±4.66^{a}	277.25±5.24^{ab}	278.16±5.57^{ab}	275.33±8.11^{ab}
GPT (IU/L)						
d 21	4.50±0.42^{ab}	3.50±0.67^{ab}	4.50±0.42^{ab}	5.00±0.85^{a}	2.66±0.66^{b}	3.66±0.33^{ab}
d 42	3.00±0.32	2.33±0.55	2.33±0.47	1.83±0.74	2.66±0.52	2.66±0.52

$^{a, b, c}$ Means within a row with different superscripts differ (P<0.05).

group COS, BG and Or.Zn plus BG on d 42 (P<0.05). Serum LDL-cholesterol concentration was significantly lower only in group Or.Zn plus COS than group Or.Zn plus BG (P<0.05), however, there were no significant differences among other groups on day 21 (P>0.05). On day 42, LDL-cholesterol concentrations of group COS and Or.Zn plus COS were lower compared with control group (P<0.05). With respect to FFA levels, there were no significant differences between groups on day 21, whereas control group and group Or.Zn plus COS had lower FFA levels than those of group Or.Zn plus BG on day 42 (P<0.05). On day 21, both serum total protein and GPT levels were lower in group Or.Zn plus COS, and statistical significances were detected between Or.Zn plus COS and Or.Zn plus BG and between Or.Zn plus COS and BG for total protein and GPT, respectively (P<0.05). On day 42, there were no significant differences among the treatments for both parameters (P>0.05). Although serum GOT level was not statistically different between groups on day 21, it was lower in control group than other groups on day 42 (P<0.05).

DISCUSSION

As it was expected, there were no differences between groups for initial BW. During the experiment,

BW, ADG, ADFI and FCR of broilers were not statistically different and this indicated that feed additives used in this experiment had no positive or negative effects on performance. Rossi *et al.* (2007) reported that there were no differences between groups fed 0, 15, 30, 45 ppm organic zinc (Bioplex Zn) for performance, and this result was in agreement with results of control group and group fed Or.Zn in this study.

However, in a study conducted by Ao *et al.* (2006), the use of 5, 10, 20 and 40 mg/kg diet Bioplex Zn (chelated zinc-proteinat) in broilers increased ADFI and ADG more than control group. Similarly, Yildiz *et al.* (2005) concluded that dietary supplementation of 25, 50, 75 and 100 ppm organic zinc (zinc-proteinat) in partridges increased body weight and body weight gain more than those of control group on day 56. This might be due to different organic zinc sources or species.

In the present study, addition of chitosan oligosaccharides (COS) had no effect on performance and there were no significant differences between control and experimental groups. Similarly, Huang *et al.* (2005) reported that broilers given 50 and 150 mg/kg diet COS were not statistically different from controls for performance. Contrarily, in a study conducted by Li *et al.* (2007) who diet supplemented COS in broiler diet 50 and 100 mg/kg reported higher ADG, ADFI and FCR than controls.

In this study, supplementation of β-glucan had no significant difference for performance between groups and the findings of this study was inline with Chae *et al.* (2006) who reported that use of 0, 0.02 and 0.04% β-glucan in broilers did not cause significant difference between groups for ADG, ADFI and FCR on day 0-17, 18-34 and 0-34. Rathgeber *et al.* (2007) used β-glucan in broiler diets 0.004% for starter period (d 0-14) and 0.002% for grower (d 15-24) and finisher (d 25-38) periods and no differences for ADFI and FCR were found for all periods. BWs of experimental groups were higher than those of controls in finisher period. Except for finisher period results, our findings for starter and grower periods were parallel to the findings of Rathgeber *et al.* (2007).

During the study, the difference between groups for the serum levels of total protein, HDL-cholesterol, urea, insulin and glucose were not significant. In a study conducted by Wang *et al.* (1992) on broilers with barley β-glucan, control group was fed only corn basal diet and experimental groups were fed diets containing barley or enzyme β-glucanase (1 g/kg) and barley. When compared to control group, experimental groups had significantly lower total cholesterol and LDL-cholesterol, and higher HDL-cholesterol. These results are different from our results in group BG. Although it was reported that a lower plasma cholesterol concentration associated with higher intestinal viscosity by β-glucan and β-glucans may have also had bound to bile acids, thus interfering with digestion and affecting plasma cholesterol concentrations, surprisingly, there were no significant differences between group BG and other groups in our study.

There are several opinions regarding the mechanism of action of chitosan oligosaccharides (COS) on cholesterol metabolism. According to an argument, COS inhibit micelle formation during the lipid digestion in tract by forming ionic bond with the bile salts and acids (Remunan-Lopez *et al.,* 1998). It was suggested that chitosan and its oligomers are bound to lipids and fatty acids directly (Tanaka *et al.,* 1997).

In this study, addition of COS did not induce significant differences between control and experimental groups for total cholesterol, triglyceride and HDL-cholesterol. When it was considered the similarities of cholesterol metabolism in chicks, humans and laboratory animals (Leveille *et al.,* 1975), these results were agree with Lee *et al.* (2003) who reported that the use of 3% COS had no effect on total cholesterol, triglyceride and HDL-cholesterol in rats, and were parallel to Kim *et al.* (1998) observed no difference for HDL-cholesterol between control mice fed diet with 3% cholesterol and experimental mice fed diet with 3% cholesterol plus 1% COS. Also, Li *et al.* (2007) concluded that COS (0.05%) had no effect on triglyceride, total cholesterol and HDL-cholesterol levels in broilers on d 21 and 42. Other finding in our study related to lipid metabolism was the effect of COS on LDL-cholesterol. When compared to control group LDL-cholesterol levels were significantly lower ($P<0.05$) by the addition of 0.025% COS to diets on d 42, although there were no differences on d 21. Similarly, Li *et al.* (2007) reported that there were no differences between control and experimental group for LDL-cholesterol on d 21 even though they used two-fold level of COS, but, interestingly, they found that LDL-cholesterol level of experimental group was significantly higher than that of control on d 42.

In conclusion, the use of organic zinc, β-glucan and COS individually or combined with organic zinc in broiler diets had no significant effect on performance. However, the addition of COS to diets caused descent in LDL-cholesterol significantly without any alteration in HDL-cholesterol.

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