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Effect of chitosan oligosaccharide and valine on growth, serum hormone levels and meat quality of broilers

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Abstract

Chitosan oligosaccharide (COS) and valine (Val) supplementation will improve growth performance, carcass traits, hormonal profile and meat quality in broilers. To evaluate this hypothesis, based on a 2×4 factorial arrangement, 480 male broilers (Ross 708) were randomly placed in eight treatment groups for two levels of COS (C1: 100 mg/kg, and C2: 150 mg/kg) and four levels of valine (V1: 0.57%, V2: 0.72%, V3: 0.87% and V4: 1.02%) with three replicates (n=24) with 20 birds in each (n_i=60; *i*=1, 2, 3,…, 8.). The results showed that live bodyweight (BW), weight gain (WG), and carcass weight increased, and feed conversion ratio (FCR) decreased with increased supplemental levels of dietary Val at C1 and C2. Abdominal fat reduced linearly for both COS and Val, with a higher reduction response value at C2 and V4. The serum levels of triiodothyronine (T_3), thyroxine (T_4), testosterone and insulin-like growth factor 1 (IGF-1) presented a linear effect for COS and Val. The proximate composition of breast and leg meat showed that crude fat content decreased linearly for COS and Val with higher reduction response values at V3 for both levels of COS. The ash content rose linearly with increasing concentration of Val at C1, but showed the highest value at V2 when birds were offered C2. There was an interaction between COS and Val for T_3, T_4, IGF-1, ash content and crude fat. In conclusion, supplementation exerted a significant influence on the growth performance, hormonal profile and meat composition of broilers.

Key words: abdominal fat, ash content, carcass, feed conversion ratio
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Introduction

The widespread use of antibiotics as growth promoters had adverse effects on poultry and on antibiotic resistance in microbes. Another harmful aspect of such practices was that traces of antibiotics became part of animal meat and ultimately human food (Qu *et al.*, 2019; Amirdahri *et al*., 2020). To avoid these undesirable consequences, poultry breeders and researchers are working to develop feed formulations that have potential antibiotic alternatives or at least to adopt an integrated approach to minimize toxicity and hazardous effects (Janardhana *et al.*, 2009; Nazir *et al.*, 2020). For this purpose, the inclusion of amino acids and prebiotics in poultry diets has been widely accepted as alternatives to antibiotics because of their growth- and health-promoting effects (Shi *et al.*, 2005).

Chitosan oligosaccharide (COS) is an oligomer that is derived from chitin through de-acetylation and hydrolysis and has several biological properties that make it an attractive replacement for applications in medical and nutrition sciences. Chitosan oligosaccharide is non-toxic, biocompatible, biodegradable, and antifungal (Haque *et al*., 2017). The effect of COS in promoting an immune response has aroused interest, because COS could enhance the migratory activity of macrophages, the cells that play a vital role in the defence mechanisms of host immune responses by promoting the release of cytokines (Qureshi *et al*., 2000; Xiong *et al.*, 2015). Research findings posited that chitosan had a significant influence on the improvement of average daily weight gain, and a decrease in the percentage of dissected abdominal fat, subcutaneous fat, inter-muscular fat depth, and the levels of triglycerides and cholesterol (Liu *et el*., 2008; Swiatkiewicz *et al*., 2015).

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Essential amino acids are fundamental dietary requirements for the growth and metabolism of all organisms. Among the nine essential amino acids there are three branched-chain amino acids (BCAAs), namely leucine, isoleucine and Val. The supplementation of specific amino acids in corn and soybean basal diets for poultry has gained importance among researchers in attempting to provide the least possible amount of crude protein in formulations of broiler feed (Liu *et al.*, 2019; Sterling *et al*., 2003). Various dietary formulations have been employed in poultry practices to attain an ideal balance among amino acids in broiler diets (Aftab, 2009; Ayasan *et al.*, 2009; Ayasan & Okan, 2014). It has also been determined that Val is an important nutritional supplement in broiler diets to encourage growth performance and to induce health benefits (Duarte *et al*., 2014; Ferreira *et al*., 2016).

The use of COS could promote growth by increasing digestibility and enhancing microbial activity in the gut, whereas Val could enhance muscle protein synthesis (Miranda *et al*., 2015). Dietary formulations that contained various levels of crude protein supplemented with COS and Val would increase growth performance (Ospina-Rojas *et al*., 2014; Zhao *et al*., 2015) and carcass yield in broilers, and lead to an improvement in FCR and efficiency of meat production because broilers are a quick, accessible and cheap source of meat (Sedghi *et al*., 2015; Ospina-Rojas *et al.*, 2020).

Based on these arguments, there is a need to consolidate the concept that COS and Val are growth promoters and could be used as alternatives to antibiotic growth promoters. Hence, the present study was contemplated to examine the hypothesis that COS and Val will accelerate growth, and improve the carcass characteristics, hormonal profile and proximate meat composition of broilers.

Materials and Methods

Ethical approval for this research was granted by the Advanced Studies and Research Board/Committee, University of the Punjab, Lahore, on 20 October 2018.

The present study was intended to improve the growth performance and meat quality of commercially important Ross 708 broilers during the period of growth up day 42 post hatch. For this purpose, based on a 2×4 factorial arrangement, a total of 480 broilers (one day old) were randomly placed in eight treatments for two levels of COS (C1: 100 mg/kg and C2: 150 mg kg) and four levels of valine (V1: 0.57%, V2: 0.72%, V3: 0.87% and V4: 1.02%) with three replicates (n = 24) for each treatment ($n_i = 60; I = 1, 2, 3,\ldots, 8$) comprising 20 birds each. The birds were fed a corn-soybean-based diet up to 20 days with all the recommended ingredients (Rostagno *et al*., 2011) and a calculated metabolized energy (ME) value of 3110 kcal/kg (Table 1). The experimental diets were prepared by adding various concentrations of COS and dietary Val at the expense of inert filler (sand).

The experimental birds were kept in three-tiered battery cages (with a floor space of 0.7 m^2 for each bird), equipped with trough feeders and nipple drinkers, in a well-ventilated solid-walled poultry facility under standard poultry practices with lighting schedule of 20 hours light and 4 hours darkness. Throughout the research, the birds were examined physically and vaccinated accordingly. The experimental procedures were conducted at Government Post Graduate Islamia College Gujranwala, whereas the samples were preserved and analysed in the Physiology/Endocrinology Laboratory, Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore. Meat quality parameters were analysed in the Animal Nutrition Research Laboratory at University of Veterinary and Animal Sciences (UVAS), Pattoki Campus.

The BW and feed intake (FI) data were recorded weekly on the 7th, 14th, 21st, 28th, 35th, and 42nd day. Data on carcass weight, abdominal fat, and the relative weights of liver, heart, spleen, gall bladder and gizzard were recorded on day 42. Blood samples to evaluate hormonal parameters were acquired from one bird from each replicate (n = 24) at the age of 42 days. After serum isolation, tests for hormonal profile were performed with serological kits and analysed in COBAS model c111 (Roche Digitals, Basle, Switzerland).

Meat quality parameters for proximate meat composition were analysed with standard protocols and apparatus. Breast and leg samples were acquired from three birds for each treatment group, preserved and analysed chemically according to the official methods of analysis (AOAC, 2006). Dry matter and moisture content in meat samples were determined by drying at 105 ^{o}C to constant weight, followed by desiccation. To estimate crude protein, crude lipid and ash content, Kjeldahl flask apparatus, Soxtherm extraction method and muffle furnace were used.

Statistical analysis of the data was performed by applying two-way factorial ANOVA, using the general linear model procedures of SPSS (IBM Corp., Armonk, New York, USA). For comparative analysis among treatments, Duncan's multiple range test (Duncan, 1955) was used and treatment means were considered different at P <0.05.

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

Ingredients	Composition
Corn	67.30
Soybean meal, 44% crude protein	14.30
Palm oil	6.60
Fish meal, 71.7% crude protein	3.00
Molasses	3.00
Di-calcium phosphate	1.55
Limestone	1.30
NaCl	0.50
Vitamin mineral premix[1]	0.20
l-lysine HCl	0.70
DL methionine	0.20
Threonine	0.15
Filler (sand)	1.20
Nutrients (%)	
Metabolizable energy (kcal/kg)	3110
Crude protein	20.18
Available phosphorus	0.45
Calcium	0.83
Potassium	0.78
Sodium	0.18
Chloride	0.25
Crude fibre	4.40
Linoleic acid	1.00
Lysine &	1.22
Methionine + cysteine	0.90
Standard ileal digestible methionine	0.50
Standard ileal digestible cysteine	058
Standard ileal digestible tryptophan	0.15
Standard ileal digestible arginine	0.95
Standard ileal digestible threonine	0.69
Standard ileal digestible leucine	1.12
Standard ileal digestible valine	0.57
Standard ileal digestible isoleucine	0.75
Standard ileal digestible phenylalanine	0.80

[1]Vitamin A: 12000 IU, vitamin D_3: 1500 IU, vitamin E: 30 mg, vitamin K_3: 5 mg, vitamin B_1: 3 mg, vitamin B_2: 6 mg, vitamin B_6: 5 mg, vitamin B_{12}: 0.03 mg, nicotinic acid amine: 40 mg, Di-calcium 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

Results and Discussion

Supplementation with COS did not produce significant effects on live BW, WG, FI and FCR. However, live BW, WG and FI increased when broiler diets were supplemented with increasing levels of Val (*P* <0.05). The birds were most efficient at V4. The interaction of COS and Val did not approach significance (*P* >0.20) for any of these measures of performance. The results for growth performance attributes are presented in Table 2.

Table 2 Live weight, weight gain, feed intake and feed conversion ratio of broilers at 42 days old when supplemented with various levels of chitosan oligosaccharide and valine

Treatments		Response variables			
		Live bodyweight, g	Weight gain, g	Feed intake, g	Feed conversion
C1		2714.91 ± 25.68	2673.25 ± 25.40	4668.75 ± 19.06	1.75 ± 0.01
C2		2740.00 ± 26.52	2699.08 ± 26.63	4698.70 ± 21.20	1.74 ± 0.01
	V1	2635.50 ± 24.56[a]	2594.83 ± 24.24[a]	4648.33 ± 31.53[a]	1.79 ± 0.01[c]
	V2	2680.50 ± 16.08[a]	2638.83 ± 16.12[a]	4626.66 ± 18.35[a]	1.76 ± 0.00[b]
	V3	2764.66 ± 19.60[b]	2724.33 ± 19.38[b]	4683.33 ± 16.31[ab]	1.72 ± 0.01[a]
	V4	2829.16 ± 19.93[c]	2786.67 ± 21.02[b]	4716.67 ± 24.63[b]	1.70 ± 0.01[a]
C1	V1	2624.33 ± 40.99	2584.33 ± 40.61	4648.33 ± 62.87	1.79 ± 0.01
C1	V2	2659.33 ± 12.20	2618.33 ± 11.68	4626.67 ± 16.41	1.76 ± 0.00
C1	V3	2769.33 ± 29.42	2728.67 ± 29.36	4683.33 ± 06.67	1.71 ± 0.02
C1	V4	2806.67 ± 21.86	2762.00 ± 24.01	4716.67 ± 37.11	1.70 ± 0.00
C2	V1	2646.67 ± 34.80	2605.67 ± 34.23	4628.33 ± 30.32	1.77 ± 0.01
C2	V2	2701.67 ± 26.39	2659.33 ± 27.24	4673.33 ± 29.48	1.75 ± 0.01
C2	V3	2760.00 ± 32.15	2720.00 ± 31.58	4710.00 ± 33.29	1.73 ± 0.01
C2	V4	2851.67 ± 31.67	2811.33 ± 31.99	4783.33 ± 23.33	1.70 ± 0.01

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

Both carcass weight and its percentage increased linearly with increasing concentration of Val (P <0.05). Both abdominal fat and its percentage decreased linearly with the increase in supplemental Val (P <0.05) and decreased with increased COS. Again, the interaction between levels of COS supplementation and Val did not approach significance (P >0.20). The effects of COS and Val on carcass weight and abdominal fat and their relative percentages are presented in Table 3.

The main effects of various levels of COS and Val and their interaction had no significant effects (P >0.05) on the relative weights of liver, heart, spleen, gizzard and gall bladder (Table 4).

Dietary supplementation of COS and Val resulted in significant interactions that affected the levels of T_3, T_4 and IGF-1 (Table 5). The interaction effect on T_3 was due primarily to the marked effect of COS that was seen at V2, whereas the effects of COS at the other levels of Val were much less. For T_4, the interaction was due to an increasing effect at C1 as the level of Val supplementation rose, whereas at C2 the effect of Val supplementation was negligible. The interaction effect on IGF-1 was similar to that of T_4, except that at the high levels of COS and Val a marked depression was observed in the serum level. There was no significant effect of supplementation on thyroid stimulating hormone (TSH) (P >0.05), though it showed a numerical increase with rising levels of supplemental COS and Val. Serum level of testosterone rose linearly (P<0.05) with the increase in COS and more markedly with the rising levels of supplemental Val. However, there was no interaction between COS and Val that affected testosterone level.

Proximate analysis of the breast meat revealed that the meat became significantly drier as the level of Val supplementation increased (Table 6). Supplementation did not affect (P >0.05) crude protein content or the pH of the breast meat. The crude fat and ash contents were affected by the interaction of the levels of COS and Val. When C1 was provided in the diet, the crude fat content of the breast muscle decreased by 0.7% as the level of Val increased. However, it decreased by 1.2% when the broilers were fed C2. Likewise, the ash content of the breast muscle increased by 1.4% as the level of Val supplementation increased at C1, but it only increased by 0.7% at C2.

Table 3 Weights and percentages of carcass and abdominal fat of broilers of broilers at 42 days old when supplemented with various levels of chitosan oligosaccharide and valine

Treatments		Response variables			
		Carcass weight, g	Carcass, %	Abdominal fat, g	Abdominal fat, %
C1		1917.08 ± 36.44	70.55 ± 0.82	40.95 ± 0.98^{a}	1.51 ± 0.05^{a}
C2		1951.66 ± 32.98	71.19 ± 0.76	37.75 ± 1.28^{b}	1.38 ± 0.06^{b}
	V1	1792.50 ± 19.82^{c}	68.01 ± 0.43^{a}	44.18 ± 0.86^{c}	1.68 ± 0.04^{c}
	V2	1891.66 ± 20.88^{b}	70.56 ± 0.61^{ab}	39.90 ± 1.36^{b}	1.49 ± 0.05^{b}
	V3	1984.16 ± 29.90^{a}	71.77 ± 1.07^{b}	38.22 ± 1.12^{b}	1.38 ± 0.04^{b}
	V4	2069.16 ± 25.01^{a}	73.15 ± 1.00^{b}	35.12 ± 1.03^{a}	1.24 ± 0.04^{a}
C1	V1	1768.33 ± 28.92	67.38 ± 0.19	44.73 ± 1.07	1.70 ± 0.03
C1	V2	1866.67 ± 29.62	70.19 ± 1.24	42.13 ± 0.74	1.58 ± 0.02
C1	V3	1980.00 ± 55.07	71.49 ± 1.23	39.97 ± 1.44	1.44 ± 0.06
C1	V4	2053.33 ± 29.48	73.15 ± 1.61	36.97 ± 1.05	1.32 ± 0.04
C2	V1	1816.67 ± 23.33	68.63 ± 0.71	43.63 ± 1.49	1.65 ± 0.08
C2	V2	1916.67 ± 26.03	70.94 ± 0.42	37.67 ± 1.93	1.39 ± 0.07
C2	V3	1988.33 ± 37.67	72.04 ± 2.02	36.47 ± 1.04	1.32 ± 0.05
C2	V4	2085.00 ± 44.81	73.11 ± 1.55	33.27 ± 0.90	1.17 ± 0.03

C1: chitosan oligosaccharide (COS) supplemented at 100 mg/kg, C2: COS supplemented at 150 mg/kg, V1: valine supplemented at 0.57%, V2: valine supplemented at 0.72%, V3: valine supplemented at 0.87%, 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

Table 4 Relative weights of liver, heart, spleen, gizzard, and gall bladder of broilers of broilers at 42 days old when supplemented with various levels of chitosan oligosaccharide and valine

Treatments		Response variables				
		Liver, %	Heart, %	Spleen, %	Gizzard, %	Gall bladder, %
C1		2.22 ± 0.04	0.70 ± 0.01	0.12 ± 0.00	1.20 ± 0.02	0.12 ± 0.00
C2		2.12 ± 0.05	0.69 ± 0.01	0.11 ± 0.00	1.24 ± 0.02	0.12 ± 0.00
	V1	2.27 ± 0.07	0.71 ± 0.02	0.12 ± 0.00	1.26 ± 0.03	0.13 ± 0.01
	V2	2.12 ± 0.03	0.70 ± 0.01	0.12 ± 0.00	1.24 ± 0.03	0.12 ± 0.00
	V3	2.17 ± 0.07	0.68 ± 0.01	0.11 ± 0.00	1.16 ± 0.03	0.12 ± 0.00
	V4	2.12 ± 0.06	0.69 ± 0.01	0.12 ± 0.01	1.24 ± 0.03	0.11 ± 0.01
C1	V1	2.25 ± 0.06	0.70 ± 0.03	0.12 ± 0.00	1.21 ± 0.02	0.11 ± 0.00
C1	V2	2.11 ± 0.06	0.69 ± 0.02	0.12 ± 0.00	1.29 ± 0.03	0.12 ± 0.01
C1	V3	2.29 ± 0.10	0.69 ± 0.01	0.12 ± 0.01	1.13 ± 0.05	0.12 ± 0.01
C1	V4	2.22 ± 0.08	0.71 ± 0.01	0.12 ± 0.01	1.19 ± 0.05	0.12 ± 0.01
C2	V1	2.29 ± 0.15	0.72 ± 0.03	0.12 ± 0.00	1.30 ± 0.05	0.14 ± 0.00
C2	V2	2.13 ± 0.00	0.71 ± 0.02	0.12 ± 0.00	1.20 ± 0.04	0.12 ± 0.00
C2	V3	2.04 ± 0.05	0.67 ± 0.01	0.11 ± 0.01	1.19 ± 0.04	0.12 ± 0.00
C2	V4	2.02 ± 0.04	0.67 ± 0.00	0.11 ± 0.00	1.28 ± 0.00	0.11 ± 0.00

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

Table 5 Serum triiodothyronine, thyroxine, thyroid stimulating hormone, testosterone, and insulin-like growth factor 1 of 42 day-old broilers when supplemented with various levels of chitosan oligosaccharide and valine

Treatments		Response variables				
		T_3, ng/ml	T_4, µg/dl	TSH, µIU/ml	Testosterone, ng/ml	IGF-1, ng/ml
C1		5.25 ± 0.23^a	1.74 ± 0.09^b	0.03 ± 0.00	0.80 ± 0.01^b	3.61 ± 0.07^a
C2		4.50 ± 0.16^b	1.93 ± 0.03^a	0.08 ± 0.04	0.84 ± 0.02^a	3.41 ± 0.03^b
	V1	5.38 ± 0.23^c	1.65 ± 0.13^a	0.07 ± 0.02	0.77 ± 0.01^a	3.37 ± 0.03^a
	V2	5.15 ± 0.44^{bc}	1.73 ± 0.10^a	0.03 ± 0.01	0.79 ± 0.01^a	3.47 ± 0.03^b
	V3	4.87 ± 0.09^b	1.95 ± 0.05^b	0.02 ± 0.00	0.84 ± 0.02^b	3.61 ± 0.05^c
	V4	4.10 ± 0.16^a	2.00 ± 0.07^b	0.11 ± 0.07	0.88 ± 0.02^c	3.59 ± 0.15^c
C1	V1	5.75 ± 0.33^a	1.40 ± 0.12^a	0.04 ± 0.00	0.77 ± 0.01	3.31 ± 0.04^a
C1	V2	6.10 ± 0.06^a	1.53 ± 0.03^a	0.04 ± 0.01	0.78 ± 0.02	3.50 ± 0.02^b
C1	V3	4.80 ± 0.17^b	1.93 ± 0.09^b	0.02 ± 0.00	0.81 ± 0.02	3.71 ± 0.03^c
C1	V4	4.33 ± 0.22^c	2.10 ± 0.12^b	0.03 ± 0.01	0.84 ± 0.02	3.93 ± 0.04^c
C2	V1	5.00 ± 0.06^b	1.90 ± 0.06^b	0.10 ± 0.02	0.76 ± 0.01	3.43 ± 0.02^b
C2	V2	4.20 ± 0.23^c	1.93 ± 0.09^b	0.03 ± 0.01	0.81 ± 0.02	3.44 ± 0.05^b
C2	V3	4.93 ± 0.09^b	1.97 ± 0.07^b	0.02 ± 0.01	0.86 ± 0.02	3.50 ± 0.05^c
C2	V4	3.87 ± 0.18^c	1.90 ± 0.06^b	0.18 ± 0.14	0.92 ± 0.02	3.26 ± 0.07^a

C1: chitosan oligosaccharide (COS) supplemented at 100 mg/kg, C2: COS supplemented at 150 mg/kg, V1: valine supplemented at 0.57%, V2: valine supplemented at 0.72%, V3: valine supplemented at 0.87%, V4: valine supplemented at 1.02% of basal diet, T_3: triiodothyronine, T_4: thyroxine, TSH: thyroid stimulating hormone, IGF-1: insulin-like growth factor 1
[a-c] Within an effect on each response variable, values with a common superscript were not different at *P* =0.05

Table 6 Dry matter, crude protein content, crude fat, ash content, pH, and moisture content of breast meat of broilers of 42-day old broilers when supplemented with various levels of chitosan oligosaccharide and valine

Treatments		Response variables					
		Dry matter, %	Crude protein, %	Crude fat, %	Ash, %	pH	Moisture, %
C1		28.61 ± 0.27	84.21 ± 0.55	7.48 ± 0.09^a	4.60 ± 0.16^b	5.84 ± 0.04	71.39 ± 0.28
C2		28.72 ± 0.21	84.70 ± 0.38	7.20 ± 0.18^b	5.11 ± 0.09^a	5.83 ± 0.04	71.28 ± 0.34
	V1	27.97 ± 0.24^a	83.38 ± 0.86	7.93 ± 0.10^c	4.34 ± 0.19^a	5.93 ± 0.04	72.04 ± 0.22^a
	V2	28.05 ± 0.21^a	84.16 ± 0.51	7.45 ± 0.15^b	4.77 ± 0.13^b	5.87 ± 0.03	71.96 ± 0.24^a
	V3	29.10 ± 0.17^b	84.48 ± 0.51	7.00 ± 0.18^a	4.95 ± 0.16^b	5.80 ± 0.03	70.90 ± 0.40^b
	V4	29.56 ± 0.13^b	85.80 ± 0.39	6.98 ± 0.09^a	5.37 ± 0.06^c	5.74 ± 0.07	70.44 ± 0.36^b
C1	V1	27.78 ± 0.48	82.72 ± 1.63	7.80 ± 0.12^c	3.95 ± 0.12^a	5.92 ± 0.04	72.22 ± 0.28
C1	V2	27.97 ± 0.44	83.83 ± 0.59	7.65 ± 0.12^b	4.50 ± 0.06^b	5.89 ± 0.02	72.03 ± 0.08
C1	V3	29.28 ± 0.22	84.04 ± 0.05	7.35 ± 0.06^a	4.60 ± 0.07^b	5.81 ± 0.03	70.72 ± 0.10
C1	V4	29.42 ± 0.18	86.24 ± 0.66	7.10 ± 0.09^a	5.35 ± 0.12^c	5.72 ± 0.13	70.58 ± 0.39
C2	V1	28.15 ± 0.17	84.04 ± 0.77	8.05 ± 0.14^c	4.72 ± 0.10^a	5.94 ± 0.09	71.85 ± 0.38
C2	V2	28.12 ± 0.13	84.48 ± 0.91	7.25 ± 0.25^b	5.03 ± 0.07^b	5.85 ± 0.06	71.88 ± 0.53
C2	V3	28.92 ± 0.24	84.92 ± 1.05	6.65 ± 0.17^a	5.30 ± 0.09^b	5.79 ± 0.06	71.08 ± 0.88
C2	V4	29.70 ± 0.17	85.36 ± 0.35	6.85 ± 0.14^a	5.39 ± 0.08^c	5.75 ± 0.06	70.30 ± 0.69

C1: chitosan oligosaccharide (COS) supplemented at 100 mg/kg, C2: COS supplemented at 150 mg/kg, V1: valine supplemented at 0.57%, V2: valine supplemented at 0.72%, V3: valine supplemented at 0.87%, 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 interaction between the supplement levels was significant for percentage ash and pH (P <0.05). The differences among the treatments for pH were of little practical consequence. However, percentage ash increased with the level of supplemental Val at C1 and decreased with augmented levels of supplemental Val at C2. The contents of dry matter, crude protein, crude fat and moisture were all affected by the level of Val supplementation (P <0.05), but only crude fat was affected (P <0.01) by the level of COS in the diet. Increasing the level of dietary Val produced drier meat from the leg of the broilers. The leg meat also contained more protein and less fat. The crude fat content of the leg meat was reduced by increasing the level of COS supplementation from C1 to C2. Proximate composition of leg meat as presented in Table 7.

Table 7 Dry matter, crude protein content, crude fat, ash content, pH, and moisture content of leg meat of broilers of broilers at 42 days old when supplemented with various levels of chitosan oligosaccharide and valine

Treatments		Response variables					
		Dry matter, %	Crude protein, %	Crude fat, %	Ash, %	pH	Moisture, %
C1		28.32 ± 0.52	71.44 ± 0.57	15.14 ± 0.16^{a}	4.15 ± 0.10^{b}	5.71 ± 0.03	71.68 ± 0.48
C2		28.77 ± 0.43	71.94 ± 0.45	14.66 ± 0.18^{b}	4.37 ± 0.12^{a}	5.73 ± 0.03	71.23 ± 0.44
	V1	26.70 ± 0.40^{a}	70.62 ± 0.61^{a}	15.70 ± 0.15^{b}	4.29 ± 0.19^{bc}	5.83 ± 0.02^{c}	73.31 ± 0.13^{c}
	V2	27.82 ± 0.37^{a}	71.04 ± 0.41^{ab}	14.78 ± 0.20^{a}	4.36 ± 0.21^{c}	5.74 ± 0.02^{b}	72.18 ± 0.44^{b}
	V3	29.42 ± 0.31^{b}	72.38 ± 0.47^{bc}	14.68 ± 0.22^{a}	4.16 ± 0.03^{a}	5.70 ± 0.01^{b}	70.58 ± 0.34^{a}
	V4	30.24 ± 0.38^{b}	72.71 ± 0.41^{c}	14.45 ± 0.07^{a}	4.25 ± 0.18^{b}	5.60 ± 0.03^{a}	69.76 ± 0.22^{a}
C1	V1	26.44 ± 0.54	70.18 ± 0.62	15.80 ± 0.68	3.87 ± 0.01^{bc}	5.80 ± 0.03^{c}	73.56 ± 0.15
C1	V2	27.28 ± 0.62	70.80 ± 0.61	15.15 ± 0.29	3.88 ± 0.01^{c}	5.78 ± 0.01^{b}	72.72 ± 0.22
C1	V3	29.51 ± 0.64	72.16 ± 0.38	15.05 ± 0.17	4.20 ± 0.06^{a}	5.70 ± 0.03^{b}	70.49 ± 0.58
C1	V4	30.04 ± 0.66	72.60 ± 0.61	14.55 ± 0.20	4.64 ± 0.03^{b}	5.55 ± 0.03^{a}	69.96 ± 0.31
C2	V1	26.95 ± 0.66	71.06 ± 1.13	15.60 ± 0.12	4.70 ± 0.03^{bc}	5.85 ± 0.03^{c}	73.05 ± 0.06
C2	V2	28.36 ± 0.09	71.28 ± 0.65	14.40 ± 0.12	4.83 ± 0.05^{c}	5.70 ± 0.03^{b}	71.64 ± 0.80
C2	V3	29.33 ± 0.25	72.60 ± 0.95	14.30 ± 0.17	4.11 ± 0.02^{a}	5.70 ± 0.01^{b}	70.67 ± 0.48
C2	V4	30.44 ± 0.51	72.82 ± 0.62	14.35 ± 0.23	3.85 ± 0.03^{b}	5.65 ± 0.03^{a}	69.56 ± 0.31
Source of variation		*P*-value					
COS		0.250	0.347	0.002	<0.0001	0.365	0.157
Val		<0.0001	0.031	<0.0001	<0.0001	<0.0001	<0.0001
COS*Val		0.712	0.975	0.251	<0.0001	0.023	0.555

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

The increase in live BW and WG in the groups confirmed COS as a prebiotic and that Val had a growth-promoting effect on broilers by improving muscle protein synthesis and nutrient digestibility and by influencing the activity and composition of gut microbiota. An increase in FI showed that supplementation of COS and Val could improve nutrient digestibility and consequently feed efficiency and growth performance. A reduction in FCR made it evident that C1 and C2 could lower FCR and improve growth performance in broilers and in other poultry. Corzo *et al*. (2010); Lee *et al*. (2014), and Bednarczyk *et al*. (2016) also found growth-promoting influence with *Bacillus subtilis*, prebiotic, isoleucine, and Val supplementation in broilers.

Dietary supplementations of COS and Val resulted in a linear increase in carcass weight and carcass percentage, which was expected from the rise in live BW and WG. A linear reduction in abdominal fat with increasing levels of COS and Val revealed that supplementation is hypolipidemic and suppresses the fat deposition in adipocytes. It was suggested that the decrease in abdominal fat was because of lowered synthesis of mRNA (Foretz *et al.*, 1999; Amirdahri *et al.*, 2020) for rate-limiting enzymes in hepatic tissues, decreasing the fatty acid synthesis. Alternatively, supplementation possibly increased the level of mRNA for rate-limiting enzymes in hepatic tissues, increasing fatty acid oxidation, which is mediated through the regulation of the transcription of the sterol regulatory element binding protein-1c (SREBP-1C). However, no effect was observed on relative weights of liver, heart, spleen, gizzard and gall bladder (Abeer & Mosaad, 2015; Abdel-Hafeez *et al.*, 2017).

The increase in thyroid hormones (T_3 and T_4) may have been due to greater release of hypothalamic TRH, which stimulated the secretion of TSH from adenohypophysis, eventually promoting T_3 and T_4 synthesis and release. The linear and interactive effects in the levels of testosterone and IGF-1 indicated that the supplementation of amino acids had a growth-promoting effect on broilers, which may have implications for other poultry. Increased levels of T_3, T_4, testosterone, and IGF-1 influence the metabolism and may ultimately affect the performance, carcass and abdominal fat percentages. Buyse *et al*. (1992), Zhai *et al*. (2016) and Ibitoye *et al*. (2019) described similar findings resulting from supplementation of lysine at 100% to 120% of recommended levels and chitosan at 0.5 g/kg in broiler diets.

The proximate composition of breast and leg meat samples exhibited a non-significant increase in dry matter and crude protein because both amino acid and prebiotic supplementation could increase muscle weight and consequently the dry matter in the of broilers. It is generally recommended that healthy broiler meat should contain a higher proportion of protein, a lower content of fat, and adequate minerals. However, the decrease in crude fat content revealed that supplements can lessen the crude fat content in meat of broilers by inhibiting the deposition of fats (Valsta *et al*., 2005; Abdurrahman *et al*., 2016). Murakami *et al.* (2010) and Ferreira *et al*. (2015) also conducted studies and reported decreased crude fat content in meat composition of broilers supplemented with linseed oil and Val.

Moreover, the increment in ash content, a parameter to estimate the mineral amount of a foodstuff, was evident from greater crude protein content in the samples. However, the decreased pH and moisture content in breast and leg meat samples with the enhanced concentration of supplements was expected as a result of greater content of dry matter. Erwan *et al.* (2009) studied the composition of broiler meat and its sensory characteristics supplemented with varying levels of leucine and reported similar changes in pH, which affected its moisture content and water-holding capacity of meat and physical qualitative traits, including tenderness, colour and moisture absorption (Husak *et al*., 2008).

Conclusions

Dietary inclusion of C2 and V3 to V4 improved growth performance, carcass characteristics, and hormonal profiles. These findings suggest that a dietary formulation for farmers that contained these supplements would improve the meat quantitatively and qualitatively.

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Authors' Contributions

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

Conflict of Interest Declaration

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

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