TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2021) 45: 1030-1040

doi:10.3906/vet-2104-89

Effects of feed restriction on histopathological changes associated with white striping and wooden breast myopathies in pectoralis major muscle and meat quality characteristics in broiler chickens

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Received: 26.04.2021 • **Accepted/Published Online:** 25.10.2021 • **Final Version:** 13.12.2021

Abstract: The aim of this study was to investigate the effects of feed restriction on histopathological changes associated with white striping and wooden breast myopathies in pectoralis major muscle and meat quality characteristics in broiler chickens. In total, 600 male broiler chicks were randomly divided into five groups with 8 replicate pens (15 chicks/pen). Treatments consisted of control, treatment 1 (T1), treatment 2 (T2), treatment 3 (T3) and treatment 4 (T4). Control group was fed ad libitum throughout experimental period. T1 and T2 groups received feed as 80% and 70% of amount received by the control group from 11 to 24 days of age, respectively. T3 and T4 groups received feed as 80% and 70% of amount consumed by control group from 25 to 39 days of age, respectively. T2, T3, and T4 groups had lower slaughter weight, breast fillet weight, ultimate pH value, cooking loss, and intra muscular fat content than control group. T3 and T4 groups had lower myodegeneration occurence ratio and myodegenearation severity in pectoralis major muscle and also lower mean values for yellowness index, intramuscular moisture content, compared to control group. In conclusion, myodegeneration occurrence and severity in breast muscle tissue of broilers at 49 days of age could be decreased, and, thus, meat quality characteristics could be improved by feed restriction to 80% of ad libitum consumption from 25 to 39 d of age. However, this feeding program may result in some reduction in slaughter weight and breast fillet weight. Further studies in commercial conditions are required to develop the management and feeding strategies that may reduce the occurrence of these myopathies with minimal effect on production performance in broiler chickens.

Key words: Broiler, feed restriction, white striping, wooden breast, apoptosis, meat quality

1. Introduction

Poultry meat consumption has increased because of its low price, high nutritional value, sensory quality, and convenience in further processing [1]. In order to meet increasing consumer demand, the poultry industry has focused on increase in production by reducing the cost and time of production, and, thus, body weight and breast yield of broilers has greatly increased due to improvements in intensive genetic selection, nutrition, and management in recent years [2, 3]. However, intensive genetic selection applied to increase growth rate, body weight, and breast meat ratio has caused to a variety of muscle abnormalities in broiler chickens [4].

White striping and wooden breast are two important myopathies that have appeared in recent years and negatively affect the broiler breeding. White striping is the formation of white lines of different size in mainly breast muscles of broiler chickens. Wooden breast has been described as a muscle defect, which there is palpably hard, pale color, bulging at caudal end, small hemorrhages, clear exudate, and white striations in breast fillets of broilers [4, 5]. Histopathologically, both myopathies have been reported to show similar changes in breast muscle tissue, including floccular or vacuolar degeneration, loss of cross striations, variability in fiber size, necrosis, fiber lysis, mild mineralization, inflammatory cell infiltration, lipidosis, fibrosis, and regenerative changes [5, 6]. However, the higher level of fibrosis has been reported for samples of breast muscle tissues with wooden breast myopathy than those with white striping [7].

Previous studies showed that white striping and wooden breast myopathies had unfavorable effects on meat quality traits such as decrease in intramuscular protein content, increase in intramuscular fat content [8–10] and cooking loss [11–15]. These myopathies cause significant economic loss in poultry industry due to their negative effects on

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the appearance, nutritional and technological quality of meat. Impaired appearance of the breast fillets affected by these myopathies, especially severe degree white striping negatively affects the consumer choice and thus these fillets downgraded by poultry processors [11, 16].

Wooden breast and white striping muscle defects are known to be related with fast growth rate, increased breast yield [5, 7, 8, 12, 17, 18], and also increased fillet thickness [14, 17]. The occurrence of these myopathies in the heavier and the thicker breast fillets has been attributed to increase in muscle fiber size associated with the lower capillarisation, which cause not to provide adequate nutrients and oxygen to muscle cells and also inadequate elimination of intermediate products and, thus, lead to impairment of fiber functionality and homeostatic dysregulation [19–21]. Several studies have reported that feed restriction may decrease the formation of these myopathies in broiler chickens [22, 23] when it is applied throughout production period. However, information about the effects of feed restriction at different times and levels on the formation of these myopathies, especially on histopathological changes in breast muscle tissue and also meat quality characteristics, is limited. Hence, this study aimed to investigate the effects of feed restriction on histopathological changes associated with white striping and wooden breast myopathies in breast muscle tissue and meat quality traits in broiler chickens.

2. Materials and methods

2.1. Animals, management, and experimental design

The study was conducted at the Poultry Unit of the Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Turkey. All experimental procedures of this study were approved by Aydın Adnan Menderes University Ethics Committee (Approval No: 64583101/2016/188).

Six hundred one-day old male chickens of Ross 308 strain were used. Chicks were housed in floor pens measuring 150 × 110 cm. Wood shavings of 6–8 cm depth were used as litter material. Hanging feeder and nipple drinkers were used in each pen to meet feed and water needs of chickens. All broilers were fed with the same 4-phase standard diets that met the nutritional requirements of theirs based on the standard recommendations of the breeder company for Ross 308 hybrids [24]. Diets consisted of starter (3000 kcal/kg ME and 23% crude protein), grower (3100 kcal/kg ME and 21.5% crude protein), finisher (3200 kcal/kg ME and 19.5% crude protein), and withdrawal diet (3200 kcal/kg ME and 18.30% crude protein), which were used in the periods from 1 to 10, from 11 to 24, from 25 to 39, and from 40 to 49 days, respectively. Water was available free for all groups during the experimental period. All groups were provided 24 h of lighting during the first 7 days and 3 days before slaughter and an 18 L: 6D lighting program on the other days. In the first 3 days, the temperature of the rooms was approximately 32 °C, and, afterward, it was decreased 3 °C every week until day 21.

Broilers were randomly allocated to 5 treatments, each consisting of 8 replicate pens (15 chicks/pen) after they were wing-banded and weighed. Feed was provided freely to broilers in control group during the study. Broilers in treatment 1 (T1) and treatment 2 (T2) were fed with 80% and 70% of the amount consumed by the control group fed ad libitum from 11 to 24 days, respectively. Broilers in treatment 3 (T3) and treatment 4 (T4) were fed with 80% and 70% of the amount consumed by the control group from 25 to 39 days, respectively. The restricted birds received the 80% and 70% of the quantity consumed by the broilers in control group fed ad libitum on the previous day. Except for the restriction period, birds were fed ad libitum. The restricted birds received feed as 80% and 70% of amount consumed by those in control group on the day before.

2.2. Slaughter procedure and sampling

At 49 days of age, 40 broilers from each group, (5 broilers from each pen) were randomly selected and slaughtered to determine meat quality characteristics and histopathological changes related to wooden breast and white striping myopathies in breast muscles. Broilers having a body weight close to the pen average were selected for slaughter. Feed was withdrawal 12 h prior to slaughter. Birds were individually weighed before slaughter. Broilers were slaughtered at the Poultry Unit of the Faculty of Veterinary Medicine, Aydın Adnan Menderes University where the study was conducted. In order to prevent preslaughter stress, the animals were carefully transported to the processing unit from the rooms where they were raised by experienced researchers. Slaughter was applied by manually by cutting carotid artery and jugular vein. After defeathering and evisceration procedures, breast fillets were removed from each carcass. Breast fillets were divided into two parts as right and left. Left breast fillets were weighted and then morphometric measurements were determined in mm by using caliper according to the method reported by Mehaffey et al. [25]. In summary, length was the measure of distance from the cranial to caudal end of the fillet, whereas width was the measure taken from the widest distance in the middle of the filet. Cranial thickness was measured as the height at the thickest point, and caudal thickness also was measured as the height at 25 mm from the bottom of filet.

2.3. Meat quality measurements

Muscle pH value measurement was done in triplicate from the cranial end of the left breast filets 15 min and 24 h after slaughter with a portable pH meter (Testo 205, Testo Inc, Lenzkirch, Germany). Colour values were measured at the cranial section of the bone-side surface of left fillets

24 h after slaughter with a chromameter (minolta CR-400; Konica, Minolta, Tokyo, Japan). Cooking loss was detected in meat samples taken from the left breast fillet according to method reported by Honikel [26]. Briefly, the meat samples placed in plastic bags were cooked in a water bath until an internal temperature of 75 °C, and then they were chilled for 15 min under running tap water and reweighed. Cooking loss was calculated as following: weight before cooking-weight after cooking / weight before cooking × 100. Chemical composition of meat samples taken from cranial side of left filet were analyzed using standard analytical methods [27].

2.4. Histopathological and apoptosis examination

The pectoralis major muscle samples of 16 broilers from each group (2 broilers from each pen) were taken immediately after slaughter for histopathological examination. Samples were fixed in 10% formalin solution, dehydrated in ethanol series and embedded paraffin wax, sectioned at 4 μm and stained with hematoxylin and eosin (HE) and Masson's trichrome. In histopathological examination, myopathic lesions, lipidosis, and fibrosis were scored in degrees between 0 and 3 (0: normal, 1: mild, 2: moderate, 3: severe) according to the method reported by Radaelli et al [28].

Apoptotic cells in sections of the pectoral muscles were labeled using Terminal TUNEL (Deoxynucleotidyl Transferase-Mediated dUTP Nick End-Labeling) assay (in situ cell death detection kit, POD, Roche Diagnostics) as recommended by the manufacturer. In negative controls, there was a substitution of the TUNEL reaction mixture for label solution without terminal transferase in the sections. Apoptotic cells were determined based on the total number of positive cells in sections of the pectoral muscles. The number of apopototic cells were determined with an ocular grid of 100 (10 × 10) squares at 400 × magnification.

2.5. Statistical analysis

Statistical analyses of data were performed by using SPSS software package version 22.0 [29]. The frequency of broilers showing myodegeneration in pectoralis major muscle was assessed by using chi square test [30]. Data of myodegeneration scores in pectoralis major muscle and the number of apoptotic nuclei were analyzed by Kruskal–Wallis test and significance control of differences between groups was determined by Mann–Whitney U test. In order to determine the effects of feed restriction on the slaughter weight, breast fillet weight and dimensions, meat quality characteristics, and also the effects of myodegeneration degree on these traits one-way ANOVA was used. Significant differences among treatment means were determined by using Duncan's multiple range test [31].

3. Results

Effects of feed restriction on live weight at slaughter, breast fillet weight and dimensions in broiler chickens are presented in Table 1. T2, T3, and T4 groups had significantly lower mean values in terms of live weight at slaughter and breast fillet weight than control group ($p < 0.001$). T3 and T4 groups had lower fillet length, width, and cranial thickness than control ($p < 0.05$). Caudal thickness was lower in all restricted groups than control; however, it was the lowest in T3 and T4 groups.

Effects of feed restriction on quality characteristics of breast meat in broiler chickens are shown in Table 2. T2, T3, and T4 groups had significantly lower values in terms of ultimate pH value and cooking loss than control group ($p < 0.001$). Yellowness index (b^*) value also was determined as lower for T3 and T4 groups than control ($p < 0.05$). It was determined that T3 and T4 groups had lower values in terms of the percentage of moisture of breast meat than control ($p < 0.05$). The percentage of fat

Table 1. Effects of feed restriction on slaughter weight, breast fillet weight and dimensions in broiler chickens.

	Treatments						
Traits	Control	T1	T2	T3	T4	**SEM**	**p**
	(n = 40)	(n = 40)	(n = 40)	(n = 40)	(n = 40)		
Live weight at slaughter (g)	3830.77^{a}	3754.68^{ab}	3691.50^{b}	3579.78^{c}	3515.33^{c}	17.51	< 0.001
Fillet weight (g)	492.85^{a}	474.07^{ab}	456.04^{b}	420.79^{c}	413.15^{c}	3.94	< 0.001
Length (mm)	197.23^{a}	195.75^{ab}	195.10^{ab}	192.38^{b}	192.06^{b}	0.64	0.042
Width (mm)	104.25^{a}	102.33^{a}	101.00^{ab}	97.88^{b}	97.60^{b}	0.60	0.003
Cranial thickness (mm)	44.51^{a}	43.30^{ab}	42.96^{ab}	41.75^{b}	41.10^{b}	0.36	0.028
Caudal thickness (mm)	30.39^{a}	28.82^{b}	28.09^{b}	23.57^{c}	23.03^{c}	0.28	< 0.001

$^{a, b,c,:}$ Means shown with different superscript letter in the same row are statistically differ ($p < 0.05$). SEM = pooled standard error of the mean.

Table 2. Effects of feed restriction on quality characteristics of breast meat in broiler chickens.

	Treatments						
Traits[1]	Control	T1	T2	T3	T4	**SEM**[2]	**p**
	(n = 40)	(n = 40)	(n = 40)	(n = 40)	(n = 40)		
pH_{15}	6.59	6.57	6.53	6.52	6.55	0.02	0.233
pH_{24}	5.89[a]	5.86[ab]	5.84[bc]	5.82[cd]	5.81[d]	0.02	< 0.001
L^*	51.27	51.50	51.10	51.34	50.87	0.17	0.790
a^*	2.90	2.78	2.70	2.64	2.58	0.07	0.586
b^*	9.90[a]	9.84[ab]	9.65[ab]	9.33[bc]	9.16[c]	0.08	0.013
Cooking loss (%)	29.68[a]	28.44[ab]	27.28[b]	25.14[c]	24.09[c]	0.38	< 0.001
Chemical composition (%)							
Moisture	75.18[a]	74.80[ab]	74.73[ab]	74.36[b]	74.29[b]	0.10	0.029
Protein	20.86[c]	21.59[bc]	21.81[b]	22.64[a]	22.72[a]	0.16	< 0.001
Fat	2.79[a]	2.43[ab]	2.20[b]	1.68[c]	1.63[c]	0.09	< 0.001
Ash	1.14	1.17	1.23	1.27	1.31	0.03	0.247

[1] pH_{15}: pH value measured at 15 min post mortem, pH_{24}: pH value measured at 24 h post mortem.
L^*: Lightness, a^*: redness, b^*: yellowness index values.
[2] SEM = pooled standard error of the mean.
[a, b, c, d:] Means shown with different superscript letter in the same row are statistically differ ($p < 0.001$).

was lower for T2, T3 and T4 groups than control, whereas the percentage of protein ratio was higher for these groups than control ($p < 0.001$).

In histopathological examination, in normal pectoralis major muscles which myodegeneartion score is 0, muscle fibers had a regular diameter and were filled with longitudinally arrayed myofibrilles; cross-striations in each fiber nuclei were located peripherally under the plasma membrane. In sections with mild myodegeneration score 1, there were mild degenerative necrotic lesions in muscle tissue, and hyper-eosinophilic muscle fibers with loss of cross-striations and lyses of nuclei were observed. Severe degenerative necrotic muscle bundles, inflammatory cell infiltrations, fibrous tissue proliferations and adipose tissue lobules were observed in sections of pectoralis major muscle samples with moderate myodegeneration (score 2). Severe degenerative, necrotic muscle bundles, inflammatory cell infiltrations, severe fibrous tissue proliferations and widespread adipose tissue lobules were observed in sections of pectoralis major muscles with severe myodegeneration (score 3) (Figure 1).

The frequency of broilers having myodegeneration in pectoralis major muscle is shown in Table 3. The lower ratios ($p < 0.05$) of myodegeneration occurrence in pectoralis major muscle were obtained for broilers in T3 and T4 groups than those in control group.

The myodegeneration scores of pectoralis major muscle are presented in Table 4. T3 and T4 groups had lower ($p < 0.05$) myodegeneration scores than control.

Apoptosis positive cells were seen as nuclear staining in myofibrils (Figure 2). No apoptosis was detected in control slides. The number of apoptotic nuclei was lower ($p < 0.001$) for T2, T3, and T4 groups than control and T1 groups. (Figure 3).

Breast fillet weight and fillet dimensions in different groups of myodegeneration severity are shown in Table 5. Fillets exhibiting moderate (score 2) and severe degree (score 3) myodegeneration had significantly higher weight, length, and width, compared to normal fillets ($p < 0.05$). Cranial thickness of all fillets exhibiting myodegeneration was higher, compared to normal fillets. However, caudal thickness of fillets having moderate and severe degree myodegeneration were higher compared to those of normal and fillets with mild myodegeneration ($p < 0.001$).

Breast meat quality characteristics in different groups of myodegeneration severity are presented in Table 6. Breast fillets exhibiting moderate and severe degree myodegeneration had higher mean values in terms of cooking loss ($p < 0.01$), ultimate pH ($p < 0.001$) value, and yellowness index ($p < 0.05$) than normal fillets. Fillets exhibiting moderate and severe degree myodegeneration had also significantly lower protein ratio but the higher fat

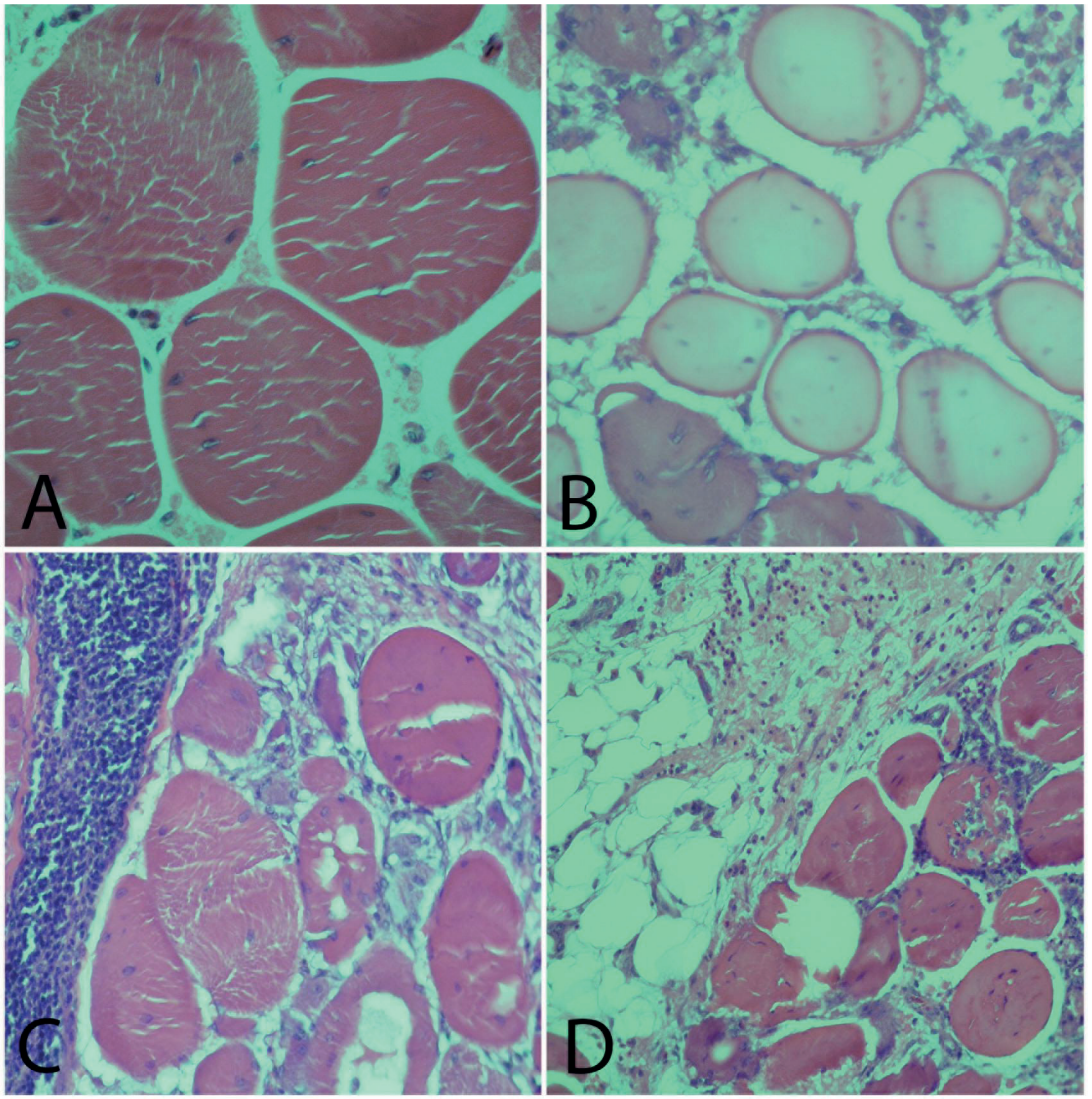

Figure 1. Histopathological changes in pectoralis major muscle in broiler chickens (H&E, X 20). Normal pectoralis major muscles (score 0): Muscle fibers with a regular diameter and longitudinally arrayed myofibrilles, cross-striations and located peripherally nuclei (A). Mild myodegeneration (score 1): degenerative necrotic lesions including hyper-eosinophilic muscle fibers with loss of cross-striations and lyses of nuclei (B). Moderate myodegeneration (score 2): severe degenerative necrotic muscle bundles, inflammatory cell infiltrations, fibrous tissue proliferations (C). Severe myodegeneration (score 3): severe degenerative, necrotic muscle bundles, inflammatory cell infiltrations, severe fibrous tissue proliferations and widespread adipose tissue lobules (D).

and moisture ratio than normal fillets ($p < 0.001$). Redness index (a^* value) of fillets having only severe degree myodegeneration was higher ($p < 0.05$) than normal fillets.

4. Discussion

In the study, it was determined that broilers in T2, T3, and T4 groups had significantly lower slaughter weight and breast fillet weight compared to control. These results indicate that the broilers in T2, T3, and T4 groups could not compensate the growth until slaughter age at 49 d of age. Similar to this study, Livingston et al. [22] determined that breast meat weight was lower in the group restricted 8 h a day from 8-day age until the end of trial than ad libitum fed group. However, Urdaneta-Rincon and Leeson [32] determined that broilers in groups received feed as 90% of ad libitum for different periods starting at the age of 14 days, and those fed ad libitum had similar breast meat weight at 49 days of age. Differences between the studies may be related to time, duration, and level of feed restriction applied in the studies. Regarding breast fillet dimensions, the lower values were obtained for fillet length, width, and cranial thickness of broilers in T3 and T4 groups than control group. Caudal thickness was lower in all restricted groups than control; however, the lowest

Table 3. The frequency of broilers having myodegeneration in pectoralis major muscle in histopathological examination.

Treatment		**The occurrence of myodegeneration in pectoralis major muscle**			
	n	N*	%	X^2	p
Control	16	16	100.00[a]		
T1	16	14	87.50[ab]	10.95	0.022
T2	16	13	81.25[ab]		
T3	16	11	68.75[b]		
T4	16	9	56.25[b]		

* Number of the broilers having myodegeneration in pectoralis major muscle.
[a,b]: Values shown with different letter are statistically differ ($p < 0.05$).

Tablo 4. Mean myodegeneration scores of pectoralis major musle of broilers in diffrent groups[1,2].

Treatments	n	Myodegeneration score of pectoralis major muscle
Control	16	2.31 ± 0.22[a]
T1	16	1.75 ± 0.27[ab]
T2	16	1.63 ± 0.27[ab]
T3	16	1.19 ± 0.26[b]
T4	16	1.06 ± 0.28[b]
P		0.013

[1]Values represent mean ± SE.
[2]Data of myodegeneration scores in pectoralis major muscle were analyzed by Kruskal–Wallis test and significance control of differences between groups was determined by Mann–Whitney U test.
[a,b:] Different superscript letters show statistical significant different among the groups ($p < 0.05$).

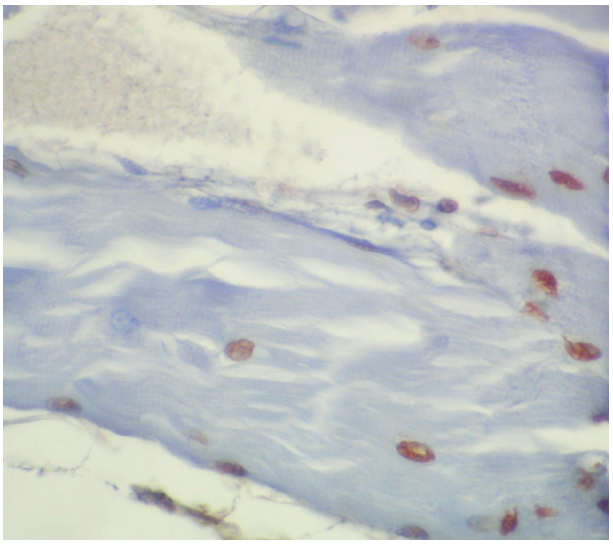

Figure 2. Apoptotic nuclei of pectoralis major musle section. TUNEL assay. 40 X.

mean values in terms of this trait was obtained for T3 and T4 groups. These results are excepted because fillet weight was lower in these groups than control. Fillets showing moderate and severe degree myodegeneration had higher values for heavy, length and width, compared to normal fillets. These results are similar to those of Xing et al. [33]. Cranial thickness of fillets showing mild, moderate, and severe degree of myodegeneration was higher than normal ones. Similarly, some studies showed that fillets with white striping myopathy had the higher weight and thickness than normal fillets [6, 12]. Caudal thickness of fillets showing moderate and severe degree myodegeneration was higher than normal fillets and fillets with mild myodegeneration. This result could be explained that there is bulge at the caudal end of fillets affected by wooden breast myopathy [5]. Mudalal et al. [12] also found that the fillets affected by wooden breast had the higher caudal thickness than normal and white striped fillets.

Feed restriction significantly affected meat quality characteristics in broiler chickens. Broilers in T2, T3, and T4 groups had significantly lower ultimate pH value than those in control. Similar to this study, Livingston et al. [22] also found that the lower pH value in breast muscles of broilers restricted fed, compared to those of ad libitum fed. Previous studies have indicated that breast meats with severe degree white striping and wooden breast had higher ultimate pH value than normal breast meats [11, 15, 34]. Similarly, in this study breast fillets with moderate and

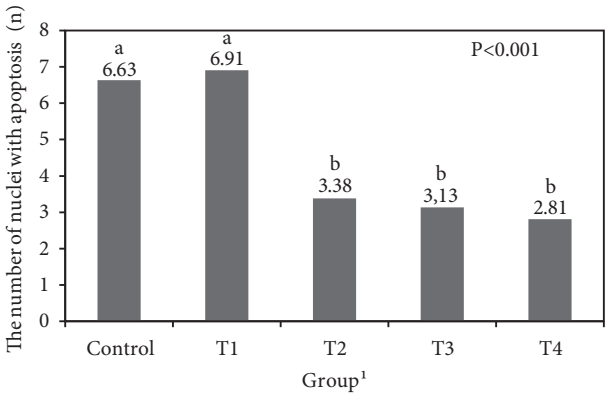

Figure 3. The number of nuclei with apoptosis in pectoralis major muscle of broilers in different groups. [1]Control: Feed was provided freely to broilers in this group during the study. T1: Broilers in this group received feed 80% of amount consumed by control group fed ad libitum from 11 to 24 d. T2: Broilers in this group received feed 70% of amount consumed by control group fed ad libitum from 11 to 24 d. T3: Broilers in this group received feed 80% of amount consumed by control group fed ad libitum from 25 to 39 d. T4: Broilers in this group received feed 70% of amount consumed by control group fed ad libitum from 25 to 39. a, b: Values shown with different letter are statistically differ ($p < 0.001$).

severe degree myodegeneration had significantly higher ultimate pH value than normal fillets ($p < 0.001$). This result is could be due to the fact that fillets with moderate and severe degree myodegeneration were heavier than normal fillets. It has been reported that there is negative correlation between breast meat weight and muscle glycogen level; thus, glycolytic potential is lower in heavier breast muscles, and, consequently, this leads to higher ultimate pH value [14, 15, 35].

Regarding the color values of breast meat, T3 and T4 groups had significantly lower b^* value than control ($p < 0.05$). However, no statistically difference was recorded among the groups in terms of L^* and a^* values. Similar results were obtained by Lippens et al. [36] for feed restricted broilers. On the other hand, Poltowicz et al. [37] found that color values of breast meat in broiler chickens were not affected by feed restriction. In this study, the lower b^*value obtained for broilers in T3 and T4 groups may be explained that breast fillets of broilers in these groups had the lower myodegeneration score than those in control. In this study, it was also determined that fillets with moderate and severity degree myodegeneration had higher b^*value than normal fillets. Similarly, some studies have reported that b^* value of breast muscles exhibiting wooden breast and white striping was higher than normal ones [11, 12, 14, 34, 38]. The higher b^* value in fillets affected these myopathies has been attributed to the higher fat content of these fillets [6, 8, 38, 39]. The presence of adipose tissue lobules in sections of pectoralis major muscles with moderate and severe degree myodegeneration in this study confirm this assumption. In this study, no significant difference was observed between fillets having different degree of myodegeneration and normal ones for lightness. This is in line with previous studies, which have reported that there was no significant difference between fillets with wooden breast and white striping myopathies and normal fillets in terms of L^* value [11, 12, 14]. However, fillets with severe myodegeneration had higher redness index than normal fillets. This result supports the reports of Mutryn et al. [40] who determined that, in wooden breast myopathy, expression of myoglobin genes is high due to fiber type switching, which causes the muscle to become redder. Compared to normal fillets, the higher a^*value for fillets affected by wooden breast was previously reported by some researchers [14, 15, 33].

Breast meat of broilers in T2, T3, and T4 groups had significantly lower cooking loss than control group. This result could be explained that the percentage of breast meats with myodegenration and also myodegeneration severity was lower in these groups. The higher cooking loss values determined in this study for fillets with moderate and severe degree myodegeneration, compared to normal ones, support this result. It is well known that myofibrillar proteins are responsible for the water holding capacity of meat and this value decreases in case of severe myodegeneration [41, 42]. Similar results were previously reported by Petracci et al. [11] for breast meats with severe degree of white striping. Many studies also determined that breast muscles affected by wooden breast abnormality had the higher cooking loss, compared to normal breast muscles [12–15]. It has been reported that the higher cooking loss in wooden breast case could be associated with muscle fiber degeneration followed by fibrosis and lipidosis. Degeneration of muscle fibers can affect water holding capacity of the meat and the changes in fiber membrane integrity may cause fluid loss during cooking [9].

With regards to the chemical composition of breast meat, broilers in T3 and T4 groups had significantly higher protein content but the lower fat and moisture content compared to control group. Similar results have been reported in previous studies [43, 44]. This result could be due to the lower percentage of broilers showing myodegeneration in pectoralis major muscle in these groups than in control group. Similary, many studies have determined that the percentage of protein was lower, whereas the percentage of fat was higher in breast muscles with wooden breast and white striping myopathies than normal breast muscles [9, 10, 39, 41, 45]. Our study also suggested that the percentages of protein were lower,

Table 5. Breast fillet weight and fillet dimensions in different groups of myodegeneration severity.

	Myodegeneration severity[1]					
Traits	Score 0	Score 1	Score 2	Score 3	**SEM**[2]	**p**
	(n = 17)	(n = 21)	(n = 20)	(n = 22)		
Weight (g)	384.33[b]	425.21[ab]	480.25[a]	496.37[a]	12.74	0.007
Length (mm)	188.55[b]	193.42[ab]	197.08[a]	197.69[a]	1.21	0.035
Width (mm)	90.57[b]	98,85[ab]	101,98[a]	107.41[a]	1.76	0.008
Cranial thickness (mm)	37.75[b]	41.81[a]	44.10[a]	45.01[a]	0.63	< 0.001
Caudal thickness (mm)	20.61[b]	22.02[b]	29.73[a]	31.11[a]	0.59	< 0.001

[1]Score 0 = Normal fillet (muscle fibers with a regular diameter,h longitudinally arrayed myofibrilles, cross-striations, in each fiber nuclei were located peripherally under the plasma membrane), score 1 = mild myodegeneration (mild degenerative necrotic lesions in muscle tissue, hyper-eosinophilic muscle fibers with loss of cross-striations and lyses of nuclei), score 2 = moderate myodegeneration (severe degenerative necrotic muscle bundles, inflammatory cell infiltrations, fibrous tissue proliferations and adipose tissue lobules), score 3 = severe myodegeneration (severe degenerative, necrotic muscle bundles, inflammatory cell infiltrations, severe fibrous tissue proliferations and widespread adipose tissue lobules). [2]SEM = pooled standard error of the mean. [a, b,:] Means shown with different superscript letter in the same row are statistically differ ($p < 0.05$).

Table 6. Breast meat quality characteristics in different groups of myodegeneration severity.

Traits	**Myodegeneration severity**[1]				**SEM**[2]	**p**
	Score 0	Score 1	Score 2	Score 3		
	(n = 17)	(n = 21)	(n = 20)	(n = 22)		
pH_{15}	6.54	6.52	6.53	6.55	0.02	0.940
pH_{24}	5.83[c]	5.86[c]	5.92[b]	5.97[a]	0.01	< 0.001
L^*	51.39	50.98	52.05	51.90	0.46	0.835
a^*	2.05[b]	2.21[b]	2.43[ab]	2.79[a]	0.09	0.017
b^*	8.37[b]	9.48[ab]	9.81[a]	10.14[a]	0.21	0.026
Cooking loss (%)	24.69[b]	25.10[b]	27.71[a]	28.55[a]	0.47	0.005
Chemical composition (%)						
Moisture	74.20[b]	74.47[b]	74.90[a]	75.18[a]	0.08	< 0.001
Protein	22.84[a]	22.51[a]	21.33[b]	20.70[c]	0.14	< 0.001
Fat	1.68[c]	1.81[c]	2.59[b]	2.96[a]	0.08	< 0.001
Ash	1.28	1.20	1.18	1.15	0.02	0.090

[1]Score 0 = Normal fillet (muscle fibers with a regular diameter,h longitudinally arrayed myofibrilles, cross-striations, in each fiber nuclei were located peripherally under the plasma membrane), score 1 = mild myodegeneration (mild degenerative necrotic lesions in muscle tissue, hyper-eosinophilic muscle fibers with loss of cross-striations and lyses of nuclei), score 2 = moderate myodegeneration (severe degenerative necrotic muscle bundles, inflammatory cell infiltrations, fibrous tissue proliferations and adipose tissue lobules), score 3 = severe myodegeneration (severe degenerative, necrotic muscle bundles, inflammatory cell infiltrations, severe fibrous tissue proliferations and widespread adipose tissue lobules). [2]SEM = pooled standard error of the mean. [a, b,c,:] Means shown with different superscript letter in the same row are statistically differ ($p < 0.05$).

whereas the percentages of fat and moisture were higher in breast muscles having moderate and severe degree myodegeneration, compared to normal ones. It has been reported that myodegeneration in muscle fibers may cause to decrease in intramuscular protein content, whereas increasing in intramuscular fat content could be due to replacement degenerated of muscle fibers by adipose tissue as a result of lipidosis. On the other hand, the

higher moisture ratio of the fillets with wooden myopathy compared to normal fillets could be associated with edema occurrence due to inflammation [5, 9].

In the study, histopathological examination indicated that the percentage of broilers showing myodegeneration in pectoralis major muscle and also myodegeneration severity were significantly lower in T3 and T4 groups than control group. This result may be explained that the broilers in these groups had lower slaughter weight, breast fillet weight, and also the lower fillet thickness. Similarly, many studies have been reported that the incidence of white striping and wooden breast miyopathies and severity was higher in broilers having higher body weight, breast fillet weight, and fillet thickness [5, 7, 8, 12, 17, 18]. This relationship between white striping and wooden breast myopathies and high growth rate have been explained by the inadequate supply of oxygen and nutrition to muscle cells and also inadequate elimination of intermediate products due to increased muscle fiber size and decreased capillarisation [19–21].

Apoptosis, characterized with internucleosomal cleavage of DNA by a family of intracellular cysteine proteases and morphologically by chromatin condensation, nuclear shrinkage, and apoptotic body formation, is important in a variety of myopathy [46]. The present study showed that apoptotic index was generally low in all groups compared with histomorphological of necrosis, suggesting that possible inflammatory mediators and cytokines released from the inflammatory leucocytes, including nitric oxide, reactive oxygen radicals and other excitotoxic components in inflammatory muscle [28]. In this study, the number of apoptotic nuclei was lower ($p < 0.001$) for T2, T3, and T4 groups than for control group. These data suggest that feed restriction applied to these groups inhibited necrosis as well as apoptosis mechanisms in preventing the formation of myopathy.

The results showed that ad libitum feeding caused significant myodegenerative changes in pectoralis major muscle and also led to a decrease in meat quality characteristics in broiler chickens. Feed restriction to 80% and 70% of ad libitum consumption from 25 to 39 d of age resulted in a decrease in myodegeneration occurrence and severity in pectoralis major muscle at 49 d of age. Hence, these feed restriction practices improved physical and nutritional quality characteristics of breast meat, although there was some reduction in slaughter weight and breast fillet weight. Increasing feed restriction level in this period did not result in any further reduction in myodegeneration occurrence and also improvement in meat quality. Considering all the results, it was concluded that myodegeneration occurrence and severity in breast muscle tissue of broilers at 49 days of age could be decreased, and, thus, meat quality characteristics could be improved by feed restriction to 80% of ad libitum consumption from 25 to 39 days of age. However, this feeding program may result in some reduction in slaughter weight and breast fillet weight. It should be assessed whether the economic loss due to decrease in performance caused by, thus, a feeding program could be compensated by the increase in meat quality. Further studies in commercial conditions are required to develop the management and feeding strategies that may reduce the occurrence of these myopathies with minimal effect on production performance in broiler chickens.

Acknowledgments

This study was financially supported by the Scientific Research Fund of Aydın Adnan Menderes University, with project no: VTF-17027.

Conflict of interest

The authors confirm that there are no conflicts of interest.

References

1. Barbut S, Sosnicki AA, Lonergan SM, Knapp T, Ciobanu DC et al. Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. Meat Science 2008; 79 (1): 46-63. doi: 10.1016/j.meatsci.2007.07.031

2. Kuttappan VA, Hargis BM and Owens CM. White striping and wooden breast myopathies in modern poultry industry: A review. Poultry Science 2016; 95 (11): 2724-2733, doi: 10.3382/ps/pew216

3. Tumova E, Teimouri A. Fat deposition in the broiler chicken: a review. Scientia Agriculturae Bohemica 2010; 41 (2): 121-128.

4. Maiorano G. Meat defects and emergent muscle myopathies in broiler chickens: implications for the modern poultry industry. Review article. Scientific Annals of Polish Society of Animal Production 2017; 13 (3): 43-51. doi: 10.5604/01.3001.0010.5454

5. Sihvo HK, Immonen K and Puolanne E. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Veterinary Pathology 2014; 51 (3): 619-623. doi: 10.1177/0300985813497488

6. Kuttappan VA, Shivaprasad HL, Shaw DP, Valentine BA, Hargis BM et al. Pathological changes associated with stripping in broiler breast muscles. Poultry Science 2013a. 92 (2): 331-338. doi: 10.3382/ps.2012-02646

7. Trocino A, Piccirillo A, Birolo M, Radaelli G, Bertotto D et al. Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of white striping and wooden breast in broiler chickens. Poultry Science 2015; 94 (12): 2996-3004.doi: 10.3382/ps/pev296

8. Kuttappan VA, Brewer VB, Apple JK, Waldroup PW, Owens CM. Influence of growth rate on occurrence of white striping in broiler breast fillets. Poultry Science 2012a; 91 (10): 2677-2685. doi: 10.3382/ps.2012-02259

9. Soglia F, Mudalal S, Babini E, Di Nunzio M, Mazzoni M et al. Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality. Poultry Science 2016; 95 (3): 651-659. doi: 10.3382/ps/pev353

10. Baldi G, Soglia F, Mazzoni M, Sirri F, Canonico L et al. Implications of white striping and spaghetti meat abnormalities on meat quality and histological features in broilers. Animal 2018; 12 (1): 164-173. doi: 10.1017/S1751731117001069

11. Petracci M, Mudalal S, Bonfiglio A, and Cavani C. Occurence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. Poulty Science 2013; 92 (6): 1670-1675. doi: 10.3382/ps.2012-03001

12. Mudalal S, Lorenzi M, Soglia F, Cavani C, Petracci M. Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. Animal 2015; 9 (4): 728-734. doi: 10.1017/s175173111400295X

13. Tijare VV, Yang FL, Kuttappan VA, Alvarado CZ, C. Coon N et al. Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. Poultry Science 2016; 95 (9): 2167-2173. doi: 10.3382/ps/pew129

14. Tasoniero G, Cullero M, Cecchinato M, Puolanne E and Dalle Zotte A. Technological quality, mineral profile, and sensory attributes of broiler chicken breasts affected by white striping and wooden breast myopathies. Poulty Science 2016; 95 (11): 2707-2714. doi: 10.3382/ps/pew215

15. Dalle Zotte A, Tasoniero G, Puolanne E, Remignon H, Cecchinato M et al. Effect of "wooden breast" appearance on poultry meat quality, histological traits, and lesion characterization. Czech Journal of Animal Science 2017; 62 (2): 51-57. doi: 10.17221/54/2016-CJAS

16. Kuttappan VA, Lee YS, Erf GF, Meullenet JFC, McKee SR et al. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. Poultry Science, 2012b; 91 (5): 1240-1247. doi: 10.3382/ps.2011-01947.

17. Kuttappan VA, Brewer VB, Mauromoustakos A, Mckee SR, Emmert JL et al. Estimation of factors associated with the occurrence of white striping in broiler breast fillets. Poultry Science 2013b; 92 (3): 811-819. doi: 10.3382/ps.2012-02506.

18. Lorenzi M, Mudalal S, Cavani C and Petracci M. Incidence of white striping under commercial conditions in medium and heavy broiler chickens in Italy. Journal of Applied Poultry Research 2014; 23 (4): 754-758. doi: 10.3382/japr.2014-00968

19. Petracci M., Mudalal S, Soglia F, Cavani C. Meat quality in fast growing broiler chickens. World's Poultry Science Journal 2015; 71 (2): 363-374. doi: 10.1017/s0043933915000367

20. Sandercock, DA, Mitchell MA. Myopathy in broiler chickens: A role for Ca2 activated phospholipase A (2). Poultry Science 2003; 82 (8): 1307-1312. doi: 10.1093/ps/82.8.1307

21. MacRae VE, Mahon M, Gilpin S, Sandercock DA, Mitchell MA. Skeletal muscle fibre growth and growth associated myopathy in the domestic chicken (Gallus domesticus). British Poultry Science 2006; 47 (3): 264-272. doi: 10.1080/00071660600753615

22. Livingston ML, Landon C, Barnes HJ and Brake J. White striping and wooden breast myopathies of broiler breast muscle is affected by time-limited feeding, genetic bacground, and egg storage. Poultry Science 2019; 98 (1): 217-226. doi: 10.3382/ps/pey333

23. Meloche KJ, Fancher BI, Emmerson DA, Bilgili SF and Dozier III WA. Effects of quantitative nutrient allocation on myopathies of the pectoralis major muscles in broiler chickens at 32, 43 and 50 days of age. Poultry Science 2018; 97 (5): 1786-1793. doi: 10.3382/ps/pex453

24. Aviagen, 2019. Ross 308 Broiler Nutrition Specifications, Aviagen Group, Huntsville, Alabama, USA.

25. Mehaffey JM, Pradhan SP, Meullenet JF, Emmert JL, MecKee SR et al. Meat quality evaluation of minimally aged broiler breast fillets from five commercial genetic strains. Poultry Science 2006; 85 (5): 902-908. doi: 10.1093/ps/85.5.902

26. Honikel KO. Reference methods for the assessment of physical characteristics of meat. Meat Science 1998; 49 (4):447-457. doi: 0309-1740 (98)00034-5

27. AOAC (1990). Official methods of analysis (15th ed). Arlington, VA, USA: Association of Official Analytical Chemists.

28. Radaelli G, Piccirillo A, Birolo M, Bertotto D, Gratta F et al. Effect of age on the occurence of muscle fiber degeneration associated with myopathies in broiler chickens submitted to feed restriction. Poulty Science 2017; 96 (2): 309-319. doi: 10.3382/ps/pew270

29. SPSS (2013). Statistical Packages for the Social Sciences. SPSS base 22.0 user's guide, IBM United States Software Announcement.

30. Steel RGD, Torrie JH. Principles and procedures of statistics. A biometrical approach, 2nd ed. New York, USA: McGraw-Hill Book Company; 1980.

31. Duncan DB. Multiple range and multiple F tests. Biometrics 1955; 11:1-42. doi: 10.2307/3001478

32. Urdaneta-Rincon M and Leeson S. Quantitative and qualitative feed restriction on growth characteristics of male broiler chickens. Poultry Science 2002; 81 (5): 679-688.doi: 10.1093/ps/81.5.679

33. Xing T, Zhao X, Zhang L, Li JL, Zhou GH et al. Characteristics and incidence of broiler chicken wooden breast meat under commercial conditions in China. Poultry Science 2020; 99 (1): 620-628. doi: 10.3382/ps/pez560

34. Kuttappan VA, Brewer VB, Clark FD, Mckee SR, Meullenet JF et al. Effect of white striping on histological and meat quality characteristics of broiler fillets. Poultry Science, 2009; 88 (E-Suppl.1): 136-137. (Abstr.).

35. Le Bilhan-Duval E, Debut M, Berri CM, Sellier N, Sant'e-Lhoutellier V et al. Chicken meat quality: Genetic variability and relationship with growth and muscle characteristics. BMC Genetics 2008; 9: 53-58. doi: 10.1186/1471-2156-9-53

36. Lippens M, Room G, De Groote G and Decuypere E. Early and temporary quantitative food restriction of broiler chickens. 1. Effects on performance characteristics, mortality and meat quality. British Poultry Science 2000; 41 (3): 343-354. doi: 10.1080/713654926

37. Poltowicz K, Nowak J, Wojtysiak D. Effect of feed restriction on performance, carcass composition and physicochemical properties of the M. Pectoralis superficialis of broiler chickens. Annals of Animal Science 2015; 15 (4): 1019-1029. doi: 10.1515/aoas-2015-0036

38. Kuttappan VA, Owens CM, Coon C, Hargis BM, and Vazquez-Anon M. Incidence of broiler breast myopathies at 2 different ages and its impact on selected raw meat quality parameters. Poultry Science 2017; 96 (8): 3005-3009. doi: 10.3382/ps/pex072

39. Petracci M, Mudalal S, Babini E and Cavani C. Effect of white striping on chemical composition and nutritional value of chicken breast meat. Italian Journal of Animal Science, 2014; 13 (1): 179-182. doi: 10.4081/ijas.2014.3138

40. Mutryn MF, Brannick EM, Fu W, Lee WR and Abasht B. Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA sequencing. BMC Genomics 2015; 16: 399. doi: 10.1186/s12864-015-1623-0

41. Mazzoni M, Petracci M, Meluzzi A, Cavani C, Clavenzani P et al. Relationship between pectoralis major muscle histology and quality traits of chicken meat. Poultry Science, 2015; 94 (1): 123-130. doi. 10.3382/ps/peu043

42. Xiong, Y. L. Protein functionality. In: Jensen WK (editor). Ency-clopedia of Meat Sciences. Oxford, UK. Elsevier; 2004. pp. 218-225.

43. Zhan XA, Wang M, Ren H, Zhao RQ, Li JX et al. Effect of early feed restriction on metabolic programming and compensatory growth in broiler chickens. Poulty Science 2007; 86 (4): 654-660. doi: 10.1093/ps/86.4.654

44. Omosebi DJ, Adeyemi OA, Sogunle MO, Idowu OMO and Njoku CP. Effects of duration and level of feed restriction on performance and meat quality of broiler chickens. Archivos de Zootecnia 2014; 63 (244): 611-621. doi: 10.4321/S0004-05922014000400005

45. Mudalal S., Babini E, Cavani C, Petracci M. Quantity and functionality of protein fractions in chickens breast fillets affected by white stripping. Poultry Science 2014; 93 (8): 2108-2116. doi: 10.3382/ps.2014-03911

46. Tews DS. Role of Apoptosis in Myopathies. Basic and Applied Myology 2003; 13 (4): 181-190.