German Journal of Veterinary Research
eISSN:2703-1322

Research article

Influence of chitosan dietary supplement on growth performance, blood indices, and characteristics of meat quality in chickens

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Article History:
Received: 09-July-2024
Accepted: 07-Aug-2024
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Abstract

This research aims to assess how chitosan supplementation impacts growth rate, gut morphology, meat quality, and blood parameters in both indigenous and commercial chicken breeds. The 2×2 factorial experimental design involved two breeds of chickens (180-day-old chicks in total from each breed), Local Omani and Cobb430-type broilers, and two experimental dietary treatments. The treatments were (1) the basal diet with no chitosan supplementation (Control treatment) and (2) the basal diet supplemented with chitosan (0.05%). The findings indicated that, in both chicken breeds, feeding chitosan significantly (p<0.001) improved the feed conversion ratio and weight gain compared to the control group. When considering average body gain over a (0-42 days) period, both broiler/Omani chickens supplemented with chitosan gained approximately 11.3% and 26.3% more weight than their counterparts in the control groups, respectively. The jejunum and ileum of Cobb430 broilers and local Omani chickens fed dietary chitosan exhibited higher height of the villi and the ratio of villus height to crypt length compared (p<0.001) to the control. The levels of RBC, WBC, heterophils, lymphocytes, and total protein in Cobb430 broiler and local Omani chickens fed dietary chitosan were significantly higher (p<0.001), showing increases of 18.3%, 54.4%, 18.6%, 12.1%, and 16.9%, respectively, compared to the control groups. Dietary chitosan supplementation significantly influenced the lightness (L*), pH, and cooking loss % in the breast muscles of Cobb430 broilers and local Omani chickens (p<0.001). In conclusion, the supplementation of 0.05% chitosan as a performance enhancer improved the growth production and meat quality parameters in broiler and Omani chicken diets. Further research is recommended to determine the optimal chitosan dosage for local chickens to enhance growth performance.

Keywords: Dietary chitosan, Omani chickens, Growth, Blood, Meat, Intestinal morphology

Citation: Al-Marzooqi, W., El-Zaiat, H. M., El Tahir, Y., Al-Kharousi, K., and Hassan, S. K. 2024 Influence of chitosan dietary supplement on growth performance, blood indices, and characteristics of meat quality in chickens. Ger. J. Vet. Res. 4 (3): 49-60. https://doi.org/10.51585/gjvr.2024.3.0097

Introduction

Poultry farming stands as a vital income source for small and medium-scale farmers in developing nations like Oman. Within rural communities of Oman, small-scale poultry production plays a crucial role in ensuring food security and generating income for numerous rural families. Small-scale farming holds a substantial significance in Oman, representing 30% of the total poultry production (MAF, 2013). To strengthen the country's food security and address the protein demand-supply gap, the Omani government has launched a program dedicated to supporting small-scale poultry production (MAF, 2013).

Feed additives are substances incorporated into animal feed to enhance feed efficiency, promote poultry health, and improve growth performance (Okey, 2023). The integration of alternative and novel feed additives holds significant potential for enhancing animal performance and reducing environmental impact (Vastolo et al., 2024). These additives improve nutrient efficiency, which not only accelerates growth rates but also lessens the environmental footprint of animal production (Vastolo et al.,

2024). Numerous feed additives are regularly utilized to support the health and metabolic balance and improve the performance of intensively raised farm animals. Key additives include organic acids, feed enzymes, probiotics and prebiotics, and herbal extracts (Mantovani et al., 2022). However, adherence to safety thresholds for the use of feed additives is essential to ensure poultry health, support sustainable production practices, and safeguard public health by minimizing risks associated with residues in poultry products (Pandey et al., 2019; Ayalew et al., 2022).

Chitosan, a biocompatible polymer obtained through the deacetylation of chitin from shellfish, has well-documented applications across industries, including agriculture and medicine (Aranaz et al., 2021). A recent review by Kamal et al. (2023) highlighted positive outcomes of dietary chitosan in different farm animals, including improved growth performance observed in growing piglets (Duan et al., 2020), rabbits (Kamal et al., 2023), lambs (Pereira et al., 2020), as well as chicks and quails (El-Ashram et al., 2020; Osho and Adeola, 2020). Nonetheless, the use of chitosan in broiler chickens has drawn attention. Numerous efforts have been undertaken to investigate the impact of adding chitosan to chickens' diets and its effect on their development rate (Lan et al., 2020; Lokman et al., 2019; Zhou et al., 2009).

Numerous studies have investigated the use of chitosan as a supplement in animal feed, with varied outcomes (Swiatkiewicz et al., 2014; Uyanga et al., 2023). Chitosan supplementation in poultry has yielded both consistent and conflicting findings across various studies (Harahap et al., 2024). For instance, Osho and Adeola (2019) reported impaired growth performance at higher levels of 2.5 g/kg chitosan in the diet. In contrast, Fathi et al. (2023) demonstrated that dietary chitosan at 2 and 3 g/kg led to improved body weight gain compared to 1 g/kg feed, suggesting that higher doses may enhance broiler chickens' growth performance more effectively. Conversely, Kobayashi et al. (2002, 2006) concluded that diets containing 50 g/kg chitosan had no significant impact on body weight gain, average feed intake, or feed conversion ratio in broilers. However, conflicting findings persist regarding the optimal dosage and long-term effects of chitosan supplementation (Aranaz et al., 2021). Some studies suggest that higher doses may not always yield proportional benefits and could potentially adversely affect nutrient absorption or digestive processes in poultry (Lan et al., 2023; Uyanga et al., 2023). Additionally, variability in results across different poultry species and environmental conditions complicates the overall interpretation of chitosan's effectiveness (Kamal et al., 2023; Fathi et al., 2023). To the best of our knowledge, however, no comprehensive study has been conducted to explore how chitosan affects the development rate, intestinal structure, meat assessment, and hematological indices of local chickens under Omani conditions. Considering the potential effects of chitosan (Lokman et al., 2019), we hypothesized that chitosan supplementation could enhance the performance and health of local chickens. Thus, our current study aimed to investigate the impact of chitosan supplementation on intestinal morphology, growth performance, meat quality, and blood parameters in indigenous and commercial strains of chickens.

Materials and methods

Ethical statement

Sultan Qaboos University's Animal Research Ethics Board approved the experiment under the ethical code SQU/EC-AUR/2022-2023/7.

Birds

One hundred eighty chicks, aged one day, of each breed Cobb430 broiler and local Omani were purchased from a chicken supplier. After weighing, chicks with lower or higher body weights were excluded from the study. All birds were reared in cages within a temperature-regulated environment (closed house system), starting at 34°C on the first day and gradually decreasing until reaching 22°C. Ad-libitum food and water were provided to the birds. The lighting schedule was 23 Light:1 Dark.

Dietary treatments and experimental design

The 2×2 factorial experimental design Involved 2 breeds (Cobb430-broiler and Local Omani chickens) and two experimental dietary treatments: (1) Control, a basal diet without added chitosan (was ≥75% deacetylated, moisture 10%, and ash ≤2%; source: HIMEDIA Laboratories Pvt. Ltd., Mumbai, India), and (2) Treatment, basal diet with chitosan (0.05%). Six birds/replicate cages per breed of chickens of

similar initial weights were assigned randomly to 15 replicate suspended wire cages (90 chicks/dietary treatment). The feed composition is presented in Table 1.

Table 1: Diet ingredient composition.

Raw ingredients (%)	Starter	Finisher
Corn	52.354	59.697
Wheat flour	2	4
Soybean meal 46.5	40.55	31.21
Soya oil	1.16	1.06
Monocalcium phosphate	0.84	0.66
Limestone	1.13	1.27
Salt	0.21	0.2
Sodium bicarbonate	0.23	0.21
Choline chloride 70%	0.08	0.08
Premix*	1.446	1.613
Calculated analysis		
Metabolizable energy kcal/kg	2980	3090
Crude protein %	22.87	19.12
Ether extract %	3.23	3.28
Crude fiber %	2.6	2.47
Calcium %	0.95	0.9
Available phosphorus %	0.45	0.4

*Vitamin and mineral premix formulated to meet breed-specific recommendations, sourced from the Animal Feeds Product Development Department at Oman Flour Mills Co. SAOG

Growth rate parameters

Birds from each dietary treatment were weighed at days 0, 7, 14, 21, 28, 35, and 42, along with their respective feeds. Weekly recordings were made for all measurements, including weight increase, feed consumption, and feed efficiency. Recordings facilitated the calculation of growth rate parameters across these time intervals.

Intestinal morphology

On day 42 of age, the histological measurements of jejunum and ileum were carried out on two birds per cage from each breed/dietary treatment, following the outlined methodology by Al-Marzooqi et al. (2019). Each intestinal segment (jejunum and ileum) was dissected, and a 3 cm section was extracted from its midpoint. These sections were then preserved in 10% formalin for subsequent morphometric analysis. After formalin fixation, the intestinal wall underwent rinsing with phosphate-buffered saline (PBS), followed by embedding in paraffin wax. Cross-sections of each intestinal segment, 5 µm thick, were prepared in two steps: first, embedding in low-melt paraffin wax; second, staining with hematoxylin and eosin. Following staining, gastrointestinal morphometric parameters were assessed using Image-Pro Plus 6.0 software. Each sample underwent six measurements for each parameter, which were then averaged. The height of the villi was assessed as the distance from the apex of the villus to the lamina propria, while the depth of the crypts was measured from the base of the crypt to the crypt-villus junction. All morphological measurements were conducted at 10 µm intervals using Image-PRO® PLUS 6.0 (Media Cybernetics Inc., Bethesda, MD).

Digestive organ weights

One bird per cage was selected randomly from each breed/dietary treatment and euthanized on day 42. Various measurements were taken, including carcass weight, organ weight, and live bird weight.

Blood collection

One bird per cage was selected randomly from each breed/dietary treatment for blood collection on day 42, following the outlined methodology by Al-Aufi et al. (2024). Briefly, a 23-gauge needle was used to draw blood samples from the wing vein, with approximately 4 mL drawn into a disposable syringe. Feather removal and vein preparation preceded bleeding. Two milliliters of blood were placed in EDTA-treated tubes for complete blood count, while the remaining 2 mL were transferred to tubes without anticoagulant for serum biochemistry. After centrifugation (4000 rpm for 15 minutes), the serum was separated from the plasma and kept for future analysis at -20°C.

Blood indices

The hematological and serum biochemistry parameters were determined following the protocol described by Al-Aufi et al. (2024). Briefly, the count of white blood cells (WBC) and red blood cells (RBC) were estimated manually using a hemocytometer. Packed cell volume (PCV) was determined utilizing the microhematocrit method, while hemoglobin concentration (HB) was measured using the cyanmethemoglobin method. Additionally, cell indices were computed according to the methodology elucidated by Ritchie et al. (1994). Concurrently, serum biochemistry parameters, including creatinine (CRE), total serum protein (TP), urea, and liver enzymes (ALT-alanine aminotransferase, AST-aspartate amino transaminase), were estimated by the Cobas C111 Machin serum chemistry analyzer from Roche Diagnostics, Germany.

Meat quality assessment

One bird per cage was selected randomly from each breed/dietary treatment for the evaluation of meat quality characteristics. Fifteen carcasses per breed/dietary treatment were then individually packaged and marked, then kept in a chiller at 4°C for 24 hours before being frozen at -20°C for further analysis. Specifically, the Major (M) pectoralis muscle from the breast was dissected from each selected carcass. Cooking loss, muscle pH, (WB) Warner Bratzler-shear force, and color values a* (redness), b* (yellowness), and L* (lightness) were ascertained following the outlined methodology by Al-Marzooqi et al. (2019).

Statistical analysis

The Shapiro-Wilk test was used to assess the normal distribution of variables, all of which demonstrated normal distributions. The data were analyzed using the PROC MIXED procedure of SAS 9.4 (SAS, 2002) in a 2×2 factorial arrangement, with breed and additive levels as fixed factors and time of measurement (week) as a random effect (Heinrichs et al., 2021). Treatment effects and interactions were examined using the probability of difference (PDIFF) option of the least square means statement in the PROC MIXED procedure of SAS. Means were separated using a post-hoc Tukey's test at $p<0.05$ (Bordin et al., 2024)

Results

The growth parameters for both Cobb430 broiler and Omani chickens, presented in Table 2: daily gain (g/bird/day), feed intake (g/bird/day), and feed conversion ratio (g feed/g gain), analyzed on a weekly basis and over the entire period (0-42 days). According to our results, both breeds' body weight gain and feed conversion ratio were positively impacted by dietary chitosan supplementation in comparison to the other birds' group ($p<0.001$). Broiler birds in the chitosan-fed group exhibited weight gains exceeding those of their counterparts in the control group by 26.2%, 13.6%, 10.6%, 10.2%, 9.9%, and 10.6% during weeks 1, 2, 3, 4, 5, and 6, correspondingly. In a similar vein, Omani birds that consumed chitosan in their diet experienced weight increases of 21.9%, 35.3%, 30.6%, 29.8%, 22.3%, and 13.6% during weeks 1, 2, 3, 4, 5, and 6, respectively. During the 0-42 days monitoring period, both broiler and Omani birds fed with dietary chitosan experienced around 11.3% and 26.3% higher weight gains, respectively, compared to their counterparts in the control groups.

The intestinal morphology of Cobb430 broilers and Omani chickens fed diets supplemented with chitosan is presented in Table 3. The morphology measurements of the small intestine indicated that the chitosan supplement had a significant impact on the jejunum and ileum, altering the villus height (VH), crypt depth (CD), and villus height to crypt depth ratio (VH/CD) ($p<0.001$). The jejunum and ileum of Cobb430 broilers and local Omani chickens fed dietary chitosan exhibited significantly higher villus height ($p<0.001$). Conversely, Cobb430 broilers and local Omani chickens fed the control diet exhibited significantly higher crypt depths in both intestinal segments than birds fed the dietary chitosan supplement ($p<0.001$). Consequently, the VH/CD showed significant variation ($p<0.001$) among the different dietary groups.

The impact of dietary chitosan supplementation on the weight of internal organs and carcass yield of Cobb430 broiler and Omani chickens is presented in Table 4. The carcass yield and internal organ weights were significantly influenced by both the chicken breed and the supplementation of dietary chitosan ($p<0.001$). The internal organ weights and carcass yield of both broiler and Omani chickens that received dietary chitosan showed remarkably higher values ($p<0.001$). In contrast, when compared to the other group of birds, the Omani birds fed a basal diet without chitosan had a considerably reduced ($p<0.001$) carcass yield and internal organ weight.

The hematological and serum chemistry indices of Cobb430 broiler and Omani chickens fed diets supplemented with chitosan are presented in Table 5. The RBC, WBC, heterophils, lymphocytes, and total protein levels in Cobb430 broilers and local Omani chickens fed dietary chitosan were significantly higher ($p<0.001$) by 18.3%, 54.4%, 18.6%, 12.1%, and 16.9%, respectively, compared to the other bird groups.

Table 2: The impact of dietary chitosan supplementation on the growth performance of Cobb430 broiler and Omani chickens.

	Breed					Significance		
	Cobb broiler		Local breed					
	Chitosan (g/kg)		Chitosan (g/kg)					
Level	**0.0**	**0.5**	**0.0**	**0.5**	**SEM**	**B**	**L**	**B*L**
Week 1								
FI	18.94^{a}	21.07^{a}	11.72^{b}	11.56^{b}	0.588	***	NS	NS
DG	15.17^{b}	19.14^{a}	8.32^{d}	10.14^{c}	0.371	***	***	**
FCR	1.24^{b}	1.11^{c}	1.41^{a}	1.14^{bc}	0.028	***	***	*
Week 2								
FI	49.26^{a}	50.71^{a}	18.21^{b}	19.07^{b}	0.750	***	NS	NS
DG	33.86^{b}	38.47^{a}	11.53^{d}	15.60^{c}	1.073	***	***	NS
FCR	1.46^{ab}	1.37^{bc}	1.58^{a}	1.24^{c}	0.052	NS	***	*
Week 3								
FI	89.18^{b}	94.47 ^{a}	29.27^{c}	32.50^{c}	1.388	***	**	NS
DG	61.57^{b}	68.12^{a}	14.93^{d}	20.88^{c}	1.406	***	***	NS
FCR	1.46^{bc}	1.39^{c}	1.96^{a}	1.57^{b}	0.038	***	***	***
Week 4								
FI	120.14^{a}	123.29^{a}	42.66^{b}	44.84^{b}	3.070	***	NS	NS
DG	74.48^{b}	82.08^{a}	18.46^{d}	26.28^{c}	2.022	***	***	NS
FCR	1.62^{bc}	1.51^{c}	2.32^{a}	1.72^{b}	0.046	***	***	***
Week 5								
FI	157.88^{a}	159.35^{a}	58.81^{b}	64.37^{b}	1.669	***	*	NS
DG	85.05^{b}	93.50^{a}	26.20^{d}	32.05^{c}	1.050	***	***	NS
FCR	1.86^{c}	1.71^{d}	2.25^{a}	2.01^{b}	0.032	***	***	NS
Week 6								
FI	192.14^{a}	192.86^{a}	87.71^{b}	92.42^{b}	1.908	***	NS	NS
DG	94.25^{b}	104.27^{a}	36.81^{d}	41.82^{c}	1.161	***	***	*
FCR	2.04^{c}	1.86^{d}	2.39^{a}	2.22^{b}	0.046	***	***	NS
Overall								
FI	104.59^{a}	106.96^{a}	43.11^{b}	44.13^{b}	0.692	***	*	NS
DG	60.72^{b}	67.60^{a}	19.37^{d}	24.46^{c}	0.607	***	***	NS
FCR	1.73^{c}	1.59^{d}	2.14^{a}	1.81^{b}	0.019	***	***	***

$^{a-d}$ different letters within the same row showed statistically significant differences (p<0.05). FI: Feed Intake, DG: Daily, FCR- Feed Conversion Ratio. NS: Not significant. SEM: standard error of the means. B: breed, L: level. * p<0.05, ** p<0.01, *** p<0.001.

Table 3: The impact of dietary chitosan supplementation on intestinal morphological measurements in Cobb430 Broiler and Omani chickens.

Level	Breed					Significance			
	Cobb broiler		Local breed						
	Chitosan (g/kg)		Chitosan (g/kg)						
	0.0	**0.5**	**0.0**	**0.5**	**SEM**	**B**	**L**	**S**	**B*L*S**
Jejunum									
VH	1086.01^{b}	1165.90^{a}	861.26^{d}	989.46^{c}	14.670	***	***	***	***
CD	130.86^{a}	119.86^{ab}	116.33^{bc}	110.70^{bcd}	2.980	*	**	***	*
VH/CD	8.36^{cd}	9.74^{ab}	7.42d^{e}	8.96^{abc}	0.230	***	***	NS	NS
Ileum									
VH	838.49^{de}	998.02^{c}	777.89^{e}	881.27^{d}	14.670	***	***	***	***
CD	103.56^{cd}	100.45^{d}	108.90^{bcd}	100.96^{d}	2.980	*	**	***	*
VH/CD	8.24^{cd}	9.97^{a}	7.16^{e}	8.74^{bc}	0.230	***	***	NS	NS

$^{a-e}$ different letters within the same row showed statistically significant differences (p<0.05). * p<0.05, ** p<0.01, *** p<0.001. NS: Not significant. SEM--standard error of the means. VH: villus height, CD: crypts depth, B: breed, L: level, S: segment.

Table 4: The impact of dietary chitosan supplementation on the weight of internal organs and carcass yield of Cobb430 broiler and Omani chickens.

Parameters	Breed				Significance			
	Cobb broiler		Local breed					
	Chitosan (g/kg)		Chitosan (g/kg)					
	0.0	0.5	0.0	0.5	SEM	B	L	B*L
Hematological indices								
RBC (×10^{6}rbc/mm^3)	2.19^b	2.59^a	1.96^c	2.11^{bc}	0.052	***	***	*
Hb (g/dL)	10.60	11.77	10.35	11.02	0.440	NS	NS	NS
PCV (%)	26.30	26.70	25.30	25.40	0.837	NS	NS	NS
MCV (fL)	147.89	155.07	139.48	141.74	11.514	NS	NS	NS
MCH (pg)	47.08	49.24	44.21	46.68	4.362	NS	NS	NS
MCHC (g/L)	31.12	31.27	31.57	31.51	0.216	NS	NS	NS
White blood cell profile								
WBC (×10^{3}wbc/mm^3)	35.76^{bc}	55.23^a	24.00^c	38.50^b	3.670	***	***	NS
Heterophils	32.66^b	38.72^a	27.77^c	35.66^{ab}	1.188	***	***	NS
Lymphocytes	58.55^b	65.62^a	45.83^c	52.12^{bc}	1.781	***	**	NS
Monocytes	5.88	5.97	5.68	5.73	0.305	NS	NS	NS
Eosinophils	5.56	5.61	5.20	5.65	0.384	NS	NS	NS
Basophils	0	0	0	0	0			
Serum Chemistry								
Total protein (g/dL^{-1})	4.12^b	4.82^a	2.32^d	3.11^c	0.168	***	***	NS
Urea (g/dL^{-1})	2.28	2.39	2.17	2.30	0.142	NS	NS	NS
Creatinine (g/dL^{-1})	0.436	0.501	0.424	0.465	0.069	NS	NS	NS
ALT (IU/L^{-1})	11.91	12.09	11.23	11.55	1.235	NS	NS	NS
AST (IU/L^{-1})	257.39	274.12	244.80	254.15	19.432	NS	NS	NS

$^{a-d}$ different letters within the same row showed statistically significant differences (p<0.05). * p<0.05, *** p<0.001. NS: Not significant. SEM: standard error of the means. B: breed, L: level. HB: hemoglobin, RBC: red blood cell counts, PCV: packed cell volume, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin, MCV: mean corpuscular volume, ALT: alanine aminotransferase, AST: aspartate amino transaminase.

Table 5: The impact of dietary chitosan supplementation on the hematological and serum chemistry parameters of Cobb430 broiler and Omani chickens.

Level	Breed				Significance			
	Cobb broiler		Local breed					
	Chitosan (g/kg)		Chitosan (g/kg)					
	0.0	0.5	0.0	0.5	SEM	B	L	B*L
Parameters								
Carcass	1724.10^b	2033.50^a	180.50^d	335.30^c	40.489	***	***	NS
Heart	9.58^b	13.04^a	2.05^d	3.87^c	0.457	***	***	NS
Liver	41.69^b	61.71^a	11.49^d	20.93^c	2.457	***	***	*
Proventriculus	9.49^b	14.33^a	2.46^d	5.52^c	0.711	***	***	NS
Gizzard	42.40^b	58.77^a	7.10^d	18.56^c	2.712	***	***	NS
Small intestine	64.45^b	95.41^a	13.01^d	26.30^c	3.466	***	***	NS
Pancreas	4.04^b	5.02^a	1.24^d	2.14^c	0.225	***	***	NS
Cacea	14.79^b	21.54^a	2.25^d	5.82^c	0.8378	***	***	NS

$^{a-d}$ different letters within the same row showed statistically significant differences (p<0.05). * p<0.05, ** p<0.01, *** p<0.001. NS: Not significant. SEM: standard error of the means. B: breed, L: level.

The meat quality characteristics of Cobb430 broiler chickens and local Omani breast (M. pectoralis) chickens fed diets supplemented with chitosan are displayed in Table 6. The meat quality characteristics, including pH, cooking loss percentage, Warner-Bratzler shear values, sarcomere length, and color, showed no significant differences between broiler and local Omani chickens. Supplementing the diet with chitosan significantly affected the pH, cooking loss (%), and Lightness (L*) of the breast muscle in both Cobb430 broiler and local Omani chickens (p<0.001).

When fed dietary chitosan, the pH levels in the breast muscle of Cobb430 broiler and Omani chickens were significantly elevated (p<0.001) in comparison to the untreated group. The cooking loss (%) and Lightness (L*) of the breast muscle in Cobb430 broiler and local Omani chickens fed a standard diet demonstrated a notable increase (p<0.001) compared to those in the group supplemented with dietary chitosan.

Discussion

Feed additives are routinely used to improve animal health and productivity. Numerous investigations have explored how chitosan can effectively be used as a supplement in animal feed (Abdel-Ghani and Salem, 2019; Rajoka et al., 2020; Kamal et al., 2023). However, research into the influence of dietary chitosan on chickens

Table 6: The impact of dietary chitosan supplementation on the meat quality characteristics of Cobb430 broiler and local Omani breast (M. pectoralis) chickens.

Parameters	Breed				Significance			
	Cobb broiler		Local breed					
	Chitosan (g/kg)		Chitosan (g/kg)					
	0.0	0.5	0.0	0.5	SEM	B	L	B*L
PH	5.62[b]	5.88[a]	5.61[b]	5.91[a]	0.062	NS	***	NS
Cooking loss (%)	23.48[a]	20.84[b]	23.63[a]	20.80[b]	0.694	NS	***	NS
WB-shear force value (kg)	1.52	1.53	1.50	1.52	0.031	NS	NS	NS
Sarcomere length (µm)	1.63	1.65	1.59	1.62	0.030	NS	NS	NS
Lightness (L)	54.32[a]	51.67[b]	54.22[a]	51.72[b]	0.648	NS	***	NS
Redness	9.38	9.13	9.24	9.15	0.380	NS	NS	NS
Yellowness	9.15	9.21	9.24	9.02	0.406	NS	NS	NS

[a-b] different letters within the same row showed statistically significant differences (p<0.05).
*** p<0.001. NS: Not significant, SEM: standard error of the means. B: breed, L: level.

exhibiting a slower rate of growth is limited and has mostly focused on fast-growing breeds (Ayman et al., 2022). Our study investigates the impact of dietary chitosan on the growth performance of both a commercial broiler strain (430 types) and Omani chickens under Omani conditions (i.e., with a temperature range from 23.5°C to 34.0°C). The study found that adding 0.05% chitosan to the diets of broiler and Omani birds improved their growth performance and feed conversion efficiency. Birds receiving chitosan gained 11.3% more weight for broilers and 26.3% more for Omani birds compared to control groups over 42 days, with the differences being statistically significant (p<0.001).

The results align with the outcomes shown by Shi et al. (2005), who conducted a study on broilers, revealing that supplementation with chitosan at levels of 0.05% to 0.10% led to enhanced feed efficiency, body weight gain, and better nitrogen retention in comparison to the control group. Li et al. (2007) similarly observed improved growth performance in broilers when their diet was supplemented with chitosan at concentrations of 0.005% or 0.01%, attributing this enhancement to improved nutrient digestibility. Additionally, other studies (Zhang et al., 2008; Suk et al., 2004) corroborated these positive effects of chitosan on feed efficiency and body weight gain, with the most pronounced effects observed when chitosan was added to the chicks' feed from day one of age.

The findings of this study demonstrated that incorporating chitosan into the diet affected the gut morphology in both breeds. There were observable increases in VH and decreases in CD, as well as VH/CD ratio, in the ileum and jejunum of Cobb430 broilers, and local Omani chickens fed dietary chitosan in comparison with the control groups. The increased VH and VH/CD ratio observed in the segments of the intestinal of birds fed the chitosan-supplemented diet are consistent with findings from numerous studies.

Our results are consistent with recent research conducted by Lan et al. (2020), which demonstrated that feeding yellow-feathered broiler chickens a lower level of chitosan supplementation (0.02%) increased villus height in both the jejunum and ileum segments. Furthermore, Li et al. (2019) reported similar findings where dietary supplementation of chitosan oligosaccharides in broiler chickens resulted in increased duodenal VH and VH/CD ratio in both the duodenum and jejunum, along with decreased CD in these segments. Ayman et al. (2022) also reported that varying doses of chitosan oligosaccharides in the diet of broiler chickens enhanced growth performance and improved intestinal histological structures.

The aforementioned studies established that longer intestinal villi, shallower crypt depths, and an increased villus height ratio are associated with enhanced absorption capacity of the villi for various nutrients, which should contribute to better chicken performance (Laudadio et al., 2012; Rysman et al., 2023). Additionally, other studies have reported the beneficial effects of chitosan supplementation on intestinal morphology, villus structure, and ileal digestibility of nutrients in various types of animal farms, including ruminants, monogastric animals, and poultry (Huang et al., 2005; Świątkiewicz et al., 2015; Magalhaes et al., 2019; Osho and Adeola, 2020). However, lower crypt depth values indicate a decreasing metabolic rate of intestinal epithelium turnover (Floc'h and S`eve et al., 2000; Xue et al., 2018), which may be reflected by the lower feed conversion ratio observed in the dietary treatment group of both

breeds of chickens in the current study. Various studies (Van Nevel et al., 2005; Nutautaite et al., 2021) have demonstrated that a reduced turnover rate of the intestinal epithelium reduces maintenance requirements, ultimately promoting higher growth rates in animals and birds. The improved intestinal histomorphometry structures in both broiler and Omani chickens compared to the control group may result from dietary chitosan's positive effects on nutrient digestibility and absorption. Consequently, this has contributed to the effective improvement of the birds' digestive process, leading to enhanced body weight gain and better performance.

Based on the present results, the depth of crypts in the jejunum and ileum of Cobb430 broilers and local Omani chickens fed basal diet without chitosan was noticeably greater (p<0.001) than in the group fed the chitosan-supplemented diet. Additionally, the height of villi and the VH/CD ratio of Cobb430 broilers and local Omani chickens fed the control diet were significantly lower compared to those in other dietary chitosan groups. Intestinal villi that are shorter in length compared to deeper crypts are associated with fewer absorptive cells and more secretory cells. (Prakatur et al., 2019). Furthermore, alterations in the composition of the intestinal mucosal surface could potentially decrease nutrient absorption or elevate the energy needed for intestinal maintenance (Apalowo et al., 2024). A previous study showed that deeper crypts promote increased multiplication of crypt cells and reduce the synthesis and secretion of digestive enzymes (Xu et al., 2014). A larger crypt accelerates tissue turnover, leading to heightened nutrient demand for new tissue resulting in insufficient nutrient absorption (Li et al., 2019). The lower performance observed in Cobb430 broilers and local Omani chickens on the control diet may be attributed to significant differences (p<0.001) in the histological parameters of intestinal segments than the chitosan-fed groups. The results of this study were consistent with the findings of Rysman et al. (2023) and Ringenier et al. (2021), who examined the correlations between histological measurements and broiler performance parameters under field conditions. Both concluded that broiler chickens with poor performance have shorter villi, larger crypt depth, and a lower VH/CD ratio.

In this study, it was noted that the poor growth rate of local chickens is mainly due to their lower feed consumption. These chickens often behave like scavengers, which leads to feed wastage despite efforts to modify feeders to minimize losses. Changes in digestion and feed absorption have been observed to correlate with the development of intestinal absorptive capacity (Montagne et al., 2003). The presence of feed stimulates the growth of villi in young chicks, expanding their surface area and thereby increasing absorptive capacity Ayman et al. (2022). To enhance the growth efficiency of the local Omani chicken breed, it is recommended to incorporate a crossbreeding program, alongside considering the developmental rates of the intestine and histological alterations related to intestinal function.

Measurements of blood parameters are used to assess how birds physiologically respond to their environment, the type of feed they consume, and feeding practices (Esonu et al., 2001). Furthermore, according to Muneer et al. (2021), the quality of the diet has a direct correlation with serum biochemical constituents. Chitosan supplementation has shown a potential to affect hematological parameters in broiler chickens positively. In this study, blood and serum indices were consistently similar across both breeds, remaining within normal ranges and consistent with values reported in the literature for broiler chickens (Campbell et al., 2003). The RBC, WBC, heterophils, lymphocytes, and total protein levels in Cobb430 broilers and local Omani chickens fed dietary chitosan were significantly higher (p<0.001) than those of their counterparts in the control birds. The increases in RBC counts associated with chitosan supplementation can enhance oxygen-carrying capacity and thereby improve the overall physiological functions of the birds (Campbell et al., 2003). Elevated hemoglobin levels were also noted, suggesting improved oxygen delivery to tissues (Campbell et al., 2003), which is essential for the growth and development of chickens. Additionally, the increase in WBC can be attributed to the role of chitosan in enhancing chickens' immunity by increasing the weight of the main immune organs (Osho and Adeola, 2019). Increased WBC counts indicate heightened immune activity, potentially leading to better resistance against pathogens and reduced incidence of infections in the flocks. The findings in this study are consistent with

those reported by Yan et al. (2010), who observed that supplementation with chitosan oligosaccharides led to a linear increase in RBC and WBC counts as well as lymphocyte concentration. These results suggest that chitosan oligosaccharides may positively influence the immune system. Similarly, Zhang et al. (2008) and Zhou et al. (2009) demonstrated that supplementation with chitosan oligosaccharides linearly increased lymphocyte numbers and RBC counts in pigs and broilers, respectively.

Our results showed that chitosan supplements increased the serum total protein of birds from the dietary chitosan groups significantly compared with the control groups. This increase was probably arbitrated through the effects of chitosan on improved the growth of villus height and consequently enhanced small intestine digestibility and nutrient absorption. This could lead to better absorption of amino acids and peptides from the diet, which are the building blocks of proteins. Increased absorption of these nutrients could contribute to higher levels of circulating proteins in the bloodstream. This result suggests that dietary supplementation of chitosan can enhance overall protein synthesis in growing broilers and local Omani chickens. El-Ashram et al. (2020) found that adding chitosan to the diet of Japanese quails at a dose of 0.2 g/kg enhanced total serum protein. Ayman et al. (2022) noted a similar observation, reporting that the higher dose rate of chitosan positively influenced the protein metabolism of broiler chicken's oligosaccharides. This was attributed to the enhanced digestion capacity provided by the chitosan inclusion in the diet, thus increasing the availability of protein to the birds.

According to Sklan (2001) early access to feed led to faster intestinal development after hatching. Our current findings indicate that differences in feed consumption between breeds became noticeable at an early age, regardless of the specific dietary treatments used. The enlargement of the digestive tract and the increase in enzyme activity associated with digestion and metabolism were found to correspond with higher feed intake (Huang et al., 2022) In our study, we observed significant differences in the sizes of internal organs between Cobb430 broilers and local Omani chickens. Specifically, internal organs in Cobb430 broilers were notably larger than those in local Omani chickens (p<0.001), which supports the findings of Khalid et al. (2010), who observed significant variations in the relative weights of intestinal sections and other internal organs across different chicken breeds. In contrast to slow-growing chickens, these observations support the idea that the rapid development of supply organs instantly after hatching is essential for the constant muscle growth in fast-growing broilers (Ravindran et al., 2021).

The findings of the present study show that the dietary addition of chitosan significantly affected the pH, cooking loss (%), and Lightness (L*) in the breast muscle of local Omani and Cobb430 broiler chickens (p<0.001). The pH of the breast muscle in chickens supplemented with dietary chitosan was significantly higher (p<0.001) than in untreated birds, improving water-holding capacity and color. Mir et al. (2017) identified pH as a key quality indicator for poultry meat, affecting water-holding capacity, juiciness, tenderness, and color. Moreover, the cooking loss (%) and Lightness (L*) in the breast muscle of chickens on the control diet were notably elevated (p<0.001) in comparison to the group supplemented with dietary chitosan. Cooking loss serves as an essential indicator of water-holding capacity, reflecting the meat's juiciness. Meat color is a critical quality attribute for both cooked and raw chicken meat, as consumers associate it with freshness, attractiveness, and choice of preference Mir et al. (2017). The present study's results demonstrate higher pH levels in the breast muscle, resulting in enhanced color and water-holding capacity. Decreased lightness values show meat that exhibits a lighter color (Jiang et al., 2014). The improved water-holding capacity may be attributed to the enhanced antioxidant properties of meat and reduced cooking loss (Jiang et al., 2014). Consistent with our findings, Wang et al. (2022) reported a decreased cooking loss in yellow-feathered chickens with chitosan supplementation. Chang et al. (2020) also found that dietary chitosan lowered pH and minimized cooking loss in yellow-feathered chickens exposed to heat stress. These findings indicate that including dietary chitosan enhances the quality characteristics of chicken meat.

While chitosan shows promise in improving growth performance, blood indices, and meat

quality in chickens, more research is needed to understand its mechanisms fully. The effects of chitosan can vary depending on chicken breeds and environmental conditions, such as housing and climate. Findings from one breed or environment may not universally apply. Factors such as sample size and the extent to which findings can be generalized across different chicken breeds or environmental conditions should be considered.

Conclusion

The findings of our research demonstrated that supplementing the diet with chitosan from day 1 to day 42 improved intestinal morphology, growth performance, blood indices, and the quality of chicken breast meat. Therefore, the diet of both breeds can be supplemented with 0.05% chitosan as a performance enhancer. Poultry farmers considering the use of chitosan should contemplate incorporating it into their feed regimen, particularly in situations where enhancing growth performance and improving meat quality are desired. It may be particularly beneficial in environments where pathogen control and nutrient utilization optimization are critical. However, further studies with graded doses of chitosan in the diet for Omani chickens are recommended to identify the optimal level for maximizing growth performance. Future research should focus on expanding the current understanding of chitosan's mechanisms of action in poultry. This includes exploring its effects on gut microbiota composition, immune system dynamics, and nutrient metabolism pathways in different breeds of chickens.

Article Information

Funding. The study received financial support from Project No. 49/2/1 of the Agriculture and Fisheries Development Fund and Project No. (IG/AGR/ANVS/22/01) Sultan Qaboos University Research Fund.

Conflicts of Interest. The authors have no conflicts of interest to disclose.

Authors contributions. W.A.-M., Conceptualization, experiment design, project administration, funding acquisition, figure/table preparation, and original draft writing, H.M.E.: data curation, writing-editing, and review, resources, validation, methodology, investigation. Y.E.T., validation, methodology, investigation, and editing of the draft. K.A.: sample collections, formal analysis, methodology. S.K.H. Experimental diet formulation and methodology. All authors have reviewed and approved the final version of the manuscript for publication.

Acknowledgments. The authors would like to thank the staff at the Poultry Unit at the Agricultural Research Station and the Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University for their valuable technical assistance and support.

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