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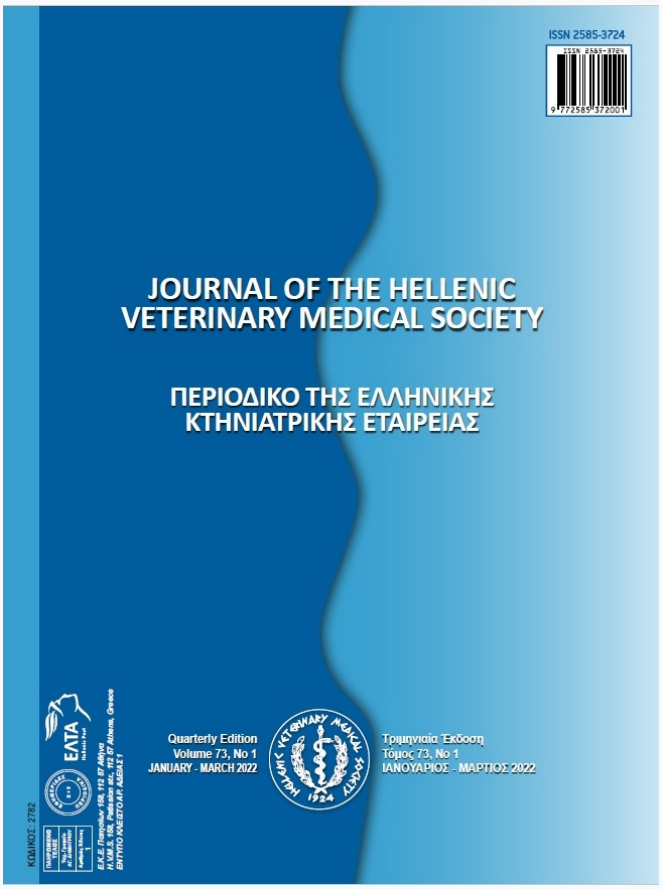

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Research article
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Effects of 25-hydroxycholecalciferol supplementation on breast meat quality and histomorphometric characteristics in broiler chickens

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ABSTRACT: This experiment was carried out to elucidate the influences of 25-hydroxycholecalciferol (25-OH-D_3) status in the diet and the sex of chickens on the sensory and histomorphometric characteristics of the breast muscle (pectoralis major) of broilers. We used a randomized design consisting of a 2×2 factorial arrangement with 25-OH-D_3 (3000 and 5000 IU kg/diet) and two sexes. Breast weight was not affected by either dietary 25-OH-D_3 status or bird sex. Cooking loss was decreased in female chickens but not in male chickens with additional 25-OH-D_3 supplementation. However, increasing 25-OH-D_3 levels in the diet reduced drip loss in male chickens but no change was observed in female chickens. A significant increase in breast meat pH_{24} was observed with increasing dietary levels of 25-OH-D_3; however, the squeezable water ratio and lightness (L^*), redness (a^*) and yellowness (b^*) values were comparable among the groups. The histomorphometric characteristics of the chicken breast meat throughout the growth period, except for the cross sectional area and number of fibres, were interactively influenced by 25-OH-D_3 and the sex of the bird. In conclusion, an improvement in 25-OH-D_3 status could be a practical tool to increase chicken breast meat quality with beneficial effects on its water holding capacity.

Keywords: Broiler, *Pectoralis major* Muscle Histology, Sensory Properties, Sex, Vitamin D.

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INTRODUCTION

Studies over the last decade have shown that increasing the level of 25-OH-D_3 in the diets of broiler chickens has beneficial implications for the yield and quality of breast meat. Enhancing the broiler chicken vitamin D status by feeding additional 25-OH-D_3 resulted in positive changes in the water holding capacity (WHC), pH and colour of the breast meat (Hutton et al., 2014; Starkey, 2014; Vignale et al., 2015; Bozkurt et al., 2017). However, almost all of these observations were obtained from male broiler chickens, and there are few studies using female chickens (Santiago et al., 2016). A recent exceptional one by Bozkurt et al. (2017) indicated significant sex and 25-OH-D_3 interactions for some meat quality traits. This finding stressed that the magnitude of the response to muscle development could differ between male and female chickens when the 25-OH-D_3 status of the diet changed. In addition, to the best of our knowledge, there are rare studies that have reported the effect of 25-OH-D_3 status on the histomorphometric characteristics of the pectoralis major muscle in broiler chickens (Chou et al., 2020). There are significant growth rate differences between fast-growing male and female broiler hybrids in association with different breast muscle development potentials (Ross, 2014; Cobb, 2018). Although there is clear evidence that 25-OH-D_3 modulates the structural characteristics and the development of the breast meat, the most valuable carcass cut-up part contributing over 30% of the overall broiler carcass weight, how male and female chickens differ in response to changes in dietary 25-OH-D_3 levels remains elusive.

There are reports that demonstrate a relationship between meat sensory characteristics including drip loss, cooking loss and pH, that determines WHC in pigs (Kim et al., 2007), cattle (Holmes and Ashmore, 1972) and sheep (Kadim et al., 1993) with different observations from male and female subjects. The effectiveness of adipose tissue in the activation of 25-OH-D_3 to 1α-25 (OH) $_2$-D_3, the most active form of Vitamin D_3, has been reported in studies with human subjects (Wamberg et al., 2012; Ryynänen et al., 2014). It is obvious that male chickens with more muscle and less adipose tissue than females will have a higher vitamin D requirement. Therefore, it is assumed that male broilers would have more responsive to increases in vitamin D levels in the feed than those the females in terms of lean muscle mass production with implications on sensory qualities of breast meat (Ceglia 2009; Ding et al., 2012). However, in broiler chickens, there is a dearth of studies using both male and female subjects in a comparative model.

We hypothesized that male chickens with a greater breast meat yield than females would benefit more from an addition of 25-OH-D_3 to their diet. Thus, our primary objective was to determine whether the improvement of 25-OH-D_3 status with the addition of 25-OH-D_3 would further impact the sensory attributes and histomorphometric characteristics of breast meat in male chickens compared with female chickens grown up to 42 days of age.

MATERIALS AND METHODS

Ethical approval

All procedures involving animals were approved by the Intuitional Animal Care and Use Committee of Aydın Adnan Menderes University, Aydın, Turkey (Ethical Committee approval no:64583101/2018/40).

Management

This experiment included a total of 720 one-day-old sexed chicks. Infectious bursal disease virus and Newcastle disease vaccines were implemented at 12 and 16 days of age, respectively. The environmentally controlled broiler test house was equipped with automatically adjustable heating, cooling, ventilation and lighting systems. Twenty-four wire-net pens were prepared, each having a floor space of 2.2 m^2 (125 x 177 cm) with five nipple drinkers and a tube-type feeder. The stocking density was 14 birds per m^2 floor space. The pens had concrete floor bedding and wood shavings litter with a thickness of approximately 6 cm.

The room temperature was maintained at 33 ºC from day 1 to 3 and thereafter gradually reduced until reaching 22 ºC on d 21 and then remained constant until the end of the trial. The light was provided by fluorescent bulbs for a period of 23 h/d from day 0 to 3, and then diminished 1 h every day until day 8, giving 18 h light per day during the remaining growth stage (i.e., from days 9 to 42). The experiment was terminated when the birds were 42 days of age.

Birds and experimental design

The experiment had a 2×2 factorial arrangement of treatments with 2 dietary regimens (diets with 3000 or 5000 IU 25-OH-D_3/kg) and 2 gender (male and female). Equal numbers of male (360) and female (360) 1-day-old broiler chicks (Ross 308) with an initial body weight of 43.6±2.9 g and 43.2±3.1 g, respec-

tively, were allocated into 4 equal groups, 180 chicks per group, with six replicates of 30 birds. Each pen was an experimental unit. The experiment was divided into two phases: a starter phase (1 to 28 days), and a finisher phase (29 to 42 days).

Experimental diets

The basal diet was a typical corn-soybean diet. The ingredient composition and nutrient content of the basal starter and grower diets are presented in Table 1. These diets contained no performance enhancer feed additives and were formulated to be nutritionally adequate in all essential nutrients. The diets were arranged as follows: (1) Control diet: The starter (from day 1 to 28) and finisher (from days 29 to 42) diets were formulated according to recommendations by the breeder (Ross, 2014). The concentrations of all macro and micronutrients, except for vitamin D_3, were chosen to represent the limitations published in the rearing guidelines for the strain (Nutrition Specifications of Ross 308 Broilers; Ross, 2014). Control diets contained 3000 IU/kg (75 μg/kg) cholecalciferol (vitamin D_3) to represent recent commercial applications in the field which coincides with the cholecalciferol content of vitamin premixes used in the feed industry for broiler chicken feed. (2) The control diet was further supplemented with 2000 IU/kg (50 μg/kg) 25-OH-D_3 to assess the effect of dietary vitamin D_3 intake by broilers over traditional levels of use in the field. All diets were mashed and were freshly prepared every two weeks. The diets and drinking water were available for ad libitum consumption throughout the experiment. The experimental diets were analysed three times to guarantee identical chemical compositions. The chemical compositions were determined according to protocols as outlined by the AOAC (1990). All feed samples were analysed for dry matter (934.01), ash (942.05), nitrogen (Kjeldahl procedure: 988.05), ether extract (920.39), and crude fibre (962.09).

Table 1. Composition of the experimental diets (as-fed basis)

Ingredient g/kg	Starter diet (d 1-28)	Finisher diet (d 29-42)
Corn, yellow, ground	367.31	431.15
Wheat	200.00	200.00
Soybean meal (48% crude protein)	357.00	286.00
Soy oil	36.50	49.00
Dicalcium phosphate	17.50	15.50
Limestone	11.00	8.50
Sodium chloride	2.40	2.64
L-Lysine HCL	1.00	0.20
DL-Methionine	2.05	2.21
L-Threonine	0.64	0.30
Vitamin-premix[a]	2.50	2.50
Mineral- premix[b]	1.00	1.00
Sodium bicarbonate	0.60	0.50
Anticoccidial[c]	0.50	0.50
Contents by analysis, %		
Dry matter	89.08	89.82
Crude protein	22.83	19.92
Ether extract	5.83	7.56
Crude fiber	3.23	3.14
Crude ash	6.52	5.99
Ca	1.18	0.99
P (total)	0.72	0.63
Calculated Contents %		
P (Available)	0.46	0.42
Lysine	1.29	1.17
Methionine	0.55	0.51
Methionine + Cysteine	0.96	0.94
Threonine	0.89	0.73
Linoleic acid	2.85	3.78
ME (kcal/kg)	3044	3234

[a]Provides per kg of diet: trans-retinol (vitamin A) 3.6 mg; vitamin D_3 (cholecalciferol) 75 mg; α-tocopherol acetate (vitamin E) 80 mg; menadione (vitamin K_3) 3.2 mg; thiamine (vitamin B_1) 3.2 mg; riboflavin (vitamin B_2) 8.6 mg; pyridoxine (vitamin B_6) 4.3 mg; cyanocobalamin (vitamin B_{12}) 0.02 mg; nicotinic acid 65 mg; Ca-D-pantothenate20 mg; folic acid 2.2 mg; D-biotin 0.22 mg.[b]Provides per kg of diet: Mn (MnO) 120 mg; Fe ($FeSO_4$) 20 mg; Zn (ZnO) 110 mg; Cu ($CuSO_4$) 16 mg; I ($CaIO_3$) 2 1.25 mg; Se (Na_2SeO_3) 0.30 mg; antioxidant (butyl hydroxy toluene) 125 mg.[c]Provides 100 mg monensin sodium per kg diet.

Dietary supplementation with 25-OH-D_3 was performed as follows. The feed grade 25-OH-D_3 used in this study (Hy-D; DSM Nutritional Products, Ltd., 4002 Basel, Switzerland) contained 69 µg/kg 25-OH-D_3, calculated to be equal to 2760 IU/kg provided by cholecalciferol (vitamin D_3) based on the conversion of 0.025 µg of cholecalciferol to 1 IU (NRC, 1994). The vitamin premix used in this study supplies 3000 IU (75.0 µg) vitamin D_3 per kg diet. Thus, a 725 g 25-OH-D_3 preparation was included per ton of basal diet to supply 50.0 µg 25-OH-D_3 per kg in the starter and finisher diets. The preparation of 25-OH-D_3 (i.e., 725 g) was balanced to 1 kg with finely ground soybean meal and homogenized using a laboratory type mixer for 30 seconds. Then, the premixture was supplemented with one ton of basal diet. Briefly, the control starter and finisher diets contained similar levels (i.e., 3000 IU/kg; 75 µg/kg) of cholecalciferol (vitamin D_3), while the die for the treatment group contained 5000 IU/kg (125 µg/kg) cholecalciferol (vitamin D_3).

Data collection

At days 14, 28 and 42, after 10 h feed withdrawal, two birds from each replicate representing average weight of the group (± 4%) were selected at random and slaughtered. Chickens were electrically stunned and exsanguinated via severing the jugular vein, bled for 45 second, scalded, mechanically defeathered and eviscerated. After weighing the absolute breast with bone and skin, the average 50 g of right half of breast muscle from each carcass at each age period were collected for histomorphometric analysis. At day 42, the breast muscles were placed in plastic bags and transported to the laboratory on ice to perform sensory characteristics of breast meat.

Meat quality analyses

pH_{24} and colour: All measurements were performed on the middle of the cranial section of the breast muscle at 24 h postmortem. For the determination of the pH of the meat samples, a pH meter (Hanna Instruments, HI 2211, Woonsocket, 02895 RI) was used. Colour measurements (lightness (L^*), redness (a^*) and yellowness (b^*)) were evaluated using a Minolta (Konica Minolta CR-400, Sensing INC. Sakai Osaka, 590-8551 Japan) at 24 h postmortem (Bozkurt et al., 2017).

Water holding capacity (WHC) : Drip loss, squeezable water and cooking loss were the main characteristics that determined the water holding capacity in this study. At 24 h postmortem, meat samples weighing between 5 and 8 g were placed between two filter papers and pressed at 10 kg for 5 minutes. The samples were reweighed, and the squeezed water weight (%) was calculated as follows: [(initial sample weight-final weight) /initial sample weight × 100]. Meat samples, approximately 4 cm in diameter, from each breast muscle were weighed, suspended on a hook inside a plastic bags and stored at 4 °C for 3 days. Each sample was reweighed, and the drip loss was calculated: [(initial sample weight-final weight) /initial weight × 100]. Fillets of approximately 60 g were weighed and cooked to an end-point temperature of 75 °C, cooled to room temperature and reweighed to determine the cooking loss: [(initial sample weight-final weight) / initial weight × 100] (Bozkurt et al., 2017).

Histomorphometry

For histological analysis, samples of the pectoralis major muscle were collected from 48 birds, 12 males and 12 females, from each group of chickens slaughtered at 42 days of age. The samples were *s*elected at 3 h post mortem from chickens having an average weight of 2.7 kg. Pectoralis major muscle samples were the “superficial layer” defined from approximately 0.2 cm to 1.2 cm below the breast muscle surface. The samples were immediately fixed in 10% buffered neutral formalin solution for 24 h, dehydrated in alcohol, cleared in xylene, infiltrated and finally embedded in paraffin (Khoshoii et al., 2013). The sections were cut at 5 µm thickness and stained with haematoxylin and eosin for general histological study (Bruck, 1975). Stained cross sections were viewed and photographed with a light microscope (Olympus BX53; Olympus, Tokyo, Japan) with a 10× objective lens and a 10× eyepiece (Tuma et al., 1962). Three photographs were taken from different cross-sections of each muscle. The samples were determined by using labSens software (Olympus Soft Imaging Solutions, Hamburg, Germany). A total of 144 preparations of pectoralis major muscle were used to determine the microstructure. Cross sectional area (CSA, μm^2) ; total fibres (TF) ; total number of fibres (NF; 1 μm^2) ; fibre diameter (FD; µm) ; FA: fibre area (μm^2) ; perimysium thickness (PT; µm) and endomysium thickness (ET; µm) were the criteria used for determining the microstructural characteristics of the pectoralis major muscle.

Statistical analysis

All data were subjected to two-way ANOVA using the JMP Statistical Package (SAS, 2018). Pen was

considered as an experimental unit for all measurements. The main effects of diet, sex and their interaction were assessed for all variables. Significant main effects were interpreted by comparing means using Student's t-tests. Means were considered significantly different when P<0.05, while P<0.10 was considered tendency.

RESULTS

The effects of 25-OH-D_3 status of the diet and the sex of the chickens on sensory characteristics of the breast meat (pectoralis major) are shown in Table 2. There was a significant 25-OH-D_3 by sex interaction for cooking loss in breast meat (P<0.05). Increasing the dietary 25-OH-D_3 levels from 3000 to 5000 IU decreased the cooking loss in the breast meat of female chickens, whereas no such effect was observed in males. The drip loss from breast muscle was slightly lower (P=0.067) in male chickens fed 5000 IU 25-OH-D_3 than in males fed 3000 IU 25-OH-D_3. However, no significant difference was found between females with different 25-OH-D_3 statuses, suggesting a 25-OH-D_3 by sex interaction for drip loss. The squeezable water ratio from the breast meat was not affected by the 25-OH-D_3 status or the sex of the bird. Dietary 25-OH-D_3 status and the sex of the chicken did not affect the lightness (L^*), redness (a^*) or yellowness (b^*) of the breast meat. An increase of 2000 IU in 25-OH-D_3 concentration in the diet increased the breast meat pH_{24} from 5.80 to 5.94 (P<0.05). No significant sex by 25-OH-D_3 interaction was found for any of the variables studied except for cooking loss. The absolute breast meat weight, CSA, TF and NF in the breast muscle were not affected by dietary 25-OH-D_3 status or the bird sex at any time point (i.e., 14, 28 and 42 days of age).

However, with regard to FD and FA, and PT and ET, no consistent tendencies were detected between dietary levels of 25-OH-D_3 and bird sex through the age periods of 14, 28 and 42. However, at d 42, in terms of FD, male birds benefited more from the increase in 25-OH-D_3 levels than females, indicating a significant 25-OH-D_3 by sex interaction (P=0.035). ET and PT were interactively affected by 25-OH-D_3 and bird sex at all age periods (P<0.01). While ET was similar between male and female chickens when they were fed a diet with 5000 IU 25-OH-D_3, female chickens had a higher ET than males treated with 3000 IU 25-OH-D_3 in the diet (P<0.01). However, with respect to PT, birds exhibited variable responses to alterations in 25-OH-D_3 status and bird sex all stages of the growth period.

Table 2. The effects of 25-OH-D_3 (IU/kg diet) status of the diet and gender of the chicken on sensory characteristics of the breast meat (Pectoralis major)

25-OH-D_3	Gender	Cooking loss %	Squeezable %	Drip loss %	pH_{24}	L^*	a^*	b^*
3000	Male	16.34[a]	9.90	3.27	5.80	58.77	1.58	7.87
	Female	17.24[a]	10.82	3.04	5.81	57.86	1.88	8.42
5000	Male	16.38[a]	9.66	2.63	5.90	58.28	1.57	8.43
	Female	14.96[b]	10.10	3.19	5.98	57.61	1.34	7.79
Pooled SEM[1]		0.265	0.560	0.106	0.031	0.320	0.120	0.150
	25-OH-D_3							
	3000	16.79	10.36	3.15	5.80[b]	38.31	1.73	8.15
	5000	15.67	9.88	2.91	5.94[a]	37.95	1.46	8.11
	Gender							
	Male	16.36	9.78	2.95	5.85	38.52	1.57	8.15
	Female	16.10	10.46	3.11	5.89	37.74	1.61	8.11
Source of variation[2]				Probability				
25-OH-D_3		0.041	0.646	0.256	0.037	0.410	0.257	0.911
Gender		0.628	0.740	0.437	0.455	0.081	0.880	0.887
25-OH-D_3 x Gender		0.035	0.275	0.067	0.535	0.785	0.275	0.053

[1]SEM: pooled standard error mean.
[2]Means in the same column within a treatment with no common superscript differ significantly (P<0.05).

Table 3. Effects of supplementation diet with 25-OH-D_3 (IU/kg diet) on muscle histomorphometric characteristics of Pectoralis major in broilers at 14 days age

25-OH-D_3	Gender	BW[3]	CSA	TF	NF	FD	FA	PT	ET
3000	Male	110	133	167	94	21.52^{c}	859^{b}	17.22^{c}	6.86^{c}
	Female	89	145	156	90	24.63^{a}	914^{a}	23.94^{b}	8.19^{a}
5000	Male	103	157	184	94	23.33^{a}	885^{b}	27.77^{a}	7.80^{b}
	Female	94	147	167	92	22.43^{b}	913^{a}	22.44^{b}	7.49^{b}
Pooled SEM[1]		2.451	6.100	6.503	2.450	0.108	6.652	0.519	0.081
	25-OH-D_3								
	3000	100	139	161	92	23.07	887	20.58	7.52
	5000	99	152	176	93	22.88	899	25.10	7.64
	Gender								
	Male	107	145	175	94	22.42	872	22.49	7.33
	Female	92	146	162	91	23.53	914	23.19	7.84
Source of variation[2]						Probability			
25-OH-D_3		0.902	0.314	0.285	0.879	0.368	0.001	0.000	0.002
Gender		0.158	0.930	0.306	0.497	0.000	0.348	0.646	0.456
25-OH-D_3 x Gender		0.461	0.372	0.823	0.878	0.000	0.037	0.000	0.000

[1]SEM: pooled standard error mean.
[2]Means in the same column within a treatment with no common superscript differ significantly (P<0.05).
[3]BW: Breast weight (g) ; CSA: Cross sectional area (μm^2) ; TF: Total number of fibres; NF: Number of fibres (1 μm^2) ; FD: Fibre diameter (μm) ; FA: Fibre area (μm^2) ;
PT: Perimysium thickness (μm) ; ET: Endomysium thickness (μm).

Table 4. Effects of supplementation diet with 25-OH-D_3 (IU/kg diet) on muscle histomorphometric characteristics of Pectoralis major in broilers at 28 days age

25-OH-D_3	Gender	BW[3]	CSA	TF	NF	FD	FA	PT	ET
3000	Male	429	217	87	43	37.88	1977	23.94^{b}	8.23^{c}
	Female	379	219	93	46	35.40	2049	30.60^{a}	11.86^{a}
5000	Male	419	209	95	42	37.08	2023	24.79^{b}	9.53^{b}
	Female	382	224	96	49	33.57	2251	21.49^{c}	9.86^{b}
Pooled SEM[1]		8.832	14.716	4.098	1.411	0.229	16.965	0.586	0.113
	25-OH-D_3								
	3000	404	218	90	44	36.64^{a}	2014^{b}	27.27	10.04
	5000	401	217	96	45	35.33^{b}	2136^{a}	23.14	9.70
	Gender								
	Male	424	213	92	42	37.48^{a}	2001^{b}	24.37	8.88
	Female	381	222	96	47	34.49^{b}	2149^{a}	26.04	10.86
Source of variation						Probability			
25-OH-D_3		0.962	0.957	0.161	0.899	0.004	0.000	0.152	0.125
Gender		0.078	0.762	0.703	0.145	0.000	0.000	0.000	0.000
25-OH-D_3 x Gender		0.844	0.831	0.315	0.363	0.268	0.453	0.000	0.000

[1]SEM: pooled standard error mean
[2]Means in the same column within a treatment with no common superscript differ significantly (P<0.05).
[3]BW: Breast weight (g) ; CSA: Cross sectional area (μm^2) ; TF: Total number of fibres; NF: Number of fibres (1 μm^2) ; FD: Fibre diameter (μm) ; FA: Fibre area (μm^2) ;
PT: Perimysium thickness (μm) ; ET: Endomysium thickness (μm).

Table 5. Effects of supplementation diet with 25-OH-D_3 (IU/kg diet) on muscle histomorphometric characteristics of Pectoralis major in broilers at 42 days age

25-OH-D_3	Gender	BW[3]	CSA	TF	NF	FD	FA	PT	ET
3000	Male	741	280	70	31	36.11^{c}	3216	21.06	8.29^{c}
	Female	701	295	58	28	45.30^{b}	3629	22.11	11.39^{ab}
5000	Male	737	279	71	31	47.45^{b}	3423	24.81	12.34^{a}
	Female	714	301	68	25	53.35^{a}	3774	27.80	10.62^{b}
Pooled SEM[1]		13.953	9.623	2.593	1.481	0.389	45.970	0.462	0.148
	25-OH-D_3								
	3000	721	287	64	30	40.71	3423	21.58^{b}	9.84
	5000	726	290	69	28	50.40	3598	26.31^{a}	11.48
	Gender								
	Male	739	279	70	31	41.78	3319^{b}	22.93	10.32
	Female	708	297	63	27	49.33	3701^{a}	24.96	11.00
Source of variation[2]						Probability			
25-OH-D_3		0.862	0.992	0.302	0.427	0.000	0.539	0.029	0.021
Gender		0.416	0.220	0.174	0.064	0.000	0.007	0.168	0.821
25-OH-D_3 x Gender		0.388	0.792	0.435	0.460	0.035	0.664	0.294	0.000

[1]SEM: pooled standard error mean.
[2]Means in the same column within a treatment with no common superscript differ significantly (P<0.05).
[3]BW: Breast weight (g) ; CSA: Cross sectional area (μm^2) ; TF: Total number of fibres; NF: Number of fibres (1 μm^2) ; FD: Fibre diameter (μm) ; FA: Fibre area (μm^2) ;
PT: Perimysium thickness (μm) ; ET: Endomysium thickness (μm).

DISCUSSION

Findings related to the sensory characteristics of chicken breast meat indicated that male and female chickens showed significant responses to dietary 25-OH-D_3 status in terms of cooking loss and drip loss, respectively. Cooking loss, a reliable indicator of WHC, was reduced in the carcasses of female birds with increasing 25-OH-D_3 supplementation, while no such effect was observed in males, suggesting a 25-OH-D_3 by sex interaction (P=0.035). With regard to cooking loss from breast meat, there has been no previous study that revealed the mechanism by which the sex of the bird affected their response to 25-OH-D_3. The data from the present experiment clearly indicate that higher levels of 25-OH-D_3 supplementation than the recommendations by the breeder (i.e., 4000 IU for the finisher period; Ross, 2014) and experimentally derived estimates, (i.e., 3000 IU) are beneficial to improve the WHC of chicken breast meat by decreasing cooking loss. Likewise, Bozkurt et al. (2017) indicated that slightly higher 25-OH-D_3 supplementation over the recommendations for the breed (i.e., 10%) markedly decreased squeezable water and cooking loss in male and female chickens at 38 days of age. Indeed, there are no specific nutrient recommendations chickens specific to sex regarding the ideal supplemental 25-OH-D_3 levels, only recommendations for both sexes (Ross, 2014; Cobb, 2018). Reduced cooking loss with increased 25-OH-D_3 status could be associated with increases in FD and FA, which are both indicative of an increase in myofibril area. This indicates a reduction in the size of the connective tissue, which facilitates the migration of intracellular water in the muscle (Hussein et al., 2019). The reduced drip loss and cooking loss in males and females, respectively, fed 5000 IU 25-OH-D_3 may be associated with the beneficial effects of 25-OH-D_3 on muscle fibre radial growth (Starkey, 2014; Świątkiewicz et al., 2017) since myofibrillar proteins are considered to be mainly responsible for the WHC of the meat (Ooizumi and Xiong, 2004). Therefore, it is reasonable that increasing the supplemental 25-OH-D_3 indirectly enhances the WHC in chicken breasts. Another plausible explanation for the decreased drip loss and cooking loss, reliable indicators that determine the WHC and consequently the raw meat quality with increased 25-OH-D_3 intake are the observed increases in FD and FA. As the CSA of the myofibres expands, the area occupied by the adipose and connective tissue would be limited, suggesting that the ability to retain liquid during refrigerated storage and cooking would be enhanced (Mazzoni et al., 2015) due to the increased diameter of the myofibrils. This is in parallel with the observations obtained from the current experiment that supplementation of the diet with 25-OH-D_3 at the level of 5000 IU increased the

CSA, FD and FA in the breast muscles of both sexes without increasing the TF and NF.

An increasing number of studies have shown that 25-OH-D_3 supplementation in the diet had positive effects on the bone (Zhang et al. 2020) and muscle development of broiler chickens (Hutton et al., 2014; Vignale et al., 2015; Bozkurt et al., 2017; Alves et al., 2018). However, until now, there have been no studies assessing the sex-specific characteristics of breast muscle for broiler chickens fed diets with different 25-OH-D_3 concentrations. In a recent example, Bozkurt et al. (2017) investigated the effects of superdosing 25-OH-D_3 (i.e., 5500 IU/kg diet) on the meat quality characteristics of broiler chickens. Moreover, to the authors' knowledge, no other study has investigated the interactions between sex and 25-OH-D_3 levels with regard to the structural characteristics of muscle meat in broiler chickens. Considering these facts mentioned above, it is challenging to elucidate the mechanism of sex based differences in breast muscle microstructure as a response to 25-OH-D_3 supplementation levels. A similar effect is expected for the sensory characteristics of breast meat because it was demonstrated that WHC in breast and thigh meat differently interact with muscle structural characteristics in male and female broilers, presumably due to the differences in their growth rate (Fanatico et al., 2005; Steczny and Kokoszynski 2019).

Muscle structural characteristics, including CSA, TF and NF, were not significantly affected by 25-OH-D_3 supplementation or the sex of the bird at any age period. In the present study, FD and FA in female chickens measured at day 42 were significantly higher than those in male chickens. However, despite the significant increases in FD in female chickens, the CSA was similar between male and female chickens in association with higher TF and NF in males than in females. This is not surprising and concurs with the findings by Scheuermann et al. (2003) that male chickens show higher myofibre density in the pectoralis major muscle than female chickens. In the present study, FD was increased with additional 25-OH-D_3 supplementation (3000 vs. 5000 IU/kg diet) ; however, the male birds benefited more from the 25-OH-D_3 supplementation. Regardless of sex, this is in agreement with earlier studies reporting the benefits of 25-OH-D_3 on muscle size (Starkey, 2014; Vignale et al., 2015). The beneficial effects of 25-OH-D_3 supplementation on FD and to a lesser extent on FA and CSA appear to be reflected in an improved WHC with significant decreases in cooking loss in female chickens. This suggests that it is possible to improve the WHC in female chicken breasts with additional 25-OH-D_3 supplementation, as already demonstrated by Bozkurt et al. (2017).

It was previously indicated that acidic meats, which are characterized by ultimate pH values lower than 5.7, have negative implications on WHC (Berri et al., 2005; Duclas et al., 2007). However, in the current experiment, squeezable water and drip loss, highly useful measurements to estimate WHC, were not influenced sex or dietary 25-OH-D_3 status. In this study, the pH of the breast meat was approximately 5.8, a value that is regarded as optimal at 24 h post-mortem. One peculiar finding from this experiment is that higher 25-OH-D_3 supplementation resulted in 0.14 increments in pH_{24} (i.e., 5.80 vs. 5.94).

Nevertheless, cooking loss, another indicator of WHC in edible meat, was significantly decreased with 25-OH-D_3 supplementation in the breast muscle of female chickens. The mechanism by which 25-OH-D_3 supplementation (3000 vs. 5000 IU) causes such a dramatic decrease in cooking loss is thought to be due to an increase in myofibriller protein accretion with a concomitant decrease in connective tissues and fat accumulation.

CSA, TF and NF were unaffected by either sex or 25-OH-D_3 status or their interactions at any time point. On the other hand, in regard to FD, FA, PT and ET measurements, no consistent responses to sex or 25-OH-D_3 status were found at the initial growth periods (i.e., 14 and 28 days of age). One possible explanation for this is that the beneficial effect of 25-OH-D_3 on muscle size in broilers is more prevalent from 5 weeks onwards when hypertrophy of the muscles speeds up to achieve a daily weight gain over 100 g (Scheuermann et al., 2003; Hutton et al., 2014; Vasquez et al., 2018). Muscle fibres with large diameters are dark, although the meat has a higher pH (Choi and Kim, 2009). In addition, Allen et al. (1997) found contrasting relationships between breast muscle pH and lightness (L^*) and yellowness (b^*) in broilers, whereas a positive correlation with redness (a^*) was noted. However, in the present experiment, despite greater FD and FA with additive 25-OH-D_3 supplementation, the colour of the breast meat was not influenced by the 25-OH-D_3 status of the diet regardless of the sex of the bird. This is in accordance with the findings of Lopez et al. (2011), who noticed that sex does not influence breast meat colour values (L^*, a^* and b^*)

in broilers. However, Hussein et al. (2019) reported higher a^* but lower b^* values in female chickens than in male chickens in terms of breast muscle colour. A contradictory pattern to that reported by Hüssein et al. (2019) was observed by Bozkurt et al. (2017) when evaluating the breast muscle colour response to supplemental 25-OH-D_3 in male and female Ross 308 broiler chickens.

CONCLUSIONS

In brief, some relationships between muscle structural characteristics and dietary 25-OH-D_3 status do exist in male and female broiler chickens at any time point, but the effects are much more pronounced at slaughter age (i.e., 42 days of age) than at earlier time points. The lack of significant 25-OH-D_3 by sex interactions for muscle structural characteristics shows that both sexes are quite similar in their response to 25-OH-D_3 levels in the diet, suggesting that no more than the typical nutritional 25-OH-D_3 specifications are needed for either male or female broiler chickens. Breast meat pH_{24} was significantly responsive to alterations in 25-OH-D_3 status but the meat colour was unchanged. The beneficial effects of increased 25-OH-D_3 supplementation on cooking loss are conclusive.

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CONFLICT OF INTEREST

The author declares that there are no conflicts of interest.

REFERENCES

Allen CD, Russell SM, Fletcher DL (1997) The relationship of broiler breast meat color and pH to shelf-life and odor development. Poultry Sci 76:1042-1046. DOI:10.1093/ps/76.7.1042.

Alves ODS, Calixto LFL, Araujo AHB, Torres-Cordido KAA, Reis TL, Calderano AA (2018) Decreased levels of vitamin D_3 and supplementation with 1, 25-dihydroxyvitamin D_3-glycoside on performance, carcass yield and bone quality in broilers. Ciência Rural 48 (8) :1-9. DOI: 10.1590/0103-8478cr20170705.

AOAC (1990) Official Methods of Analysis.15th ed., Association of Official Analytical Chemists, Washington DC.

Berri C, Debut M, Sante-Lhoutellier V, Arnould C, Boutten B, Sellier N, Baéza E, Jehl N, Jégo Y, Duclos M.J, LeBihan-Duval E (2005) Variations in chicken breast meat quality: implications of struggle and muscle glycogen content at death. Brit Poultry Sci 46:572-579. DOI:10.1080/00071660500303099.

Bozkurt M, Yalçın S, Koçer B, Tüzün, AE, Akşit H, Özkan S, Uygun M, Ege G, Güven G, Yildiz O (2017) Effects of enhancing vitamin D status by 25-Hydroxycholecalciferol supplementation, alone or in combination with calcium and phosphorus, on sternum mineralisation and breast meat quality in broilers. Brit Poultry Sci 58:452-461. DOI:10.1080/ 00071668.2017.1327703.

Burck HC (1975) Histological technology. Warszawa: Panstwowy Zakład Wydawnictw Lekarskich Poland.

Ceglia L (2009) Vitamin D and its role in skeletal muscle. Curr Opin Clin Nutr Metab Care 12 (6) :628-633. DOI:10.1097/MCO.

Choi YM, Kim BC (2008) Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. Livest Sci 122:105-118. DOI:10.1016/j.livsci.2008.08.015.

Chou PC, Chen YH, Chung TK, Walzem RL, Lai LS, Chen SE (2020) Supplemental 25-hydroxycholecalciferol alleviates inflammation and cardiac fibrosis in hens. Int J Mol Sci 21: 1-12 8379; DOI:10.3390/ijms21218379.

Cobb (2018) Cobb broiler nutrition guide, The Cobb Vantress Company.

Ding C, Gao D, Wilding J, Trayhurn P, Bing C (2012) Vitamin D signalling in adipose tissue. Br J Nutr 108:1915-1923. DOI:10.1017/S0007114512003285.

Duclas MJ, Berri C, Le Bihan-Duval E (2007) Muscle growth and meat quality. J Appl Poultry Res 16:107-112. DOI:10.1093/japr/16.1.107.

Fanatico AC, Cavitt LC, Pillai PB, Emmert JL, Owens CM (2005) Evaluation of slower-growing broiler genotypes grown with and without outdoor access: meat quality. Poultry Sci 84:1785-1790. DOI: 10.1093/ps/84.11.1785.

Holmes JHG, Ashmore CR (1972) A histochemical study of development of muscle fiber type and size in normal and ‘‘double muscled’’ cattle. Growth, 36:351-372.

Hutton KC, Vaughn MA, Litta G, Turner BJ, Starkey JD (2014) Effect of vitamin D status improvement with 25-hydroxycholecalciferol on skeletal muscle growth characteristics and satellite cell activity in broiler chickens. J Anim Sci 92:3291-3299. DOI:10.2527/jas.2013-7193.

Hussein EOS, Suliman GM, Al-Owaimer AN, Ahmed SH, Abudabos AM, Abd El-Hack ME, Taha AE, Saadeldin IM, Swelum AA (2019) Effects of stock, sex, and muscle type on carcass characteristics and meat quality attributes of parent broiler breeders and broiler chickens. Poultry Sci 98:6586-6592. DOI:10.3382/ps/pez464.

Kadim IT, Purchas RW, Davies AS, Rae AL, Barton RA (1993) Meat quality and muscle fibre type characteristics of south down rams from high and low back fat selection lines. Meat Sci 33:97-109. DOI:10.1016/0309-1740 (93) 90097-2.

Khoshoii AA, Mobini B, Rahimi E (2013) Comparison of chicken strains: muscle fibre diameter and numbers in Pectoralis superficialis muscle. Glob Vet 11:55-58. DOI:10.5829/idosi.gv.2013.11.1.73205.

Kim JM, Lee YJ, Choi YM, Kim BC, Yoo BH, Hong KC (2008) Possible muscle fiber characteristics in the selection for improvement in porcine lean meat production and quality. Asian Austral Anim Sci 21:1529-1534. DOI:10.5713/ajas.2008.80050.

Lopez KP, Schilling MW, Corzo A (2011) Broiler genetic strain and sex effects on meat characteristics. Poultry Sci 90:1105-1111. DOI:10.3382/ps.2010-01154.

Mazzoni M, Petracci M, Meluzzi A, Cavani C, Clavenzani P, Sirri F (2015) Relationship between pectoralis major muscle histology and quality traits of chicken meat. Poultry Sci 94:123-130. DOI: 10.3382/ps/peu043.

NRC (1994) National Research Council. Nutrient Requirements of Poultry, 9th ed., National Academy Press, Washington DC.

Ooizumi T, Xiong YL (2004) Biochemical susceptibility of myosin in chicken myofibrils subjected to hydroxyl radical oxidizing systems. J Agr Food Chem 52:4303-4307. DOI:10.1021/jf035521v.

Ross (2014) Broiler nutrition specifications, Newbrigde, Scotland, Aviagen.

Ryynänen J, Neme A, Tuomainen TP, Virtanen JK, Voutilainen S, Nurmi T, Mello VDF, Uusitupa M, Carlberg C (2014) Changes in vitamin D target gene expression in adipose tissue monitor the vitamin D response of human individuals. Mol Nutr Food Res, 58: 2036-2045. DOI 10.1002/mnfr.201400291.

Santiago M, David S, Alexandra N, Eduardo A, Jimmy QG (2016) Effect of 25-hydroxycholecalciferol (25-OH-D3) on productive performance and bone mineralization in broiler. Open J Anim Sci, 6:180-184. DOI:10.4236/ojas.2016.63023.

SAS (2018) Institute. JMP® User's Guide: Statistics Version 15.0 Edition. Cary, SAS Institute, North Caroline.

Scheuermann GN, Bilgili SF, Hess JB, Mulvaney DR (2003) Breast muscle development in commercial broiler chickens. Poultry Sci 82:1468-1658. DOI: 10.1093/ps/82.10.1648.

Starkey JD (2014) A role of vitamin D in skeletal muscle development and growth. J Anim Sci 92:887-892. DOI:10.2527/jas.2013-7087.

Steczny K, Kokoszynski D. (2019). Effects of probiotics and sex on physicochemical, sensory and microstructural characteristics of broiler chicken meat. Italian Journal of Animal Science, 18: 1385-1393. DOI:10.1080/1828051X.2019.1667269.

Świątkiewicz S, Arczewska-Włosek A, Bederska-Lojewska D, Józefiak D (2017) Efficacy of dietary vitamin D and its metabolites in poultry - review and implications of the recent studies. World Poult Sci J, 73 (1) :57-68. DOI:10.1017/S0043933916001057.

Tuma HJ, Venable JH, Wuthier PR, Henrickson RL (1962) Relationship of fiber diameter to tenderness and meatiness as influenced by bovine age. J Anim Sci 21:33-36. DOI:10.2527/jas1974.38124x.

Vazquez JR, Gomez GV, Lopez CC, Cortes AC, Díaz AC, Fernandez SRT, Rosales EM, Avila AG 2018. Effects of 25-hydroxycholecalciferol with two D3 vitamin levels on production and immunity parameters in broiler chickens. J Anim Physiol Anim Nutr 102:493-497. DOI: 10.1111/jpn.12715.

Vignale K, Greene ES, Caldas JV, England JA, Boonsinchai N, Sodsee P, Pollock ED, Dridi S, Coon CN (2015) 25-Hydroxycholecalciferol enhances male broiler breast meat yield through the mTOR pathway. J Nutr 145:855-863. DOI:10.3945/jn.114.207936.

Wamberg L, Christiansen T, Paulsen SK, Fisker S, Rask P, Rejnmark L, Richelsen B, Pedersen, SB (2012) Expression of vitamin D-metabolizing enzymes in human adipose tissue—the effect of obesity and diet-induced weight loss. Int J Obes, 37 (5) :651-657. DOI:10.1038/ijo.2012.112.

Zhang LH, He TF, Hu JX, Li M, Piao XS (2020) Effects of normal and low calcium and phosphorus levels and 25-hydroxycholecalciferol supplementation on performance, serum antioxidant status, meat quality, and bone properties of broilers. Poultry Sci 99:5663-5672. DOI:10.1016/j.psj.2020.07.008.