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Effect of Supplementation of Prebiotic Oligosaccharides to Diets Containing Zinc Propionate on Some Serum Enzymes, Metabolites and Electrolytes in Broilers

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ABSTRACT

***Background*:** Zinc is needed as essential for many physiological functions in humans and animals. Chitosan is a natural substance produced in the body from glucose and some biological effects including anti-microbial, immune modification mechanisms. β-glucans are polysaccharides of D-glucose monomers and have important roles in antitumor and antimicrobial activity in animals. Inulin is used for its effects on immune function, bioavailability of minerals, lipid metabolism and gastrointestinal tract health. The aim of the study was to investigate the effects of chitosan oligosaccharide (COS), β-glucan, and inulin on some serum enzymes, metabolites and electrolytes in broilers.

***Materials, Methods & Results*:** A total of 96 male and 1-day-old Ross broiler chicks were used in the study. The ambient temperature was maintained at 33ºC for the first 3 days, which was gradually reduced by 3ºC a week until reaching 24ºC. Chicks had free access to food and tab water. Basal diets containing the soybean meal and corn were prepared for starter (day 1 to 21) and grower (day 22 to 42) periods. Chicks were equally assigned to eight dietary treatment groups: Control (basal diet); Zn (zinc propionate, 1%); Chitosan (Chitosan, 0.025%); β-glucan (β-glucan, 0.05%); Inulin (inulin, 1%); Zn+Chitosan (zinc propionate, 1% + Chitosan, 0.025%); Zn+ β-glucan (zinc propionate, 1% + β-glucan, 0.05%) and Zn+Inulin (zinc propionate, 1% + inulin, 1%). Blood samples were collected from V. brachialis before feeding in the morning on days 21 and 42. Serum K levels were found lower in Zn+COS group than in COS and control groups on day 21 ($P < 0.05$). They were insignificantly different among all groups on day 42 in the study. Serum sodium levels were significantly higher in inulin group on day 21 and in control group on day 42 than other groups. Serum phospholipid levels were insignificantly different among control, COS and Zn+COS groups on days 21 and 42 in the present study. Serum creatine kinase (CK) activities were higher in β-glucan group and in Zn+Inulin group than other groups on day 42 ($P < 0.05$). Serum creatinine levels were significantly higher in Zn+Inulin group than control group, Zn group and Chitosan group on day 42. Serum gamma-glutamyl transferase (γ-GT) activities were the lowest in Chitosan group on day 42.

***Discussion*:** According to literature, antibiotics could be hazardous especially to human health when residues occurs and bacteria gains resistance against antibiotics. Chitosan and glucans have antibacterial functions. Therefore, they can be used as an alternative against antibiotics. Because of chitosan gels bind minerals in the intestines, which is an undesirable biochemical effect, a lack of absorption of minerals occurs. In the study, all blood parameters remained within their normal ranges. All experimental groups showed a significant decrease in serum Na levels at the end of the study. Serum K levels decreased after 21 days in all Zn added groups, but raised to their normal levels at the end of the study. The addition of Zn plus inulin to diets caused increase in CK and creatinine, significantly. Additional researches are needed to elucidate the negative effects and the action mechanisms of Zn plus inulin on muscle tissue in broilers. This study may also show the potential protective effect of COS on liver enzymes, and COS may play a role in modulation of liver enzymes.

Keywords: broiler, chitosan oligosaccharide, β-glucan, inulin, zinc.

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INTRODUCTION

Zinc (Zn) is needed as essential for many physiological functions such as immune and antioxidant functions in animals [28].

Addition of antibiotics can lead to resistance of bacteria and antibiotic residues may be hazardous to human health. Antibiotic supplementation should be limited and alternative sources with equal effectiveness should be evaluated [4]. There have been many problems remain unresolved regarding prebiotic oligosaccharides. Therefore the effects of prebiotics on animal's colon microflora composition and gastrointestinal health should be investigated [10,33].

Chitosan has anti-ulcerogenic, anti-microbial, osteogenetic effects and can be used as a bio-tissue engineering scaffold allowing the skin or bone cell growth. [29,30].

Glucans have antitumor and antimicrobial functions [4,13]. It stabilizes the toxic effects of bacterial endotoxins, scavenges free radicals and has a regression effect on liver cirrhosis [8,15]. Studies showed that β-1,3/1,6-glucan can be useful as an alternative to antibiotics that promote growth in poultry [1,11].

Inulin is a natural reservoir of non-structural carbohydrates found in many vegetables [7]. Nowadays, inulin is used because of its effects on the colonic microflora, gastrointestinal physiology, immune function, bioavailability of minerals, lipid metabolism and gastrointestinal tract health [16,24,33].

The aim of the study was to investigate the effects of chitosan oligosaccharide (COS), β-glucan, and inulin on some serum enzymes [gamma-glutamyl transferase (ɣ-GT) and creatine kinase (CK)], metabolites (phospholipid and creatinine) and electrolytes [sodium (Na) and potassium (K)] in broilers.

MATERIALS AND METHODS

Animals

A total of 96 male, 1-year-old Ross broiler chicks were used in the study and equally assigned to eight dietary treatment groups.

Experimental Groups and Study Design

Room temperature was maintained at 33ºC for the first 3 days, which was gradually reduced by 3ºC a week until reaching 24ºC. Basal diets containing the soybean meal and corn were prepared for starter (day 1 to 21) and grower (day 22 to 42) periods. The composition of basal diets and nutrient levels were presented in Table 1. Groups in the study were control (basal diet); Zn (zinc propionate[1], 1%); COS (COS[2], 0.025%); β -glucan (β-glucan[3], 0.05%); Inulin (inulin[3], 1%); Zn+COS (zinc propionate, 1% + COS, 0.025%); Zn+ β-glucan (zinc propionate, 1% + β-glucan, 0.05%) and Zn+Inulin (zinc propionate, 1% + inulin, 1%). Chicks were provided *ad libitum* access to feed and water. Blood samples were collected from all chicks on days 21 and 42. The samples were collected from brachial vein by using vacutainer tubes before feeding in the morning. The samples were centrifuged at 3500 *g* for 10 min for separating serum. The serum samples were stored at -20ºC until analyzed. Serum phospholipid, K, Na, creatinine concentrations and ɣ-GT, CK activities were determined using commercially available spectrophotometric kits[4].

Statistical Analysis

Data were compared using analysis of variance (Duncan's multiple range test) between groups within each blood sampling day for all serum indices. Results are presented as mean and standard error (Mean ± SE). All statistical analyses were performed using software package program[5]. A significance level of $P < 0.05$ was employed in the analysis of data from groups.

RESULTS

Serum phospholipid, K, Na, creatinine concentrations and ɣ-GT, CK activities are shown in Table 2.

Serum phospholipid level was the highest in Zn+β-glucan group on day 21. The levels were significantly higher in inulin and Zn+β-glucan groups than control, Zn and β-glucan groups on day 21. Serum phospholipid concentrations were insignificantly different among all groups on day 42. Serum ɣ-GT activities were significantly higher in control and Zn groups than COS and Zn+Inulin groups on day 21. Serum ɣ-GT activity was the lowest in COS group and were insignificantly different among all groups except for COS group on day 42.

Serum CK activity was the highest in Zn group on day 21. The activities were significantly higher in control, COS and β-glucan groups than inulin and Zn+COS groups on day 21. Serum CK activities were higher in β-glucan and Zn+Inulin groups than other groups on day 42 ($P < 0.05$). Serum K concentrations were significantly higher in control, Zn and COS groups than Zn+COS, Zn+β-glucan and Zn+Inulin

groups on day 21. The concentrations were insignificantly different among all groups on day 42.

Serum Na levels were significantly higher in inulin group on day 21 and in control group on day 42 than other groups. The levels were insignificantly different among other groups except these two groups. Serum creatinine levels were the highest in Zn+β-glucan group on day 21. The levels were higher in COS and Zn+β-glucan groups than Zn, Inulin, Zn+COS and Zn+Inulin groups on day 21 ($P < 0.05$). Serum creatinine concentrations were significantly higher in Zn+Inulin group than control, Zn and COS groups on day 42.

Table 1. Diet ingredients and calculated content in the starter (day 0-21) and grower (day 21-42) diet.

Ingredient (%)	Starter (day 0-21)	Grower (day 22-42)
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 B_{12}, 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. Effect of supplementation of prebiotic oligosaccharides on serum phospholipid, gamma-glutamyl transferase, creatine kinase, potassium, sodium and creatinine in days 21 and 42.

	Days	Control	Zn	COS	Glu	Inu	Zn+COS	Zn+Glu	Zn+Inu
Phospholipid	21	268±15.3^{b}	262±11.4^{b}	283±14.5^{ab}	269±15.1^{b}	311±12.5^{a}	262±8.40^{b}	313±10.4^{a}	289±11.7^{ab}
	42	271±10.5	257±10.5	249±11.1	245±19.2	250±8.35	267±27.7	263±7.83	288±11.7
γ-GT	21	15.5±1.04^{a}	15.5±0.96^{a}	12.6±0.97^{bc}	13.8±0.62^{abc}	15.3±0.85^{ab}	14.3±1.11^{abc}	14.4±0.69^{abc}	12.2±0.54^{c}
	42	26.1±1.75^{a}	22.9±2.23^{ab}	17.7±2.36^{b}	22.0±2.61^{ab}	26.0±2.09^{a}	29.1±2.55^{a}	24.3±1.52^{ab}	23.8±2.75^{ab}
CK	21	1522±205^{b}	2488±162^{a}	1554±275^{b}	1467±210^{b}	893±101^{c}	874±112^{c}	1026±156^{ab}	1365±121^{ab}
	42	1148±200^{b}	1583±278^{b}	1206±289^{b}	2814±104^{a}	1489±315^{b}	1413±282^{b}	1824±261^{b}	2730±278^{a}
K	21	4.33±0.87^{ab}	5.54±0.47^{a}	4.59±0.22^{ab}	2.84±0.53^{bc}	3.19±1.01^{bc}	2.20±0.73^{c}	2.28±0.73^{c}	1.56±0.33^{c}
	42	3.88±0.48	4.09±0.24	4.59±0.26	3.95±0.32	4.11±0.42	3.95±0.43	4.09±0.25	3.63±0.40
Na	21	1.07±0.03^{b}	1.03±0.01^{b}	1.02±0.06^{b}	1.04±0.03^{b}	1.16±0.06^{a}	1.00±0.01^{b}	1.03±0.01^{b}	1.04±0.01^{b}
	42	1.17±0.04^{a}	1.06±0.01^{b}	1.03±0.02^{b}	0.99±0.03^{b}	1.02±0.02^{b}	1.02±0.02^{b}	1.03±0.02^{b}	1.05±0.02^{b}
Creatinine	21	0.31±0.04^{ab}	0.24±0.03^{bc}	0.38±0.04^{a}	0.28±0.03^{abc}	0.18±0.01^{c}	0.23±0.03^{bc}	0.39±0.05^{a}	0.26±0.05^{bc}
	42	0.22±0.03^{bc}	0.17±0.01^{c}	0.25±0.04^{bc}	0.33±0.05^{ab}	0.28±0.08^{abc}	0.36±0.05^{ab}	0.30±0.04^{abc}	0.42±0.08^{a}

Mean ± Standard Error. ^{a,b,c} Different letters indicate significant differences within lines (the comparisons among groups) [$P < 0.05$].

DISCUSSION

A significant undesirable biochemical effect of chitosan treatment is to reduce the absorption of minerals. Chitosan gels formed in the intestines bind minerals. The chitosan with sodium ascorbate administered to male rats fed with high fat diet for two weeks has resulted in a significant reduction in mineral absorption associated with a decrease in bone mineral content [32]. The significant obstacle in mineral absorption and the reduction of bone mineral content during short-term (2 weeks) application of chitosan indicate potentially hazardous results after long-term ingestion of food supplement [17]. Short-term ingestion of chitin or chitosan is seen not to affect absorption of zinc. The zinc absorption did not change in young male rats fed a semipurified diet with the addition of 5% chitin or chitosan for 31 days [18]. It was reported that the zinc content of the tibia and the femur tends to be lower in animals fed with dietary fiber than the control group, but the differences are not statistically significant [17]. In the present study, serum K levels were lower in Zn+COS group than in COS and control groups on day 21 ($P < 0.05$). They were insignificantly different among all groups on day 42. Apparently, COS supplementation to diet did not affect serum K level. However, serum Na levels were higher in control group than in COS and Zn+COS groups on day 42 ($P < 0.05$). This result is similar to previous findings of another study [32].

The lipid-lowering effect of chitosan was documented in earlier studies [14,20,31].In a study it was found that dietary supplement of COS can reduce blood lipids in broilers[20]. It was reported that blood lipid levels decreased by supplementation with COS [31]. It was detected that chitosan has changed intestinal lipid absorption [2]. Serum phospholipid levels were insignificantly different among control, COS and Zn+COS groups on days 21 and 42 in the present study. However, the level was tended to be lower in COS group than control group on day 42. The results of the present study showed similarity with the previous reported results of other authors [2,20,31]. It was mentioned that chitosan reduces lipid absorption from the intestine probably by binding bile acids [35].

It was reported that 0.05% to 0.1% injection of COS did not have any effect on serum creatinine levels in rats [34]. In addition, serum creatinine levels were found as 0.60 mg/dL and 0.62 mg/dL in control group and in treatment group, respectively [34]. In a study, the authors found the decreased blood creatinine level in the highest dose group of rats fed with a diet containing 0%, 0.04%, 0.2% or 1% COS for 90 days [22]. They also reported that the dietary level of 0.2% did not induce any signs of toxicity [22]. In the present study, serum creatinine levels on days 21 and 42 were not significantly differ between control and COS group fed with 0.025% COS. According to previous reported results of other authors, our finding is an expected situation. Moreover, similar findings were found by other researchers studies [22,34]. In a study 0, 10, 30, 50, 100, 150 and 200 mg/kg/day doses of chitosan were injected to mongrel dogs subcutaneously for 1 month, and found the raised creatine kinase level (notably at 200 mg/kg/day) [21]. Similarly, in the present study, no significant difference was determined between control and COS groups in serum CK levels on days 21 and 42. In the same study it was reported that they could not found the raised CK level in low doses [21]. Low dose of COS was also used in the present study.

It has been reported that addition of βglucan has improved growth performance in nursery pigs [25] and improved immunity, nutrient retention and growth performance in broilers [4]. In a study 20 mg/kg or 40 mg/kg glucanic extract were added to the diet of broiler chickens, and found 0.65 mg/dL or 0.64 mg/dL serum creatinine levels, respectively and control group's creatinine level was 0.87 mg/dL [27]. They also reported that serum creatinine levels were within the physiological limits in all groups and the differences were insignificant. Similarly in a study of fishes fed diet containing 0.1% β-glucan, and found that β-glucan-treated group (0.58 mg/dL) was insignificantly differed from control group (0.61 mg/dL) for serum creatinine levels [9]. Also in another study of rats fed rats with 65 mg β-glucan + 100 mg Zn bisglycinate/kg body weight/day [8]. Researchers found 0.30 mg/dL serum creatinine level in control group and 0.28 mg/dL in experimental group. The levels were insignificantly difference between two groups. In the present study, serum creatinine levels were 0.31 and 0.22 mg/dL in control group, 0.28 and 0.33 mg/dL in β-glucan group

and 0.39 and 0.30 mg/dL in Zn+β-glucan group on days 21 and 42, respectively. The differences between groups were insignificant. These findings were similar with the previous reported results of other authors [8,9,27].

108 IU/L serum CK activity was found in rats fed with 65 mg β-glucan + 100 mg Zn bisglycinate/kg body weight/day and 110 IU/L in control group [8]. The difference among two groups was insignificant. In the present study, serum CK activities were 1522 and 1148 IU/L in the control group, 1026 and 1824 IU/L in Zn+β-glucan group on days 21 and 42, respectively. The differences between control and Zn+β-glucan groups were insignificant on days 21 and 42. The results of the present study is in agreement with previous reported results of another study [8]. β-glucan ingestion has been shown to improve the lipids in humans and in experimental animals [23]. Serum phospholipid concentrations were insignificantly different between control and β-glucan groups on days 21 and 42 in the present study. However, Zn+β-glucan group had higher serum phospholipid concentrations than control and β-glucan groups on day 21. It was reported that β-glucans help the fermentation of lipids by intestinal bacteria to short-chain fatty acids which are easily absorbed [8]. Whereas, dietary β-glucan did not effectively change serum phospholipid levels in the present study.

ɣ-GT, is a membrane-bound enzyme that initiates the degradation of extracellular glutathione (GSH) forming ɣ-glutamyl amino acid and cysteinylglycine. Cysteinylglycine is then degraded by dipeptidases into cysteine and glycine, both of which can be taken up by cells and used for de novo GSH synthesis [8]. The utilization of extracellular cysteine has been shown to be dependent on the ɣ-GT activity in human endothelial cells [5]. Significant difference was not found in the serum ɣ-GT activity between control group and rats fed with β-glucan plus Zn bisglycinate [8]. Similarly serum ɣ-GT activity was insignificantly different between control and Zn+β-glucan groups on days 21 and 42 in the present study.

It was reported that data obtained from animal studies suggests the inhibition of de novo lipogenesis as the primary mode of the effects of fructans [12]. Their study supports findings in animals that fructans can affect the formation and degradation of triglyceride rich lipoprotein particles. Whereas, in the present study, serum phospholipid levels were insignificantly different between control and inulin groups on day 42, although they were significantly higher in inulin group than control group on day 21. It was mentioned that inulin may play a role in modulation of hepatic enzymes, intestinal characteristics and blood metabolites [33]. They also reported that studies should be continued to determine the most effective feeding period, dose and diet according to animal species. It was suggested that at higher levels than 80 g/kg diet inulin may play a role in the modulation of blood metabolites [6]. A lower dose (1%) than 80 g/kg diet of inulin was used in the present study, and similarly to the previous studies serum ɣ-GT and CK activities and creatinine concentration were not found significantly different between control and inulin groups on day 42. Although this, serum CK activity and creatinine concentration were significantly lower in inulin group than control group in day 21. Hence, it was seemed that dietary inulin affected some blood parameters as short-term.

Inulin-type fructans improve mineral content of bone by stimulating mineral absorption. This is mediated by the microbial production of short-chain fatty acids in the large intestine (mainly in the cecum) which reduce the luminal pH and, as a result, increase the soluble fraction of minerals. Moreover, the increase of the cecum weight and villus height shows an increase in absorbent surface mediated mainly by butyrate [19]. This improved mineral transport is also provided with a marked osmotic action as discussed by another study [3]. Oligofructose particularly increases the solubility of minerals. The mediating mechanism of inulin-type fructans to this effect is suggested that include direct effects such as an increase in soluble minerals, growth of the absorbent surface, and acidification of intestinal lumen by short-chain fatty acids. Indirect effects such as the improving of intestinal health, the stabilization of intestinal flora and the stimulation of immune defense are also discussed [26]. Contrarily to findings of these researchers, dietary inulin insignificantly affected serum K and Na levels in the present study. These levels were insignificantly different between control

and inulin groups on day 42. Although serum Na level was higher in inulin group than control group on day 21 ($P < 0.05$), serum K level was insignificantly different between control and inulin groups on day 21.

In conclusion, all blood parameters remained within their normal ranges. The addition of Zn plus in to diets caused increase in CK and creatinine, significantly. Additional researches are needed to elucidate the negative effects and the action mechanisms of Zn plus inulin on muscle tissue in broilers. This study may also show the potential protective effect of COS on liver enzymes, and COS may play a role in modulation of liver enzymes.

MANUFACTURERS

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