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Impact of chitosan oligosaccharide and valine supplementation on blood biochemical profile of broilers

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Abstract

It was hypothesized that dietary supplementation of chitosan oligosaccharide (COS) and valine will ameliorate the biochemical profile of Ross 708 broilers between days 21 and 42 after hatching. To investigate this hypothesis, 480 male broilers were randomly placed in eight treatment groups with two dietary levels of COS (C1: 100 mg/kg and C2: 150 mg/kg) and four dietary levels of valine (V1: 0.57%, V2: 0.72%, V3: 0.87% and V4: 1.02 %) with three replicates having 20 birds in each. The blood samples for serological and haematological profiles were collected and analysed at the age of 42 days to evaluate the effects of supplementation. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased with the lowest response values when birds were supplemented at V3 at both C1 and C2. Serum triglycerides (TG) and cholesterol (CHO) levels reduced linearly with increasing supplemental levels of valine at both regimen of COS with the lowest values of 61.07 ± 1.12 and 125.33 ± 1.40 for diet supplemented at V4 and C2, respectively. Serum uric acid (UA) and total proteins (TP) increased linearly with increasing supplemented levels of valine at both C1 and C2. There was a linear increase in total leucocyte count (TLC) and red blood cell (RBC) count for increasing supplemental levels of valine at both C1 and C2. An interactive effect of COS and valine was observed for the changes in ALT, albumin (ALB), TLC and haemoglobin (Hb). In conclusion, COS and valine supplementation significantly affected the liver, renal, lipid and haematological profiles of broilers.

Key words: alanine aminotransferase, aspartate aminotransferase, cholesterol, triglycerides
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Introduction

Antibiotic growth promoters (AGPs) are routinely used in poultry practices to improve the growth performance but have produced many undesirable effects, including antibiotic resistance, toxicity and environmental hazards (Nazir *et al.*, 2020; Sugiharto *et al.*, 2020). There is a need to look for feed formulation that improve the utilization of dietary energy and use nutraceuticals as antibiotic alternatives to accomplish future goals regarding food demands. For this purpose, the inclusion of prebiotics and essential amino acids as alternatives to AGPs is recommended because of their benefits in improving the growth and health of poultry (Qu *et al.*, 2019).

Branched chain amino acids which include leucine, isoleucine and valine, are among nine essential amino acids that are fundamental dietary requirements for growth and metabolism of all organisms. They are not degraded by liver enzymes, and can readily diffuse into body tissues (Miranda *et al.*, 2015; Lee *et al.,* 2020). Dietary deficiency of valine causes the formation of lipid droplets in the liver, hypo albuminemia, leukopenia, reduced food intake, and weight loss. Such dietary deficiencies can also reduce meat yield and quality in poultry (Duarte *et al.*, 2014; Ferreira *et al.*, 2015; Ospina-Rojas *et al.*, 2020). Under clinical settings a fatty liver may be prevented by delivering a small amount of valine directly to the portal vein of the liver (Nishihira *et al.,* 1995). The minimum crude protein content (CP) of broiler diets varies and is dependent on

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the fourth limiting amino acid, namely valine (Kidd & Hackenhaar, 2005; Corzo *et al.*, 2010; Ospina-Rojas *et al.*, 2014; Mohammed, 2019).

Chitosan oligosaccharide is a safe nontoxic degraded product of chitosan produced by enzymatic deacetylation and depolymerization. It possesses biocompatible and biodegradable characteristics (Bednarczyk *et al.*, 2016). Being a prebiotic, COS could promote growth, improve daily weight gain, enhance apparent digestibility (Abdel-Hafeez *et al.*, 2017). However, Xiong *et al.* (2015) observed no effects of COS on promoting growth performance. Further, Arslan & Tufan, (2020) also observed no effect of COS supplementation of broiler diets on growth performance, carcass traits and serum parameters. Among other desirable biological properties, COS is known for its anti-inflammatory, antihypertensive, antioxidant, antimicrobial, antidiabetic and hypocholesterolemic effects (Naveed et *al.*, 2019).

The supplementation of COS and valine together in 2 × 4 factorial design could magnify the benefits of the two supplements and improve the blood biochemical profile, leading to improved growth of broilers. Hence, the current study was planned to evaluate the influence of COS and valine on blood biochemistry and haematological parameters, ultimately promoting the performance of broilers.

Materials and Methods

The current study was part of an earlier research trial (Ahmed *et al.*, 2021), which was approved by the Advanced Studies and Research Board of University of the Punjab, Lahore, Pakistan. The study was designed to assess the effects of various levels of COS and valine on performance, serological characteristics, and haematological indices of broilers. To explore the study objectives, Ross 708 male broilers (n=480) were randomly placed in eight treatments for two dietary concentrations of COS (C1: 100 mg/kg and C2: 150 mg/kg) and four supplemental levels of valine (V1: 0.57, V2: 0.72, V3: 0.87, V4: 1.02%) in a factorial design with three replicates (n=24) per treatment, each comprising 20 birds (n_i=60; i=1, 2, 3,…, 8). A basal diet was prepared by Ahsan Feeds Industries (Pvt.), Gujranwala, Pakistan, containing all the recommended ingredients (Rostagno *et al*., 2011). All birds were offered with same basal diet up to 20 days. The eight treatments were prepared by adding various levels of COS and valine to the basal diet at the expense of inert filler (sand). These treatments were then offered to birds from day 21 to 42 (Table 1).

Table 1 Ingredients and nutrient composition of the basal experimental diet for broilers

Ingredients	%	Nutrient	%, except as noted
Corn	67.30	Metabolizable energy (kcal/kg)	3110
Soya bean meal (44% CP)	14.30	Crude protein	20.18
Palm oil	6.60	Available phosphorus	0.45
Fish meal (71.7% CP)	3.00	Calcium	0.83
Molasses	3.00	Potassium	0.78
DCP	1.55	Sodium	0.18
Limestone	1.30	Chloride	0.25
NaCl	0.50	Crude fibre	4.40
Vitamin mineral premix[1]	0.20	Linoleic acid	1.00
l-lysine HCl	0.70	Lysine	1.22
DL methionine	0.20	Methionine + cysteine	0.90
Threonine	0.15	Ileal digestible methionine	0.50
Filler (sand)	1.20	Ileal digestible cysteine	0.58
		Ileal digestible tryptophan	0.15
		Ileal digestible arginine	0.95
		Ileal digestible threonine	0.69
		Ileal digestible leucine	1.12
		Ileal digestible valine	0.57
		Ileal digestible isoleucine	0.75
		Ileal digestible phenylalanine	0.80

[1]Vitamin A: 12000 IU; vitamin D3: 1500 IU; vitamin E: 30 mg; vitamin K3: 5 mg; vitamin B13 mg; vitamin B2: 6 mg; vitamin B6: 5 mg; vitamin B12: 0.03 mg; nicotinic acid amine: 40 mg; D-Ca-pantothenate: 10 mg; folic acid: 0.075 mg; choline: 370 mg; manganese: 85 mg; iron: 80 mg; copper: 8 mg; iodine: 0.5 mg; cobalt: 0.25 mg; selenium: 0.10 mg

Blood samples were collected on day 45 of the study, and subjected to standard serological and haematological protocols for evaluation. For serology, serum was separated, stored at -20 ^{o}C and analysed for liver enzymes (ALT and AST), lipid profile (serum CHO, TG and HDL levels), renal profile (serum urea, UA and creatinine concentration) and TP and ALB concentration to determine the effects of the dietary supplementations. Serological kits of Roche diagnostics were used for analysis of serological parameters. All the procedures were performed with chemistry analyser COBAS model c111. For haematological analysis, blood samples were collected in anticlotting EDTA coated tubes and subjected to the microhaematocrite method using capillary glass tubes in a haematology analyser (Sysmex Model KX 21, Sysmex Corp., Japan).

The procedures were conducted at the Government Post Graduate Islamia College Gujranwala and the samples were preserved and analysed in Physiology and Endocrinology Laboratory, Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore. The birds were reared in three-tiered cages (with floor space of about 0.5 m^2 for each bird). The cages were fitted with nipple drinkers and trough feeders, and were maintained in a well-ventilated poultry facility under standard poultry production practices. The birds were monitored throughout the research and vaccinated prophylactically.

Data were analysed statistically with an analysis of variance for a 2 x 4 factorial arrangement of treatments using the general linear model procedures of SPSS version 21 (IBM Corp., Armonk, New York, USA). Duncan's multiple range test (Duncan, 1955) was applied to compare treatment means, and differences were considered significant at P <0.05.

Results & Discussion

Table 2 shows the effects of dietary supplementation of varying levels of COS and valine on serum levels of ALT, AST, urea, UA and creatinine of broilers. Supplementation of COS and valine caused a significant reduction in serum ALT levels, with the lowest mean values of 32.23 ± 0.94 U/L and 30.63 ± 0.55 U/L at V3 at both C1 and C2 levels. Interaction between COS and valine was significant for the decrease in serum ALT levels (P <0.05). The supplementation also resulted in reduction in serum AST levels, with lower mean values of 249.70 ± 2.56 U/L and 250.27 ± 5.07 U/L at V3 level of valine at both C1 and C2. However, the decrease was significant for valine (P <0.05) only, and the interaction of COS with valine for the decrease in serum AST levels was not detected. The serum level of urea showed an increase in linear manner with increasing levels of valine when birds were fed at C2, but showed the highest mean value of 36.07 ± 0.38 mg/dl at V3 and C1. The supplementation of both COS and valine caused a significant elevation in serum UA level linearly with increasing levels of valine when birds were fed at both levels of COS. No interaction between COS and valine was observed for increased serum UA. However, supplementation of both COS and valine in the diet had little influence on serum creatinine levels, which could not achieve significance (P >0.05).

The blood biochemical profile is an important parameter to evaluate the animal health conditions, including poultry. The liver utilizes aminotransferases for glycogen synthesis, which is a stored form of glucose, a monosaccharide that is used in cellular respiration in tissues as a source of energy. The amount of glucose in excess of body needs is stored in the form of glycogen, primarily in the liver, and is reserved for future needs. High levels of serum AST and ALT are indicators of damage to the liver (Yin & Tong, 2009). However, in the present study both enzymes decreased (P <0.05), which was a sign of healthy liver for both protocols. Serum levels of AST and ALT are a significant cardio-metabolic risk factor (Skrypnik *et al.,* 2018) for probiotic supplementation on liver function and lipid profile status in rats. The present study revealed a significant reduction in serum AST and ALT concentration with increasing levels of COS and valine, which indicated that the supplementation was potentially hepatoprotective and prevented liver damage.

Regarding the renal profile, serum level urea and uric acid increased in linear (P <0.05) terms as protein and amino acid supplemented diets are expected to increase urea and uric acid level in blood. The major nitrogenous waste produced as a result of protein metabolism in birds is excreted as uric acid, which is the least toxic of all nitrogenous wastes. Birds are uricotelic in that ammonia is converted metabolically to uric acid, resulting in an increase in serum level of uric acid, which could be an important factor in reducing the toxic levels of ammonia (Namroud *et al.*, 2008). Rosebrough *et al.* (1996) and Zhai *et al.* (2016) also discussed increased serum levels of uric acid for dietary crude protein, lysine and methionine supplementation in broilers from 21 to 42 days old. COS and valine supplementation may have increased serum concentrations of uric acid by decreasing possible toxic levels of ammonia, leading to improved broiler performance. The decrease in plasma concentration of creatinine showed that these supplementations in experimental diets might improve the excretory functions of animals. However, plasma creatinine level was not affected significantly because of supplementation.

Table 2 Mean values of serum alanine aminotransferase, aspartate aminotransferase, urea, uric acid and creatinine of broilers with dietary supplementation of various levels of chitosan oligosaccharide and valine at the age of 42 days

Treatments		Response variables				
		Alanine aminotransferase, U/L	Aspartate aminotransferase, U/L	Urea, mg/dl	Uric acid, mg/dl	Creatinine, mg/dl
C1		36.72 ± 1.11^{a}	257.20 ± 2.25	34.75 ± 0.32	5.60 ± 0.05	0.05 ± 0.01
C2		32.65 ± 0.44^{b}	257.86 ± 2.55	35.75 ± 0.24	5.71 ± 0.05	0.07 ± 0.01
	V1	35.80 ± 1.25^{c}	265.32 ± 2.42^{c}	34.60 ± 0.28	5.46 ± 0.04^{a}	0.07 ± 0.01
	V2	33.83 ± 0.64^{b}	255.67 ± 2.98^{ab}	35.20 ± 0.43	5.59 ± 0.05^{a}	0.06 ± 0.02
	V3	31.43 ± 0.60^{a}	249.98 ± 2.54^{a}	36.00 ± 0.26	5.76 ± 0.06^{b}	0.06 ± 0.02
	V4	37.68 ± 1.71^{d}	259.15 ± 2.24^{bc}	35.23 ± 0.61	5.81 ± 0.05^{b}	0.04 ± 0.01
C1	V1	38.33 ± 0.99^{c}	265.33 ±3.44	34.27 ± 0.32	5.41 ± 0.05	0.06 ± 0.02
C1	V2	34.93 ± 0.72^{b}	256.53 ± 4.87	34.50 ± 0.50	5.53 ± 0.07	0.06 ± 0.02
C1	V3	32.23 ± 0.94^{a}	249.70 ± 2.56	36.07 ± 0.38	5.66 ± 0.06	0.05 ± 0.04
C1	V4	41.40 ± 0.87^{d}	257.23 ± 2.27	34.20 ± 0.78	5.82 ± 0.02	0.04 ± 0.01
C2	V1	33.27 ± 0.63^{c}	265.30 ± 4.17	34.93 ± 0.43	5.52 ± 0.06	0.08 ± 0.02
C2	V2	32.73 ± 0.55^{b}	254.80 ± 4.48	35.90 ± 0.44	5.67 ± 0.07	0.07 ± 0.03
C2	V3	30.63 ± 0.55^{a}	250.27 ± 5.07	35.93 ± 0.42	5.86 ± 0.05	0.07 ± 0.02
C2	V4	33.97 ± 0.27^{d}	261.07 ± 3.74	36.27 ± 0.44	5.79 ± 0.10	0.06 ± 0.02

C1: chitosan oligosaccharide supplemented at 100 mg/kg, C2: chitosan oligosaccharide supplemented at 150 mg/kg, V1: valine supplemented at 0.57% of basal diet, V2: valine supplemented at 0.72% of basal diet, V3: valine supplemented at 0.87% of basal diet, V4: valine supplemented at 1.02% of basal diet

^{a,b,c,d} Within an effect on each response variable, values with a common superscript were not different at P =0.05

Table 3 presents the effects of COS and valine supplementation on lipid profile. Supplementation of COS and valine caused a linear decline in serum levels of TG as the level of valine supplementation rose at C1 and C2 with the lowest mean value of 61.07±1.12 mg/dl when birds were offered V4 and C2. The reduction in TG levels was significant for the main effects of COS and valine (P <0.05). However, the interaction between COS and valine for decrease in TG did not attain significance (P >0.05). Similarly, serum levels of CHO reduced linearly with increasing supplemental levels of valine at both regimens of COS, with the lowest mean value of 125.33 ± 1.40 mg/dl when birds were fed V4 (1.02%) and C2 (150 mg/kg). The decrease in serum CHO attained the level of significance for valine (P <0.05), whereas COS had no significant influence on serum levels of CHO. The serum HDL levels presented an increase with rising dietary concentrations of valine, with the highest response value of 76.92 ± 1.70 mg/dl when birds were offered diet supplemented with C1 and V4. The main effect on increased serum level of HDL was significant for varying levels of Val. Serum level of TP elevated in linear manner for dietary levels of valine at C1 and C2. The increase in serum TP concentration was significant for varying levels of COS and Val. The serum levels of ALB rose at C1 and presented a decline at C2 with increased supplementation of valine (Table 3).

The decrease in serum levels of TG and CHO (Tufan & Arslan, 2020) and increase in high density lipoproteins (Corzo *et al*., 2010; Li *et al.,* 2007) showed that supplementation of leucine, valine and COS could improve the lipid profile. Prebiotic and amino acid supplementation probably suppressed hydroxy-methyl-glutaryl-coenzyme A (HMG CoA), an enzyme necessary for pathways involved in cholesterol synthesis, hence decreased CHO synthesis (Fukushima & Nakano, 1995; Zhu *et al.*, 2020). Other studies showed that antibiotic growth promoters increased the total CHO and TR levels significantly (P<0.05) because of the degraded effect of such antibiotics on the absorption of fat in the gastro-intestinal tract, which suggested that AGPs should be replaced with nutritional supplements such as prebiotics, probiotics and amino acids (Li *et al.,* 2007). Another possible mechanism for lowering serum cholesterol levels might be the formation of fibres produced by binding of bile acids in the small intestine, which resulted in increased excretion of faecal bile acids, ultimately decreasing fat and CHO absorption in the gut. Moreover, it decreased the absorption of carbohydrate, lowering serum levels of insulin, and suppressing the stimulation for CHO and lipoprotein synthesis. The lower levels of serum insulin could retard the activity of HMG-CoA, suppressing and slowing the synthesis of CHO.

Table 3 Mean values of serum triglycerides, cholesterol, high density lipoprotein, total protein and albumin of broilers with dietary supplementation of various levels of chitosan oligosaccharide and valine at 42 days old

Treatments		Response variables				
		Triglycerides, mg/dl	Cholesterol, mg/dl	High density lipoprotein, mg/dl	Total protein, g/dl	Albumin, g/dl
C1		68.92 ± 1.64[a]	132.41 ± 2.16	71.57 ± 1.78	3.64 ± 0.07[b]	1.66 ± 0.02[b]
C2		65.51 ± 1.66[b]	129.93 ± 1.58	72.09 ± 1.19	3.86 ± 0.08[a]	1.78 ± 0.02[a]
	V1	73.62 ± 1.71[c]	137.78 ± 2.46[c]	68.68 ± 1.36[a]	3.56 ± 0.06[a]	1.74 ± 0.06
	V2	68.01 ± 1.77[b]	132.92 ± 2.12[bc]	69.09 ± 2.61[a]	3.63 ± 0.06[a]	1.72 ± 0.04
	V3	65.52 ± 2.17[ab]	128.13 ± 1.96[ab]	72.83 ± 1.16[ab]	3.78 ± 0.07[a]	1.71 ± 0.04
	V4	61.71 ± 1.06[a]	125.85 ± 1.23[b]	76.73 ± 1.30[b]	4.04 ± 0.14[b]	1.72 ± 0.03
C1	V1	74.31 ± 2.41	140.63 ± 3.35	68.15 ± 2.84	3.44 ± 0.02	1.62 ± 0.02
C1	V2	70.85 ± 2.40	134.42 ± 4.26	68.17 ± 5.43	3.53 ± 0.09	1.64 ± 0.05
C1	V3	68.18 ± 2.85	128.22 ± 2.25	73.03 ± 1.37	3.69 ± 0.13	1.66 ± 0.05
C1	V4	62.35 ± 1.07	126.37 ± 2.31	76.92 ± 1.70	3.88 ± 0.20	1.74 ± 0.06
C2	V1	72.93 ± 2.89	134.93 ± 3.29	69.20 ± 0.91	3.67 ± 0.08	1.86 ± 0.06
C2	V2	65.17 ± 1.37	131.42 ± 1.43	70.02 ± 1.92	3.72 ± 0.04	1.80 ± 0.03
C2	V3	62.87 ± 2.87	128.03 ± 3.77	72.62 ± 2.20	3.86 ± 0.05	1.76 ± 0.04
C2	V4	61.07 ± 1.12	125.33 ± 1.40	76.53 ± 2.34	4.20 ± 0.19	1.70 ± 0.03

C1: chitosan oligosaccharide supplemented at 100 mg/kg, C2: chitosan oligosaccharide supplemented at 150 mg/kg, V1: valine supplemented at 0.57% of basal diet, V2: valine supplemented at 0.72% of basal diet, V3: valine supplemented at 0.87% of basal diet, V4: valine supplemented at 1.02% of basal diet

[a-c] Within an effect on each response variable, values with a common superscript were not different at P =0.05

The increased inclusion of amino acid and prebiotic had numerically positive effects on total protein and albumin. These phenomena might result because branched chain amino acids could promote synthesis of albumin in primary hepatocytes through the mTOR signal transduction system (Ijichi *et al.,* 2003).

Tables 4 and 5 present the effect of COS and valine supplementation on haematological indices including TLC, RBCs, Hb, packed cell volume, mean corpuscular volume mean corpuscular haemoglobin (MCH), MCH concentration and platelets. Linear upsurge was observed for TLC with increasing levels of valine at both concentrations of COS, with the highest response value of 11.53 ± 0.20 at C2 and V4. The elevation in TLC values was significant for increasing levels of COS and valine (P<0.05). Moreover, an interactive effect of COS and valine was recorded for the increase in TLC (P <0.05). Red blood cell counts showed an increase in linear manner because of the increase in supplemented level of valine at both concentrations of COS, with the highest response value of 3.11 ± 0.06 at V4 and C1. The increase in RBCs was significant for increasing level of valine only (P <0.05). However, the interaction between COS and valine for changes in RBCs (P>0.05) did not achieve significance. A linear increase was seen in Hb with varying levels of valine at C1, but presented the highest mean value of 12.40 ± 0.26 g/dl at V2 and C2. However, the main effects for variation in levels for COS and valine could not approach significance for changes in Hb for either COS or valine (P >0.05). Platelets count increased linearly with supplemental level of valine at C1 (100 mg/kg), but showed the highest mean value of 237.67 ± 2.96 at V3 (0.87%) and C2 (150 mg/kg). The increase in platelets count was significant for increasing levels of both COS and valine (P<0.05). Moreover, there was no interactive effect of COS and valine for platelet count in blood (P >0.05). Other haematological indices, which included packed cell volume, mean corpuscular volume, MC) and MCH, could not achieve the significance level (P >0.05) in linear or interactive terms (Table 5).

Table 4 Mean values of total leucocyte count, red blood cells, haemoglobin and packed cell volume of broilers with dietary supplementation of varying levels of chitosan oligosaccharide and valine at 42 days old

Treatments		Response variables			
		Total leucocyte count, 10^3/µl	Red blood cells, 10^6/µl	Haemoglobin, g/dl	Packed cell volume, %
C1		10.39 ± 0.28^{b}	2.76 ± 0.09	11.69 ± 0.23	38.71 ± 0.92
C2		10.90 ± 0.17^{a}	2.80 ± 0.06	11.82 ± 0.20	37.25 ± 1.03
	V1	9.70 ± 0.21^{a}	2.52 ± 0.08^{a}	11.72 ± 0.35	37.57 ± 1.51
	V2	10.30 ± 0.23^{b}	2.73 ± 0.08^{ab}	11.87 ± 0.35	36.73 ± 0.84
	V3	11.05 ± 0.11^{c}	2.81 ± 0.08^{b}	11.65 ± 0.29	40.07 ± 1.96
	V4	11.53 ± 0.13^{d}	3.05 ± 0.05^{c}	11.78 ± 0.30	37.55 ± 0.88
C1	V1	9.27 ± 0.12^{a}	2.50 ± 0.12	11.07 ± 0.38	38.13 ± 2.08
C1	V2	9.83 ± 0.09^{b}	2.68 ± 0.17	11.33 ± 0.50	35.97 ± 1.30
C1	V3	10.93 ± 0.15^{c}	2.76 ± 0.15	12.00 ± 0.44	42.10 ± 1.66
C1	V4	11.53 ± 0.20^{d}	3.11 ± 0.06	12.37 ± 0.26	38.63 ± 0.47
C2	V1	10.13 ± 0.12^{a}	2.55 ± 0.13	12.37 ± 0.24	37.00 ± 2.62
C2	V2	10.77 ± 0.18^{b}	2.78 ± 0.03	12.40 ± 0.26	37.50 ± 1.13
C2	V3	11.17 ± 0.15^{c}	2.85 ± 0.05	11.30 ± 0.32	38.03 ± 3.49
C2	V4	11.53 ± 0.20^{d}	3.01 ± 0.08	11.20 ± 0.21	36.47 ± 1.57

C1: chitosan oligosaccharide supplemented at 100 mg/kg, C2: chitosan oligosaccharide supplemented at 150 mg/kg, V1: valine supplemented at 0.57% of basal diet, V2: valine supplemented at 0.72% of basal diet, V3: valine supplemented at 0.87% of basal diet, V4: valine supplemented at 1.02% of basal diet

$^{a-d}$ within an effect on each response variable, values with a common superscript were not different at P =0.05

Table 5 Mean values of serum mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelets of broilers (n=24) with dietary supplementation of various levels of chitosan oligosaccharide and valine at 42 days old

Treatments		Response variables			
		Mean corpuscular volume, fL	Mean corpuscular haemoglobin, pg	Mean corpuscular haemoglobin concentration, g/dl	Platelets,10^3/µl
C1		81.43 ± 1.46	42.51 ± 0.56	29.96 ± 0.39	218.08 ± 4.43
C2		82.25 ± 0.81	41.77 ± 0.35	30.01 ± 0.36	226.33 ± 3.09
	V1	80.33 ± 2.56	42.22 ± 0.66	29.53 ± 0.29	207.00 ± 5.67^{a}
	V2	82.03 ± 1.44	41.65 ± 0.46	30.53 ± 0.38	224.83 ± 3.64^{b}
	V3	82.67 ± 1.45	41.88 ± 0.81	30.20 ± 0.70	232.33 ± 3.35^{b}
	V4	82.33 ± 0.99	42.78 ± 0.77	29.67 ± 0.63	224.67 ± 4.01^{b}
C1	V1	77.00 ± 4.04	41.77 ± 1.18	29.47 ± 0.53	196.33 ± 4.67^{a}
C1	V2	81.07 ± 2.66	42.57 ± 0.38	30.77 ± 0.54	221.00 ± 6.11^{b}
C1	V3	85.00 ± 1.73	41.90 ± 1.47	29.43 ± 0.69	227.00 ± 4.36^{b}
C1	V4	82.67 ± 1.86	43.80 ± 1.33	30.17 ± 1.32	228.00 ± 4.93^{b}
C2	V1	83.67 ± 2.33	42.67 ± 0.74	29.60 ± 0.36	217.67 ± 5.04^{a}
C2	V2	83.00 ± 1.53	40.73 ± 0.26	30.30 ± 0.61	228.67 ± 3.76^{b}
C2	V3	80.33 ± 1.45	41.87 ± 1.03	30.97 ± 1.19	237.67 ± 2.96^{b}
C2	V4	82.00 ± 1.15	41.77 ± 0.35	29.17 ± 0.09	221.33 ± 6.69^{b}

C1: chitosan oligosaccharide supplemented at 100 mg/kg, C2: chitosan oligosaccharide supplemented at 150 mg/kg, V1: valine supplemented at 0.57% of basal diet, V2: valine supplemented at 0.72% of basal diet, V3: valine supplemented at 0.87% of basal diet, V4: valine supplemented at 1.02% of basal diet

^{a,b} within an effect on each response variable, values with a common superscript were not different at P =0.05

A linear increase in TLC showed that supplementation could support the immune response of broilers, whereas the linear increase in RBCs and platelets suggested that supplements could influence haematopoiesis and stimulate blood cell formation in bone marrow stem cells (Miao *et al.*, 2020). Lokman *et al.* (2019) and Nuengjamnong and Angkanaporn (2018) found similar effects of chitin and chitosan on crickets and shrimp and on growth, carcass traits, haematological parameters and gut functions of broilers, respectively. The non-significant changes in packed cell volume, mean corpuscular volume, MCH and MCH concentration suggested that supplementation did not exert a negative effect which could affect physiological functions and ultimately retard growth and performance of animals (Gasparyan *et al.*, 2011).

Conclusions

Supplementation of COS and valine in broiler diets had a positive numeric effect on blood biochemical and haematological indices, which might lead to improved health and growth performance of male broilers. Dietary inclusion of COS at C2 and valine at V2 and V4 levels are suggested according to the present findings.

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Author's contributions

IA (ORCID: https://orcid.org/0000-0002-9858-5064) executed the experiment, statistically analysed the collected data and completed the manuscript, NR (ORCID: https://orcid.org/0000-0002-2396-5433) critically analysed, reviewed and supported in final compilation of manuscript, whereas AR (ORCID: https://orcid.org/0000-0003-1737-7843) helped in preparing and drafting the manuscript.

Conflict of Interest declaration

The authors of this work have no financial or other association with persons or organizations that could have any inappropriate influence on this article or bias the contents.

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