Restricted Feeding Regimens Improve White Striping Associated Muscular Defects In Broiler Chickens

Hammed Ayansola (Ls20193040044@cau.edu.cn)
China Agricultural University College of Animal Science and Technology https://orcid.org/0000-0002-9932-654X

Xiaoxiao Yu
China Agricultural University College of Animal Science and Technology

Jiaqi Lei
China Agricultural University College of Animal Science and Technology

Chaoyong Liao
China Agricultural University College of Animal Science and Technology

Yuming Guo
China Agricultural University College of Animal Science and Technology

Bingkun Zhang
China Agricultural University College of Animal Science and Technology

Bo Wang
China Agricultural University College of Animal Science and Technology https://orcid.org/0000-0003-0604-1607

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Abstract

Background:

Ad libitum (AD) feeding is crucial to profitable commercial broiler chicken production. However, it partly disrupts muscle development, causing myopathies like white striping in broilers' breast meat. For this reason, this study investigated the impacts of intermittent feeding (IF) and fasting strategies as potential alternatives to AD feeding. A total of 384 one-day-old broilers were randomly allotted into 4 groups - *ad libitum*, 1h-IF group (4 times/day, 1 hour each time), 1.5h-IF (4 times/day, 1.5hrs each time), and acute fasting (1-day acute fasting, 6-days free access to feed). Feed intake, weight gain, muscle structure, differential genes, and protein expressions were assessed in the broiler breast muscles.

Results:

IF and fasting significantly reduced ectopic fat deposit and muscle fiber size ($p < 0.05$). Notably, 1.5h-IF promoted $PAX7^+$ satellite cell proliferation supporting muscle growth and repair activities in fast-growth broiler chickens. Consistently, the restricted regimens downregulated the collagen protein synthesis of skeletal muscle-specific E3 ubiquitin ligases (TRIM63 and MAFBX) in 42 – days old breast muscle samples ($p < 0.05$), especially in the 1.5h-IF group. Compared to AD-fed birds, 1.5h-IF and fasting feeding significantly decreased white striping scores in the breast meat muscle ($p < 0.05$).

Conclusion

Chronic IF or acute fasting improved muscle health of broiler chickens without significant compromise on growth rate and feed efficiency compared to AD feeding. Therefore, this study presents potential feeding frequencies relevant for optimal growth pace while alleviating the occurrence of myopathic pathophysiology in broiler chickens.

1.0 Introduction

Ad libitum (AD) feed consumption exacerbates metabolic perturbations in broiler chickens (1). For example, excess energy consumed by broilers during unrestricted access to feed is converted into visceral and ectopic fats, which are harmful to health and meat quality (2, 3). As a result, researchers are currently considering alternative feeding strategies to alleviate meat defects in commercial broiler chicken production (4, 5). Therefore, it is pertinent to investigate effective feeding strategies that can optimize the lean meat qualities of broiler chickens.

Meat quality characteristics are a major concern for consumers of broiler's carcass. Previous findings revealed that increased growth rate, as seen in AD-fed birds, results in higher degrees of meat myopathies (6–9). Specifically, in past studies, muscular myopathies arise from lipidosis and collagen accretion, purportedly linked with AD feeding (10, 11). In support of this assumption, findings revealed that breast muscle myopathies, including white striping (WS) and wooden breast (WB) (4, 8), occur due to muscle hypertrophy resulting from the fast growth in birds with unstrained access to feeds (12). This voluntary feed

consumption leads to excess ectopic fat deposit and enlarged muscle fiber sizes that disrupt muscular compositions and decrease vascular density in the breast muscle region (13). Notably, these muscular defects contribute to the etiologies of WS-affected broiler's meat quality (14, 15). To solve growth-related muscular myodegeneration, Velleman and colleagues altered the timing of feeding to elucidate the fate of fat deposit and adipogenic transcription factors expression in pectoralis major muscle (3). Their result revealed that 20% feed restriction during week 2 eliminated excess fat deposit, and they concluded that appropriate feed restriction strategies could positively improve the ratio of fat to lean meat and expression of adipogenic genes.

To date, there are limited studies that have investigated the intermittent feeding strategies effects on meat quality features of broiler chickens. This is based on the assumption that restricted feeding will reduce growth performance which is the primary goal of broiler producers. However, Fondevilla et al. (16) reported that broilers restricted from eating for less than 6 hours per day showed similar body weight gain compared with those birds fed *ad libitum.* Likewise, Fargly et al. (17) previously demonstrated that 4 hours of intermittent feeding does not decrease broiler growth performance at the end of the experimental study. The prominent argument for these results is that broilers are likely to adapt quickly during restricted feeding regimens over a prolonged period, thereby attaining full compensatory growth performance (16).

In addition to improved metabolic activities, we could deduce that intermittent feeding might produce similar growth performances like AD feeding in broiler chickens if designed appropriately. Therefore, the current study investigated the impacts of intermittent feeding frequencies and acute fasting on broiler's breast muscle physiology, focusing on histomorphology, muscle growth and differentiation markers, and protein expression related to muscular defects. The findings in the current study provide relevant information to poultry farmers, meat scientists, and potential consumers about the benefits of alternative feeding strategies on the muscular health of broiler chickens.

2.0 Materials And Methods

2.1 Experimental Design and management

The research was conducted at the poultry Unit, Zhuozhou research center, China Agricultural University. All experimental procedures followed the guidelines approved by the China Agricultural University Laboratory animal welfare and experimental ethical committee.

For this research, a total of 384 1-day male old chicks were brooded and acclimatized for eight days. The chicks were raised in a controlled environment with 24 hrs light supply, and the room temperature was periodically adjusted to ensure optimum room temperature for brooding purposes. During this period, *ad libitum* feed and clean water were supplied 24 hours a day.

The birds were randomly distributed using a completely randomized experimental design into four groups with eight replicates that contained 12 birds each as *ad libitum* (AD), 1h-IF, 1.5h-IF, and fasting groups, respectively. During the experiment periods, AD feeding served as the control diet, while alternative feeding techniques comprised (1h-IF) intermittent 1-hour, (1.5h-IF) intermittent 1-hour 30 min, and 6:1-day fasting

schedules (skip a day fasting). The 1h-IF and 1.5h-IF groups were subjected to 4 times daily intermittent feeding schedules as follow: 07.00-08.00, 12.30-13.30, 17.30-18.30, 22.30 until the light was switched off at 00.00 midnight, and 07.00-08.30, 12.30-14.00, 17.30-19.00, 22.30 until the light was switched off at 00.00 respectively. All birds were fed a standard commercial diet throughout the experiment based on the National Research Council (NRC) recommendations for broiler chickens (Table 1). The data recorded during the rearing period include daily and weekly growth performance data such as daily feed intake, weekly feed intake, body weight, and weight gain, and feed conversion rate for all groups.

Table 1
Composition of Experimental diets and nutrient contents

INGREDIENTS g/100g	day 1 – 21	day 22 – 42
Corn	57.50	60.00
Wheat offal	1.00	0.0
Soybean meal	33.21	27.85
Cottonseed meal	2.00	2.50
Corn gluten meal	0.00	2.00
Soybean oil	2.00	4.0
Methionine	0.27	0.18
Lysine	0.50	0.28
98.5 L-Threonine	0.20	0.07
Calcium Hydrogen Phosphate	1.40	1.10
Limestone	1.20	1.30
50% Choline	0.10	0.10
Vitamin premix*	0.05	0.05
Mineral supplement**	0.20	0.20
Salt	0.30	0.30
Phytase	0.02	0.02
Santoquin	0.03	0.03
Antioxidant	0.02	0.02
Total	**100**	**100**
Nutrient content		
Energy AME (Mcal/kg)	2.88	3.04
Crude protein (%)	20.11	18.99
Lysine (%)	1.48	1.19
Methionine (%)	0.58	0.48

The following supplements are included/kg in diet: *Vitamin premix provides following per kg diet: Vit. A, 9500 IU; Vit. D3, 62.5 ug; Vit. E, 30 IU; Vit. K3, 2.65 mg; Vit. B1, 2 mg; Vit B6, 2 mg; Vit. B12, 0.025 mg; Biotin, 0.0325 mg; Folic Acid, 1.25 mg; Pantothenic Acid, 12 mg; Nicotinic Acid, 50 mg. **Mineral premix - Cu, 8 mg ($CuSO_4 \cdot 5H_2O$); Fe, 80 mg ($FeSO_4$); Mn, 100 mg ($MnSO_4 \cdot H_2O$); Se, 0.15 mg (Na_2SeO_3); I, 0.35 mg (KI). The feeds were formulated based on the recommendation by the NRC (1994)

INGREDIENTS g/100g	day 1 – 21	day 22 – 42
Ca (%)	0.89	0.84
Available Phosphorus (%)	0.39	0.33
The following supplements are included/kg in diet: *Vitamin premix provides following per kg diet: Vit. A, 9500 IU; Vit. D3, 62.5 ug; Vit. E, 30 IU; Vit. K3, 2.65 mg; Vit. B1, 2 mg; Vit B6, 2 mg; Vit. B12, 0.025 mg; Biotin, 0.0325 mg; Folic Acid, 1.25 mg; Pantothenic Acid, 12 mg; Nicotinic Acid, 50 mg. **Mineral premix - Cu, 8 mg ($CuSO_4 \cdot 5H_2O$); Fe, 80 mg ($FeSO_4$); Mn, 100 mg ($MnSO_4 \cdot H_2O$); Se, 0.15 mg (Na_2SeO_3); I, 0.35 mg (KI). The feeds were formulated based on the recommendation by the NRC (1994)		

2.2 Sample collections

Before tissue sample collections, two birds per replicates were fasted overnight, selected based on average body weight from each cage, tagged, and weighed individually. On days 21 and 42, the selected birds fasted overnight were slaughtered for sample collection, including blood, leg muscle, thymus, gizzard, glandular stomach, spleen, bursa of fabricus, abdominal fat, and breast muscles. The samples were frozen with liquid nitrogen and stored at -80 ^{0}C for real-time PCR assay or in 4% paraffin solution for histomorphometry indices used for further experimental analysis. In addition, on day 42, we took images of sampled broiler breast fillets for further visual white striping scoring in accordance to Kuttappan et al. (6) with adjustments such that the grading scale adopted here represented normal (0-1), mild (2–3), moderate (3–4), and severe scores (5).

2.3 RNA extraction and Real-Time quantitative PCR

According to (Invitrogen Life Technologies, Carlsbad, CA, USA) protocol, the tissue samples stored in -80 ^{0}C were homogenized for total mRNA isolation in Trizol reagent; subsequently, the mRNA concentration and quality were measured with a Nano-300 spectrophotometer. The total mRNA (~1000 ng) was reverse transcribed using the Beyotime biotechnology cDNA synthesis kit. Finally, the MyIQ2 real-time PCR machine was carried out using SYBR – Green Supermix from Beyotime using *GAPDH* as the reference gene. The relative expressions of the targeted genes were calculated in fold changes to the AD group (18), while the gene primer sequences designed with Primer-BLAST are provided in Table 2.

Table 2
List of primer sequences for quantitative real-time PCR

Gene names	Primer ID	Primer Sequence (5’ – 3’)	
MYOD	NM_204214.2	**F**: CACGGAATCACCAAATGACCCA	**R**: GCAGTTGGTGGGGGAAGGAAT
MYF5	NM_204184.1	**F**: CTCCGATGTGATGGCGGACT	**R**: TCCACGATGCTGGAGAGGCA
MYOG	NM_204184.1	**F**: ACCACAACCTGCTGCACCCA	**R**: TCCACGATGGAGGAGAGCGA
PAX7	NM_205065.1	**F**: TCAGCTACCGGACACGAGAGA	**R**: GGGTGGACACTTCCAAAGGGA
HIF-A	NM_204297.1	**F**: ACCAGCAGTTCCTCATGCAATA	**R**: CATCTTTGGCCGGCGGTAGT
EGF	NM_001001292.1	**F**: CCCGTTGCTTTCTTGCCAGT	**R**: GGAATGGTGCAGGGTCATTTACG
VEGFR-1	NM_204252.1	**F**: TTTTCCTTGGGCGCCTCTCC	**R**: CTTGCCTTCCTGTTGCACGC
IL1B	NM_204524.1	**F**: TGCCTGCAGAAGAAGCCTCG	**R**: TCGAAGGACTGTGAGCGGGT
IL18	NM_204608.1	**F**: ATGAGCTGGAATGCGATGCCT	**R**: TGAAGGCGCGGTGGTTTTGT
IL6	NM_204628.1	**F**: GCTGCAGGACGAGATGTGCAA	**R**: TCTGAAAGGCGAACAGGCCG
FABP4	NM_204290.1	**F**: ATATGAAAGAGCTGGGTGTGGGG	**R**: CTGCTTCAGTGTGCCACTGTCT
CEBPα	NM_001031459.1	**F**: TCGGCGACATCTGCGAGAAC	**R**: CGTGCATGCCGTGGAAATCG
CEBPβ	NM_205253.2	**F**: CAGTAGCGGCGGCCAAGATT	**R**: TCTCGAAGACCGGCTCCACT
ZNF423	XM_025154318.1	**F**: GACAGCAGTGCTCCCAAGTGT	**R**: CTCCCAGGCCACCTGATTGA
PDGFR-α	NM_204749.2	**F**: GCGCGTTATAAAGGAGGAGCTGT	**R**: GGCCACTGTTGTTCTCCTCGT
PPAR-γ	XM_015292931.2	**F**: AGGGAACAGTTTCTCCGGCTG	**R**: GCTCCATTTTGATTGCACTTTGGC
GAPDH	NM_204305.1	**F**: TGACGTGCAGCAGGAACACTA	**R**: GCGGCCAATACGGCCAAATC

2.3 Histomorphology and Immunohistochemistry

The fixated breast muscles were subsequently embedded in paraffin wax, cut at 5μm using Leica microtome, then mounted on slides. Next, the mounted tissue slides were dewaxed, dehydrated, stained with Hematoxylin and Eosin for H&E staining (histology composition and structure image) (19) or in Picrosirius red solution for collagen fiber evaluations (20), then dehydrated, and cleared in xylene and subsequently quantified using ImageJ software. Immunohistochemistry staining was used for $PAX7^+$ density in the breast muscle tissues (21). Briefly, after deparaffinization, sectioned tissues were blocked in 10% goat serum in TBST containing 0.5% Triton X-100. The blocked tissues were incubated overnight at 4 ^{0}C with anti-$PAX7^+$ antibody (1:40, Developmental Studies Hybridoma Banks) and subsequently counterstained with

corresponding secondary antibody for 1hr at room temperature. Finally, sections were mounted in a fluoroshield mounting medium with DAPI (Cat No: C1002, Beyotime Biotechnology, China), then immediately viewed using an EVOS enabled fluorescence microscope (ECHO model: RVL-100-G).

2.4 Triglyceride assay

The triglyceride (18) content levels in the frozen breast samples were used as a marker to measure ectopic fat deposits in the chicken breast using the Folch extraction method (22). In summary, the breast muscle tissue samples (~ 50 mg) were homogenized in 2:1 chloroform: methanol solution, centrifuged at 7,000 rpm, 4 ^{0}C for 10 min, washed in 0.9% NaCl solution, then centrifuged again at low speed (2000 rpm) to separate into 2 phases – upper salt and methanol layer, while the lower phase contains the chloroform. The chloroform layer containing the lipid content was freeze-dried, resuspended in ACS-graded 2-propanol, followed by absorbance reading at 540 nm using Dongou TG reagent kit (Cat No: A0-10017).

2.5 Protein expression using the Western blotting technique

The protein content in the breast muscle was quantified according to western blot protocol. Briefly, the protein was digested using a lysis buffer containing RIPA, protease, and phosphatase inhibitors. The extracted protein lysate was separated by 10% SDS page and electroporated onto the nitrocellulose membrane. This is followed by blocking in 5% Milkfat: TBST solution for an hour and subsequently incubated in primary antibodies at 4°C overnight, including β-TUBULIN (Cat No: AF1216), NF-KB (AF5243), and CASPASE-3 (Cat No: AC030-1), purchased from Beyotime Biotechnology, while TRIM63 (Cat No: A3101), and FBXO32 (Cat No: A3193) were purchased from ABclonal Biotechnology. The membrane was washed with TBST buffer (137 mM Sodium chloride, 2.7 mM Potassium chloride, 50 mM Tris-HCl, and 0.1% Tween 20) before being incubated with HRP-labeled secondary antibody (1:5000, Shanghai Beyotime, Shanghai, China). The blotting bands were developed using a Super Enhanced Chemiluminescence (Shanghai Beyotime, Shanghai, China) and quantified using ImageJ software (NIH, USA).

2.6 Statistical Analysis

The data using one – way ANOVA tool of SPSS v20.0 software are presented as means and standard error of the mean. Duncan's test compared the statistical differences among individual means at $p < 0.05$ as the significance level. The graphical representations were designed with GraphPad Prism 8.0.2 software package.

3.0 Results

3.1 Intermittent feeding and fasting reduced white striping of chicken breast without affecting growth performance

Unsurprisingly, intermittent and fasting groups significantly decreased the average daily feed intake consumed during weeks 2 – 5 compared to the *ad libitum* (AD) group (Table 3, $p < 0.05$). Despite that, alternative feeding strategies did not impair the growth (Figure 1A and B, $p > 0.05$) or feed conversion ratio

(Table 4, $p > 0.05$) of birds when compared to the AD feeding. Overall, the data revealed that the alternative feeding strategies had statistically similar effects on growth and weight gain on broiler chickens.

Table 3
Effects of intermittent feedings and fasting on feed intake levels in broiler chickens

(g/b/d)	*Ad libitum*	1Hour	1.5Hour	Fasting	SEM	p-value
week 1	20.32	19.68	20.12	20.19	0.26	0.843
week 2	52.66^{a}	45.22^{b}	45.14^{b}	51.49^{a}	0.82	<0.001
week 3	79.35^{a}	74.19^{b}	75.04^{b}	73.34^{b}	0.78	0.028
week 4	124.75^{a}	112.56^{b}	110.18^{b}	111.04^{b}	1.46	<0.001
Week 5	158.70^{a}	149.63^{b}	149.02^{b}	138.68^{c}	1.89	0.001
Week 6	159.15	146.33	152.19	150.14	2.27	0.231

Means in the same row with different superscript letters differ significantly ($p < 0.05$, n = 8, mean ± SEM; g/b/d – gram/bird/day).

Table 4
Feed conversation ratio of broiler chickens fed different feeding strategies

	Ad libitum	1Hour	1.5Hour	Fasting	SEM	p-value
Week 1	1.24	1.19	1.22	1.25	0..02	0.444
Week 2	1.26	1.20	1.17	1.24	0..01	0.134
Week 3	1.68	1.72	1.76	1.80	0.03	0.577
Week 4	1.43	1.34	1.30	1.45	0.03	0.137
Week 5	1.64	1.62	1.76	1.63	0.04	0.628
Week 6	1.70	1.82	1.78	1.89	0.20	0.299

Means in the same row with different superscript letters differ significantly ($p < 0.05$, n = 8, mean ± SEM)

Foremost, no differences were observed in the weight of tissues or organs, including leg muscle, thymus, gizzard, glandular stomach, spleen, and bursa of fabricus at 21 or 42 days old (Table 5, $p > 0.05$). However, 1h-IF and fasting significantly reduced abdominal fat of 21-days old birds (Table 5, $p < 0.05$). While the breast muscle mass of 1h-IF, 1.5h-IF, and fasting birds was smaller than that of the AD birds on day 21 (Figure 1C, $p < 0.05$), there were no differences in day 42 samples (Figure 1D, $p > 0.05$). Using phenotypic scoring metrics for white striping (WS), only I.5-h IF and fasting significantly reduced the WS appearance on the breast muscle fillets (Figure 1E and F, $p < 0.05$). Though 1h-IF reduced the WS scores numerically, the changes were insignificant compared to the AD birds (Figure 1F, $p > 0.05$).

Table 5
Intermittent feeding and fasting effects on carcass and organs of broiler chickens

21 days (g/kg)	*Ad libitum*	1Hour	1.5Hour	Fasting	SEM	p-value
leg muscle	67.48	74.94	68.78	68.22	1.30	0.169
thymus	1.20	1.13	1.18	1.12	0.05	0.938
gizzard	15.91	15.63	14.61	16.07	0.28	0.266
glandular stomach	5.07	4.36	4.67	4.87	0.13	0.219
abdominal fat	10.10^{a}	7.08^{c}	10.47^{a}	7.64^{bc}	0.49	0.017
spleen	1.70	1.40	1.49	1.23	0.08	0.170
bursa of fabricus	1.11	1.15	1.13	0.99	0.05	0.747
42 days (g/kg)						
leg muscle	72.61	71.42	69.30	73.53	1.69	0.858
thymus	1.27	1.48	1.36	1.37	0.06	0.643
spleen	1.06	1.24	1.23	1.20	0.05	0.453
gizzard	10.27	11.04	9.99	9.44	0.25	0.152
abdominal fat	14.07	12.73	10.38	11.52	0.60	0.141
glandular stomach	3.32	3.84	3.08	3.76	0.14	0.183
bursa of fabricus	0.45	0.49	0.48	0.42	0.02	0.726

Means in the same rows with different superscript letters differ significantly – $p < 0.05$ mean ± SEM

3.2 Intermittent feeding and fasting effect on broiler chickens muscle development and growth

To understand the effect of the feeding regimens on muscle structure, the H&E staining and frequency distribution data revealed that the breast muscle samples from the AD group had the largest fiber diameter size than the rest of the trial feeding strategies (Figure 2A - C). Specifically, 1.5h-IF significantly decreased the percentage frequency distribution of larger fiber sizes compared to the AD group (Figure 2B and C, $p < 0.05$). Thus, the alternative feedings could reduce breast muscular hypertrophy in broiler chickens.

Subsequently, expressions of myogenic marker genes suggest that the 1.5h-IF strategy promotes higher myogenic capacity in broiler breast muscle. The quantifications of qPCR differential expressions data (Figure 2D), including *MYOD, MYF5, MYOG, HIF-1a, EGF*, and *VEGFR-1*, showed that 1.5h-IF-strategy significantly upregulated *MYF5* and *MYOD* genes compared to the *ad libitum* group in the day 21 breast muscle samples. Similarly, angiogenic markers in the breast muscle showed that both *HIF1A* and *VEGFR-1* genes were significantly upregulated by intermittent feeding in the 1.5hour group ($p < 0.05$), while only the EGF gene recorded no significant differences on day 21 across the group. In contrast, no significant

differences in the myo-genes expressed on day 42 in the breast muscle samples (Figure 2E, $p > 0.05$). However, by using immunohistochemistry staining for day 42 breast muscle samples, the result revealed that 1.5h-IF increased *PAX7*$^{+}$ satellite cells, demonstrating improved myogenesis rather than hypertrophic muscles observed in the AD group (Figure 3A and B, $p < 0.05$).

3.3 Intermittent feeding mitigated ectopic fat deposit in breast muscle of broiler chickens

Most significantly, the effects of intermittent feeding on TG as an indicator of lipid fractions in the breast muscle are shown in Figure 4A. The data revealed that AD feeding significantly increased TG concentration than intermittent and fasting feeding strategies in the pectoralis major region (Figure 4A, $p < 0.05$). The breast muscle samples of 1.5h-IF had the lowest TG concentration (mg/g). As presented further in Figure 4B, it was observed that lipid metabolism marker genes, including *FABP4, C/EBP-A, C/EBP-B*, are not differentially expressed in 42-day old breast muscle samples ($p > 0.05$). Also, only *ZNF423* and *PDGFR-A* involved adipogenesis were significantly downregulated in the 42-day old breast muscle samples of both 1h-IF and fasting birds (Figure 4B, $p < 0.05$).

3.4 Intermittent feeding and fasting alleviated muscle degradation and myofibrosis

As a result of the overwhelming fast growth in broiler chickens, there is a tendency for the poor repair of tissue damages, leading to cell death. The histomorphological area covered by collagen using picrosirius red staining data showed that both chronic intermittent feeding and fasting strategies alleviated collagen accretion compared to AD feeding (Figure 5A and B, $p < 0.05$). To validate the collagen staining data, the western blot protein fold changes depicted that fibrotic-related protein markers, including TRIM63 and MAFBX, were significantly decreased in the 42-day old breast muscle samples of 1h-IF, 1.5h-IF, and fasting group (Figure 5C-F, $p < 0.05$). Also, an elevated CASPASE 3 synthesis – a protein marker for apoptosis – indicates protein degradation activities in AD-fed birds, while significantly suppressed by fasting and slightly decreased by IF regimens (Figure 5G, $p < 0.05$). As such, the current data demonstrate that the alternative feeding strategies significantly reduced collagen fiber accretion in breast muscle, thereby improving the meat fillet qualities of broiler chickens.

4.0 Discussion

Feed restriction strategies are common management practices in broiler breeders (4), but there are variations in their effects in broiler chicken productions (4, 16, 23). On this account, the current study clarifies the discrepancies of intermittent feeding effects on broiler chickens for commercial production purposes. Specifically, this study explores the impacts of intermittent feedings and fasting strategies on broiler chickens' growth indices, histological and molecular mechanisms linked with WS phenotype in breast muscle meats.

The feed intake data obtained in the current study are consistent with previous research suggesting that restricted feeding may suppress feed consumption levels in broiler chickens (24, 25). Despite the reduced

feed intake observed in our study, the alternative feeding strategies did not impair growth indices. During the feed restriction period, birds could easily adjust to changes in feeding patterns by temporarily storing feeds in the crop and subsequently prolonging digestion in the proximal region of the intestine (26, 27). These results could be attributed to an adaptive mechanism deployed over time by birds fed the alternative feeding strategies, which show improved performance than Velele (23) study that reported stunted growth in restricted fed broilers. Previous findings have also suggested the possibility of compensatory growth rate reprogramming during restricted feeding strategies in broilers production over a long period (28–30). Consistently, our results further reveal that intermittent feedings did not significantly decrease the breast meat yield at the market age, i.e., 42 days of age, even though the AD feeding had the highest weight for breast meat. This observation, in fact, corresponds with the final body weight across the groups and also aligns with Jahanpour et al. (31) that previously suggested that up to 75% of restricted feed regimens had a neutral or non-detrimental effect on broiler's breast meat yield.

More importantly, consumers are concerned about breast meat quality, accounting for more than 67% of broiler carcass parts. Thus, the current study focuses on the impacts of chronic IF on breast meat quality related to the WS-associated myodegeneration (32, 33). Enlarged breast muscle fiber size, one of the precursors of WS development, has previously been linked with meat hardness and muscular defects such as fibrosis (34, 35). Several researchers agree with this proposition as observed in the AD group (36, 37). On this premise, this study shows that 1.5h-IF reduced the fiber diameter in the breast muscle region, which aligns with the meat quality criterion for improved meat tenderness. The idea has been that enlarged fiber size predisposes the breast muscle region to poor vascularization and lipidosis, consequently leading to metabolic disorders that characterize severe hypertrophic muscle in broiler pectoralis major (14). The reduced muscle fiber diameter in the 1.5h-IF group could promote vascular tone and ensure proper muscular development by increasing interstitial space for vascular tissues without significantly affecting relative breast weight in broiler chickens (38, 39).

Larger fiber sizes attributed to AD feeding predispose muscles to myodegeneration (32, 33). In the current study, the molecular analysis further supports the beneficial effects of intermittent feeding on meat quality compared to AD feeding. The analyses elucidated their roles on myogenic regulatory factors and inflammatory cytokines. The gene expression data revealed no significant differences among all the feeding strategies. These results suggest that chronic intermittent feeding had a similar regulatory effect on myogenic markers such as *MYF5, MYOD*, and *PAX7* gene expressions (40). Both *MYF5* and *MYOD* are involved in muscular cell growth and development (41), whereas *PAX7*, a skeletal muscle satellite cell, supports continuous cell proliferation for protein turnover in the pectoralis major region (42, 43). As revealed by the immunohistochemistry staining, $PAX7^{+}$ proliferation density was significantly compromised by AD feeding. With this data, we could extrapolate that decreased $PAX7^{+}$ density resulted in poor muscular differentiation and repair activities during the later phase of AD birds that caused hypertrophic muscle defects (41, 44). From the histology standpoint, IF might contribute to normal muscle growth essential for optimal muscular cell proliferation, maintenance, and repair during broiler production. Moreover, the myogenic markers examined in this research prove that, despite the lower feeding rate, chronic IF strategies had comparable effects on myogenesis (45).

To understand the effects on fat deposits, lipid metabolic markers were examined in the breast muscle samples. As reported by Papah et al. (46), functional analysis revealed that lipid metabolism-related genes are elevated in the myopathic tissues of broiler chickens. Though the lipid uptake and transport-related genes, including *PABP4, C/REP-A*, and *C/REP-B*, were not significantly altered in the current study. However, the relative upregulations of the adiposis-related genes (*ZNF423* and *PDGFR-A*) in AD birds compared to 1h-IF and fasting birds align with a previous whole-genome sequencing that reported a potential higher degree of intermuscular fat in the pectoralis major samples of fast growth broiler chickens (47). *ZNF423* and *PDGFR-A* gene expressions increased pre-adipocyte cells, contributing to muscular adiposity development during muscular disruption as detected in the current study (27, 48, 49). Consistently, the results here also reveal that both chronic intermittent feedings and fasting feeding decreased the TG level – a metabolic indicator of ectopic fat deposit in the breast muscle samples. The excess TG accumulation could exacerbate lipotoxicity in the breast muscle, which is composed mainly of Type IIB non-oxidative muscle (46, 50). From the intramuscular energy supply standpoint, decreased intramuscular TG levels in IF and fasting birds were likely mobilized to provide energy for muscular activities during the restricted feeding period (51). Note, excess TG accumulation causes lipid peroxidation in meat fillets leading to oxidative stress (52) in relation to the etiology of myopathic lesion development (11, 53, 54). These observations, therefore, corroborate lipid-laden perturbation in WS and WB-affected muscle during severe conditions (55). In light of this understanding, we suggest that the IF regimens used in the current study could alleviate lipid spoilage in broiler breast meat.

Besides, collagen accretion in the breast muscle indicates poor cell repair activities that cannot sustain the fast growth rate associated with AD feeding. Interestingly, our results show that IF and fasting feeding significantly decreased TRIM63 and MAFBX protein expressions. These atrophic proteins are responsible for muscular degradation, causing tissue fibrosis and muscle wasting during chronic muscular stress related to hypertrophy in fast-growing broiler chickens (48, 53). Moreover, birds fed restricted feeding regimens showed lower apoptotic activities, demonstrating relieved muscular repair processes. It is likely that the higher muscle protein breakdown in the AD group triggers the increased abundance of CASPASE 3, which might aggravate muscular damage in severe conditions, as reported in a previous study that provided evidence of apoptotic liver damage of birds affected by WB myopathy (56). This means that the enhanced protein degradations in the AD group profoundly contribute to higher collagen aggregate than alternative feeding regimens. Within this context, we could infer that intermittent feeding and fasting improves muscle growth and repair processes in the pectoralis major of broiler chickens (53, 57). This proposition agrees with previous findings that demonstrated timing schedules alleviate collagen modifications and fibrotic myopathy affecting abnormal breast muscles (35, 58, 59).

Although 1h-IF had an insignificant effect on the WS scoring in the current study, both 1.5h-IF and fasting strategies alleviated the WS appearance significantly compared to AD feeding. As generally believed, the fast growth rate is potentially recognized as the fundamental cause of WS (8, 53). In the past, feeding strategies such as lowering energy in diets (6), 85% *ad libitum* feed restriction (60), and 8 hours daily feed restrictions (61) have alleviated WS phenotype at the end of the studies (62). These previous findings further support why the alternative feeding strategies investigated in this study could produce breast meat fillets

with improved muscular conditions. To sum it all, when applied appropriately, chronic IF could potentially solve myopathies associated with fast growth muscle hypertrophy in the future.

Conclusion

The current study demonstrates that *ad libitum* feeding has greater detrimental effects on breast meat quality because it partly contributes to higher ectopic fat deposit and exacerbates fibrotic tissue development, potentially leading to WS myodegeneration in the pectoralis major of fast growth broiler chickens. Contrarily, the chronic intermittent feeding strategies, especially the 1.5h-IF, could serve as an alternative feeding approach to AD feeding when breast meats with less ectopic fat and softer steak are considered. Although AD had numerically higher weight gain values, the 1.5-hour IF strategy had statistically similar feed efficiency, final body weight, and relative breast muscle carcass cut at a decreased feed consumption rate. For these reasons, this study suggests that the 1.5h-IF could alleviate production concerns, particularly broiler's meat quality caused by AD feeding in broiler chickens. Hence, the results presented here would serve as a reference for future researches. Accordingly, further studies should explore the modulatory effect of 1.5h-IF with or without slight changes in nutritional diets fed to the chickens. In line with these propositions, subsequent researches should focus on elucidating the 1.5hour intermittent feeding effects at the metabolomic and proteomic levels for in-depth knowledge.

Abbreviations

AD: *Ad libitum*

WS: White striping

WB: Wooden breast

IF: Intermittent feeding

TG: Triglycerides

Declarations

Ethics approval and consent to participate

All experimental procedures, including animal management, housing, slaughtering, and laboratory analysis, followed the guidelines approved by the China Agricultural University Laboratory animal welfare and experimental ethical committee.

Consent for publication

Not applicable

Availability of data and materials

All data supporting our findings are included in the manuscript.

Competing interests

The authors declare that there are no conflicts of interest.

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Author contributions

Hammed Ayansola and Wang Bo conceptualized the study idea and experimental design. Hammed Ayansola and Jiaqi Lei conducted the animal experiment. Hammed Ayansola, Xiaoxiao Yu, and Wang Bo carried out molecular experiments. Hammed Ayansola and Wang Bo analyzed the result data. Hammed Ayansola and Wang Bo prepared the flow and structure of the figures. Hammed Ayansola wrote the original draft. Chaoyong Liao, Yuming Guo, Bingkun Zhang, and Wang Bo commented and revised the draft. Wang Bo and Bingkun Zhang provided the research funding. Wang Bo supervised the project, edited and proofread the final draft. All authors made significant contributions to the work, read and approved the final version of the manuscript.

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Authors' information

[a] State Key Laboratory of Animal Nutrition, Department of Animal Nutrition and Feed Science College of Animal Science and Technology, China Agricultural University, Beijing, 100193, China

References

1. Khurshid A, Khan AA, Banday MT, Ganai AM, Khan HM, Choudhary AR, et al. Effect of feed restriction on performance of broiler chicken. J Entomol Zool Stud. 2019;7:1054–6.
2. Mir NA, Rafiq A, Kumar F, Singh V, Shukla V. Determinants of broiler chicken meat quality and factors affecting them: a review. J Food Sci Technol. 2017;54(10):2997–3009.
3. Velleman SG, Coy CS, Emmerson DA. Effect of the timing of posthatch feed restrictions on the deposition of fat during broiler breast muscle development. Poult Sci. 2014;93(10):2622–7.

4. Arrazola A, Mosco E, Widowski TM, Guerin MT, Kiarie EG, Torrey S. The effect of alternative feeding strategies for broiler breeder pullets: 1. Welfare and performance during rearing. Poultry science. 2019;98(9):3377–90.
5. Tallentire CW, Leinonen I, Kyriazakis I. Artificial selection for improved energy efficiency is reaching its limits in broiler chickens. Sci Rep. 2018;8(1):1168.
6. Kuttappan V, Brewer V, Apple J, Waldroup P, Owens C. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult Sci. 2012;91(10):2677–85.
7. Lake JA, Dekkers JCM, Abasht B. Genetic basis and identification of candidate genes for wooden breast and white striping in commercial broiler chickens. Sci Rep. 2021;11(1):1–13.
8. Bordini M, Zappaterra M, Soglia F, Petracci M, Davoli R. Weighted gene co-expression network analysis identifies molecular pathways and hub genes involved in broiler White Striping and Wooden Breast myopathies. Sci Rep. 2021;11(1):1776.
9. Prisco F, De Biase D, Piegari G, d'Aquino I, Lama A, Comella F, et al. Pathologic characterization of white striping myopathy in broiler chickens. Poult Sci. 2021;100(7):101150.
10. Petracci M, Mudalal S, Bonfiglio A, Cavani C. Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. Poult Sci. 2013;92(6):1670–5.
11. Dalle Zotte A, Tasoniero G, Puolanne E, Remignon H, Cecchinato M, Catelli E, et al. Effect of "Wooden Breast" appearance on poultry meat quality, histological traits, and lesions characterization. Czech Journal of Animal Science. 2017;62(2):51–7.
12. Petracci M, Mudalal S, Soglia F, Cavani C. Meat quality in fast-growing broiler chickens. World's Poultry Science Journal. 2015;71(2):363–74.
13. Mazzoni M, Petracci M, Meluzzi A, Cavani C, Clavenzani P, Sirri F. Relationship between pectoralis major muscle histology and quality traits of chicken meat. Poult Sci. 2015;94(1):123–30.
14. Ayansola H, Liao C, Dong Y, Yu X, Zhang B, Wang B. Prospect of early vascular tone and satellite cell modulations on white striping muscle myopathy. Poult Sci. 2021;100(3):100945.
15. Sihvo HK, Immonen K, Puolanne E. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet Pathol. 2014;51(3):619–23.
16. Fondevila G, Archs JL, Camara L, de Juan AF, Mateos GG. The length of the feed restriction period affects eating behavior, growth performance, and the development of the proximal part of the gastrointestinal tract of young broilers. Poult Sci. 2020;99(2):1010–8.
17. Farghly MF, Mahrose KM, Ahmad EAM, Rehman ZU, Yu S. Implementation of different feeding regimes and flashing light in broiler chicks. Poult Sci. 2019;98(5):2034–42.
18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–8.
19. He Y, Yang Y, Dong Y, Yan C, Zhang B. The Effects of Flavomycin and Colistin Sulfate Pre-Treatment on Ileal Bacterial Community Composition, the Response to Salmonella typhimurium and Host Gene Expression in Broiler Chickens. Microorganisms. 2019;7(11):574.

20. Sanden KW, Böcker U, Ofstad R, Pedersen ME, Høst V, Afseth NK, et al. Characterization of Collagen Structure in Normal, Wooden Breast and Spaghetti Meat Chicken Fillets by FTIR Microspectroscopy and Histology. 2021;10(3):548.
21. Bandín S, Morona R, López JM, Moreno N, González A. Immunohistochemical analysis of Pax6 and Pax7 expression in the CNS of adult Xenopus laevis. J Chem Neuroanat. 2014;57-58:24–41.
22. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957;226(1):497–509.
23. Velele S. The effect of quantitative feed restriction on growth performance, carcass characteristics. Alice: University of Forte Hare; 2017.
24. Dissanayake D, David L. Effects of quantitative feed restriction on the performance of broiler chickens. 2017.
25. van der Klein SA, Silva FA, Kwakkel RP, Zuidhof MJ. The effect of quantitative feed restriction on allometric growth in broilers. Poult Sci. 2017;96(1):118–26.
26. Azis A, Berliana B, Afriani A. Effects of feeding time restriction during the whole rearing period on the growth performance of broiler chickens. International Journal of Poultry Science. 2019;18(1):14–20.
27. Svihus B, Lund VB, Borjgen B, Bedford MR, Bakken M. Effect of intermittent feeding, structural components and phytase on performance and behaviour of broiler chickens. Br Poult Sci. 2013;54(2):222–30.
28. Benyi K, Acheampong-Boateng O, Norris D, Ligaraba TJ. Response of Ross 308 and Hubbard broiler chickens to feed removal for different durations during the day. Trop Anim Health Prod. 2010;42(7):1421–6.
29. Ghazanfari S, Kermanshahi H, Nassiry MR, Golian A, Moussavi ARH, Salehi A. Effect of feed restriction and different energy and protein levels of the diet on growth performance and growth hormone in broiler chickens. Journal of Biological Sciences. 2010;10(1):25–30.
30. Khetani TL, Nkukwana TT, Chimonyo M, Muchenje V. Effect of quantitative feed restriction on broiler performance. Trop Anim Health Prod. 2009;41(3):379–84.
31. Jahanpour H, Seidavi A, Qotbi AAA, Van Den Hoven R, Rocha e Silva S, Laudadio V, et al. Effects of the level and duration of feeding restriction on carcass components of broilers. Archives Animal Breeding. 2015;58(1):99–105.
32. Livingston M, Ferket P, Brake J, Livingston K. Dietary amino acids under hypoxic conditions exacerbates muscle myopathies including wooden breast and white stripping. Poultry science. 2018;98(3):1517–27.
33. 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):728–34.
34. Ismail I, Joo ST. Poultry Meat Quality in Relation to Muscle Growth and Muscle Fiber Characteristics. Korean J Food Sci Anim Resour. 2017;37(6):873–83.
35. Velleman SG. Pectoralis Major (Breast) Muscle Extracellular Matrix Fibrillar Collagen Modifications Associated With the Wooden Breast Fibrotic Myopathy in Broilers. Front Physiol. 2020;11:461.

36. Dransfield E, Sosnicki A. Relationship between muscle growth and poultry meat quality. Poultry science. 1999;78(5):743–6.
37. Huang X, Ahn DU. The Incidence of Muscle Abnormalities in Broiler Breast Meat - A Review. Korean J Food Sci Anim Resour. 2018;38(5):835–50.
38. Kawasaki T, Iwasaki T, Yamada M, Yoshida T, Watanabe T. Rapid growth rate results in remarkably hardened breast in broilers during the middle stage of rearing: A biochemical and histopathological study. PLoS One. 2018;13(2):e0193307.
39. MacRae VE, Mahon M, Gilpin S, Sandercock DA, Mitchell MA. Skeletal muscle fibre growth and growth associated myopathy in the domestic chicken (Gallus domesticus). Br Poult Sci. 2006;47(3):264–72.
40. Wen C, Jiang X, Ding L, Wang T, Zhou Y. Effects of dietary methionine on breast muscle growth, myogenic gene expression and IGF-I signaling in fast- and slow-growing broilers. Sci Rep. 2017;7(1):1924.
41. Meloche KJ, Dozier WA 3rd, Brandebourg TD, Starkey JD. Skeletal muscle growth characteristics and myogenic stem cell activity in broiler chickens affected by wooden breast. Poult Sci. 2018;97(12):4401–14.
42. Mohammadabadi M, Bordbar F, Jensen J, Du M, Guo W. Key Genes Regulating Skeletal Muscle Development and Growth in Farm Animals. Animals (Basel). 2021;11(3):835.
43. Relaix F, Rocancourt D, Mansouri A, Buckingham M. A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature. 2005;435(7044):948–53.
44. Ferreira TZ, Kindlein L, Flees JJ, Shortnacy LK, Vieira SL, Nascimento VP, et al. Characterization of Pectoralis Major Muscle Satellite Cell Population Heterogeneity, Macrophage Density, and Collagen Infiltration in Broiler Chickens Affected by Wooden Breast. Front Physiol. 2020;11:529-.
45. Gratta F, Birolo M, Sacchetto R, Radaelli G, Xiccato G, Ballarin C, et al. Effect of feed restriction timing on live performance, breast myopathy occurrence, and muscle fiber degeneration in 2 broiler chicken genetic lines. Poult Sci. 2019;98(11):5465–76.
46. Papah MB, Brannick EM, Schmidt CJ, Abasht B. Gene expression profiling of the early pathogenesis of wooden breast disease in commercial broiler chickens using RNA-sequencing. PLoS One. 2018;13(12):e0207346.
47. Marchesi JAP, Ibelli AMG, Peixoto JO, Cantao ME, Pandolfi JRC, Marciano CMM, et al. Whole transcriptome analysis of the pectoralis major muscle reveals molecular mechanisms involved with white striping in broiler chickens. Poult Sci. 2019;98(2):590–601.
48. Praud C, Jimenez J, Pampouille E, Couroussé N, Godet E, Le Bihan-Duval E, et al. Molecular Phenotyping of White Striping and Wooden Breast Myopathies in Chicken. Front Physiol. 2020;11:633.
49. Kuttappan V, Shivaprasad H, Shaw D, Valentine B, Hargis B, Clark F, et al. Pathological changes associated with white striping in broiler breast muscles. Poult Sci. 2013;92(2):331–8.
50. Zambonelli P, Zappaterra M, Soglia F, Petracci M, Sirri F, Cavani C, et al. Detection of differentially expressed genes in broiler pectoralis major muscle affected by White Striping–Wooden Breast myopathies. Poultry science. 2016;95(12):2771–85.

51. Zhan XA, Wang M, Ren H, Zhao RQ, Li JX, Tan ZL. Effect of early feed restriction on metabolic programming and compensatory growth in broiler chickens. Poult Sci. 2007;86(4):654–60.
52. Salles GBC, Boiago MM, Silva AD, Morsch VM, Gris A, Mendes RE, et al. Lipid peroxidation and protein oxidation in broiler breast fillets with white striping myopathy. J Food Biochem. 2019;43(4):e12792.
53. Soglia F, Petracci M, Davoli R, Zappaterra M. A critical review of the mechanisms involved in the occurrence of growth-related abnormalities affecting broiler chicken breast muscles. Poult Sci. 2021;100(6):101180.
54. Papah MB, Brannick EM, Schmidt CJ, Abasht B. Evidence and role of phlebitis and lipid infiltration in the onset and pathogenesis of Wooden Breast Disease in modern broiler chickens. Avian Pathol. 2017;46(6):623–43.
55. Sihvo HK, Linden J, Airas N, Immonen K, Valaja J, Puolanne E. Wooden Breast Myodegeneration of Pectoralis Major Muscle Over the Growth Period in Broilers. Vet Pathol. 2017;54(1):119–28.
56. Xing T, Pan X, Zhang L, Gao F. Hepatic Oxidative Stress, Apoptosis, and Inflammation in Broiler Chickens With Wooden Breast Myopathy. Front Physiol. 2021;12:659777-.
57. Griffin JR, Moraes L, Wick M, Lilburn MS. Onset of white striping and progression into wooden breast as defined by myopathic changes underlying Pectoralis major growth. Estimation of growth parameters as predictors for stage of myopathy progression. Avian pathology. 2018;47(1):2–13.
58. Orlowski SK, Dridi S, Greene ES, Coy CS, Velleman SG, Anthony NB. Histological Analysis and Gene Expression of Satellite Cell Markers in the Pectoralis Major Muscle in Broiler Lines Divergently Selected for Percent 4-Day Breast Yield. Front Physiol. 2021;12:1248.
59. Velleman SG, Clark DL. Histopathologic and Myogenic Gene Expression Changes Associated with Wooden Breast in Broiler Breast Muscles. Avian Dis. 2015;59(3):410–8.
60. Meloche KJ, Fancher BI, Emmerson DA, Bilgili SF, Dozier WA. 3rd. Effects of quantitative nutrient allocation on myopathies of the Pectoralis major muscles in broiler chickens at 32, 43, and 50 days of age. Poult Sci. 2018;97(5):1786–93.
61. Livingston ML, Landon C, Barnes H, Brake J. White striping and wooden breast myopathies of broiler breast muscle is affected by time-limited feeding, genetic background, and egg storage. J Poultry science. 2019;98(1):217–26.
62. Gratta F, Birolo M, Sacchetto R, Radaelli G, Xiccato G, Ballarin C, et al. Effect of feed restriction timing on live performance, breast myopathy occurrence, and muscle fiber degeneration in 2 broiler chicken genetic lines. Poult Sci. 2019;98(11):5465–76.

Figures

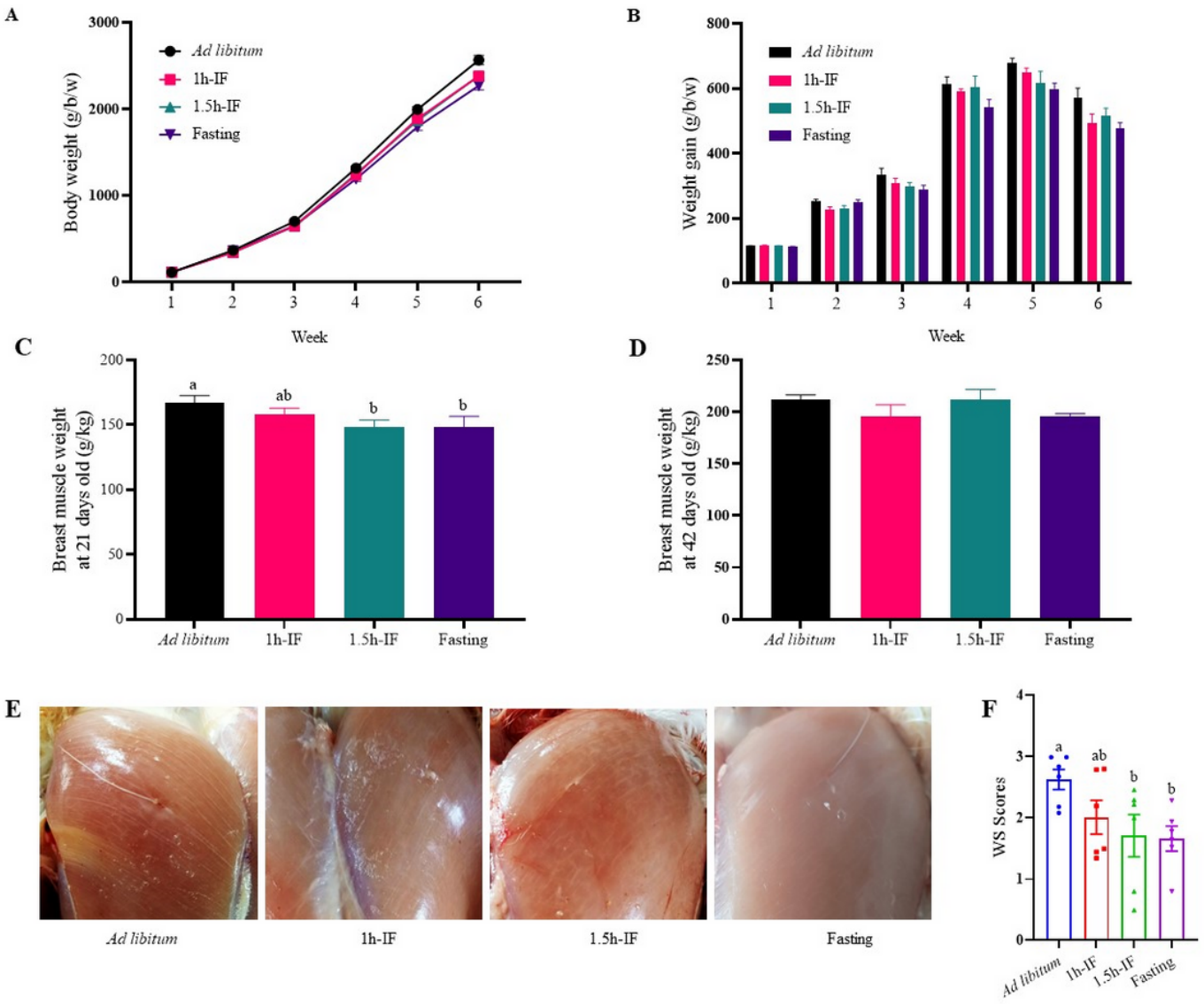

Figure 1

Restricted feeding regimens effect on breast muscle characteristics in broiler chickens.

(A) Representative graph for growth curve **(B)** relative weight gain, g/b/w – gram/bird/week **(C)** 21 days old relative breast weight **(D)** 42 days old relative breast weight, **(E)** represents the phenotypic appearance of pectoralis major muscle of 42 days old broiler chickens and (**F**) WS – white striping scores in 42 days old breast fillets. g/kg – gram/kilogram. (* - $p < 0.05$, n = 8, data representatives are means ± SEM).

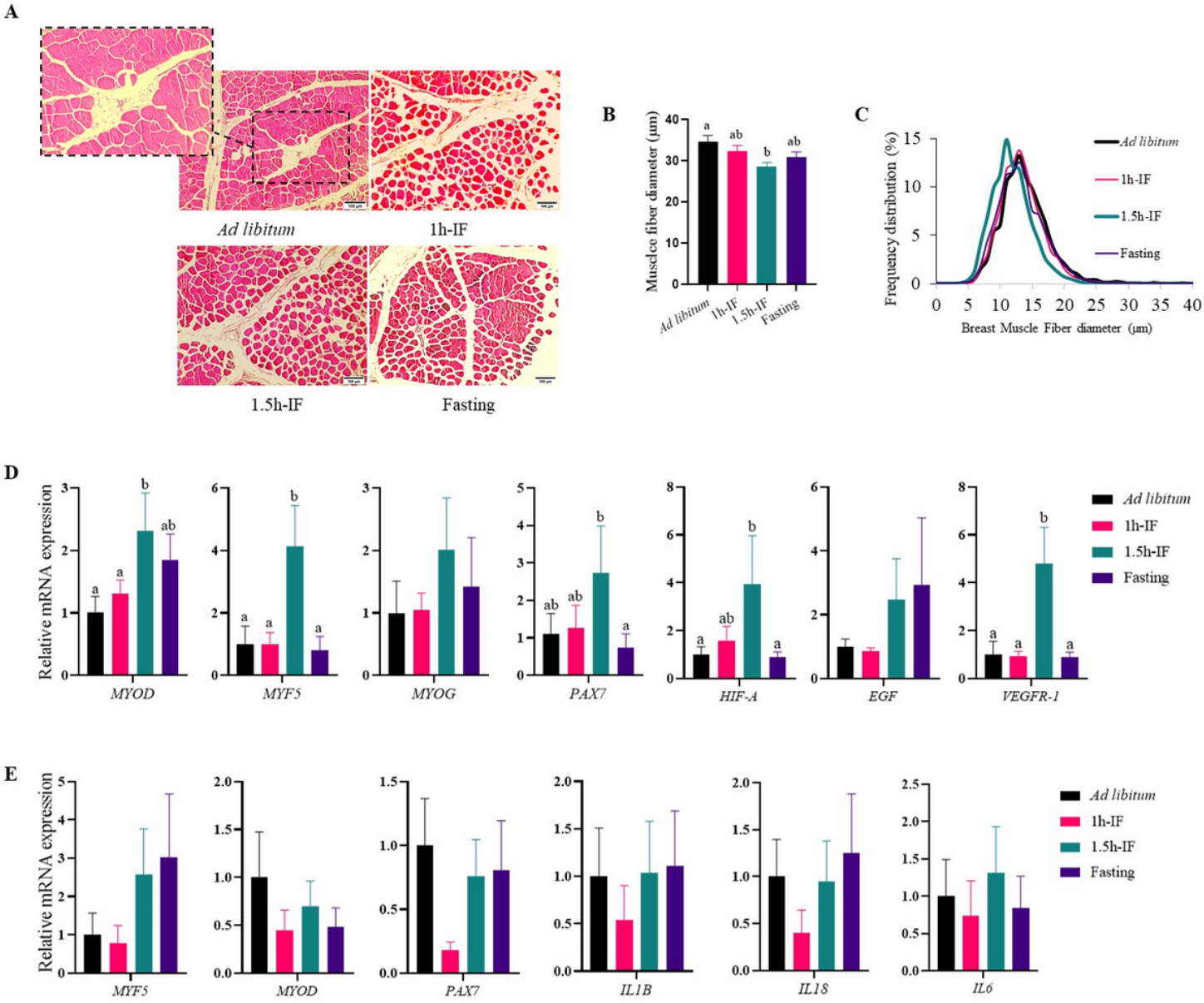

Figure 2

Intermittent feeding improves breast muscle features in broiler chickens.

(A) The H&E staining depicts 42 days old pectoralis major samples (scale bar = **100 μm)**, showing hypertrophic muscle laden with adiposity in *Ad libitum* group **(B)** Fiber diameter of breast muscle **(C)** percentage distributions of breast muscle fiber sizes **(D)** *MYOD, MYF5, MYOG, PAX7, HIF-1A, EGF,* and *VEGFR-1* mRNA levels in 21 days old pectoralis major muscle **(E)** *MYF5, MYOD, PAX7, IL1B, IL18,* and *IL6* mRNA levels in 42 days old pectoralis major muscle. (* **-** $p < 0.05$, n = 8, data representatives are means ± SEM).

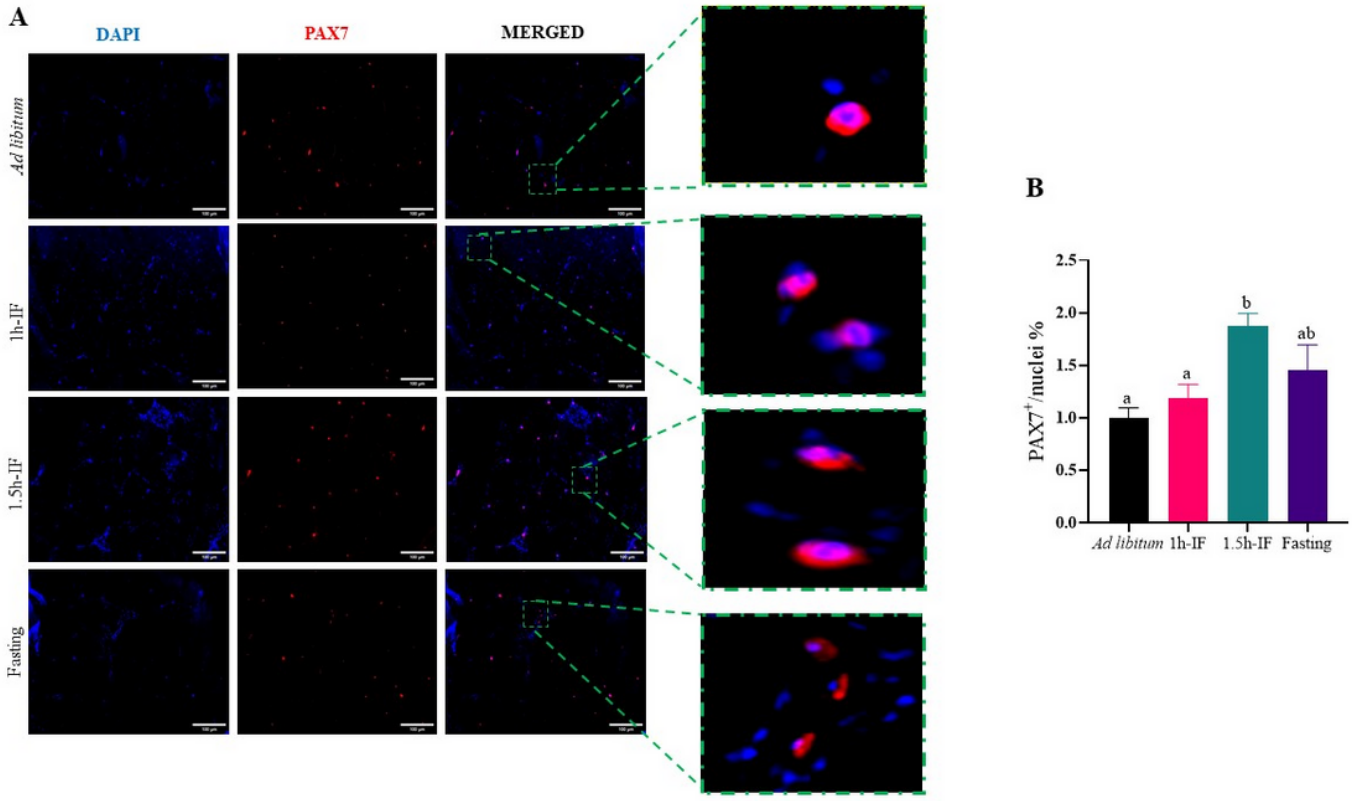

Figure 3

Intermittent feeding promotes PAX7^{+} proliferations in pectoralis major of broiler chickens.

(A) IHC -immunohistochemistry staining of broiler breast muscles (scale bar = **100 µm)** (B) is the quantifications of PAX7^{+}/nuclei % using ImageJ (* $p < 0.05$, data representatives are means ± SEM)

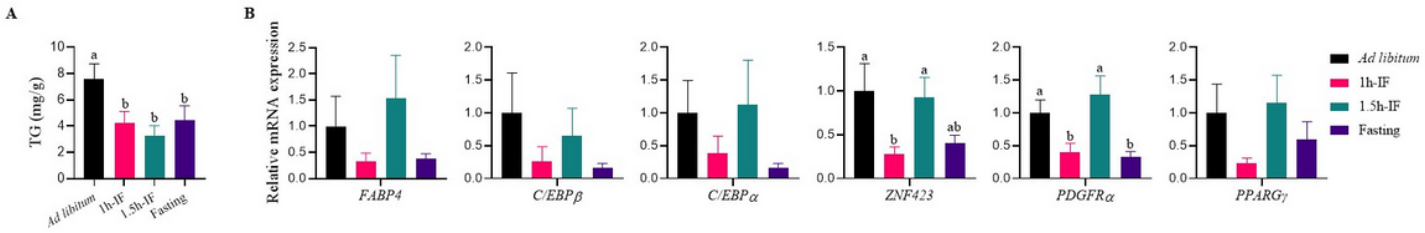

Figure 4

Restricted feeding regimens reduce ectopic fat deposits in broiler chickens

(A) Folch extraction graph for TG- Triglyceride content of 42 days old breast muscles **(B)** graph represents lipogenic, pre-adipocyte and adipocyte differentiation mRNA genes expressed in 42 days old breast muscle samples: including *FABP4, C/EBP*β, and *C/EBP*α mRNA levels involved in lipid transport and uptake, *ZNF423, PDGFR*α, and *PPAR*γ mRNA levels involved in pre-adipocyte and adipocyte respectively. (* **-** $p < 0.05$, data representatives are means ± SEM).

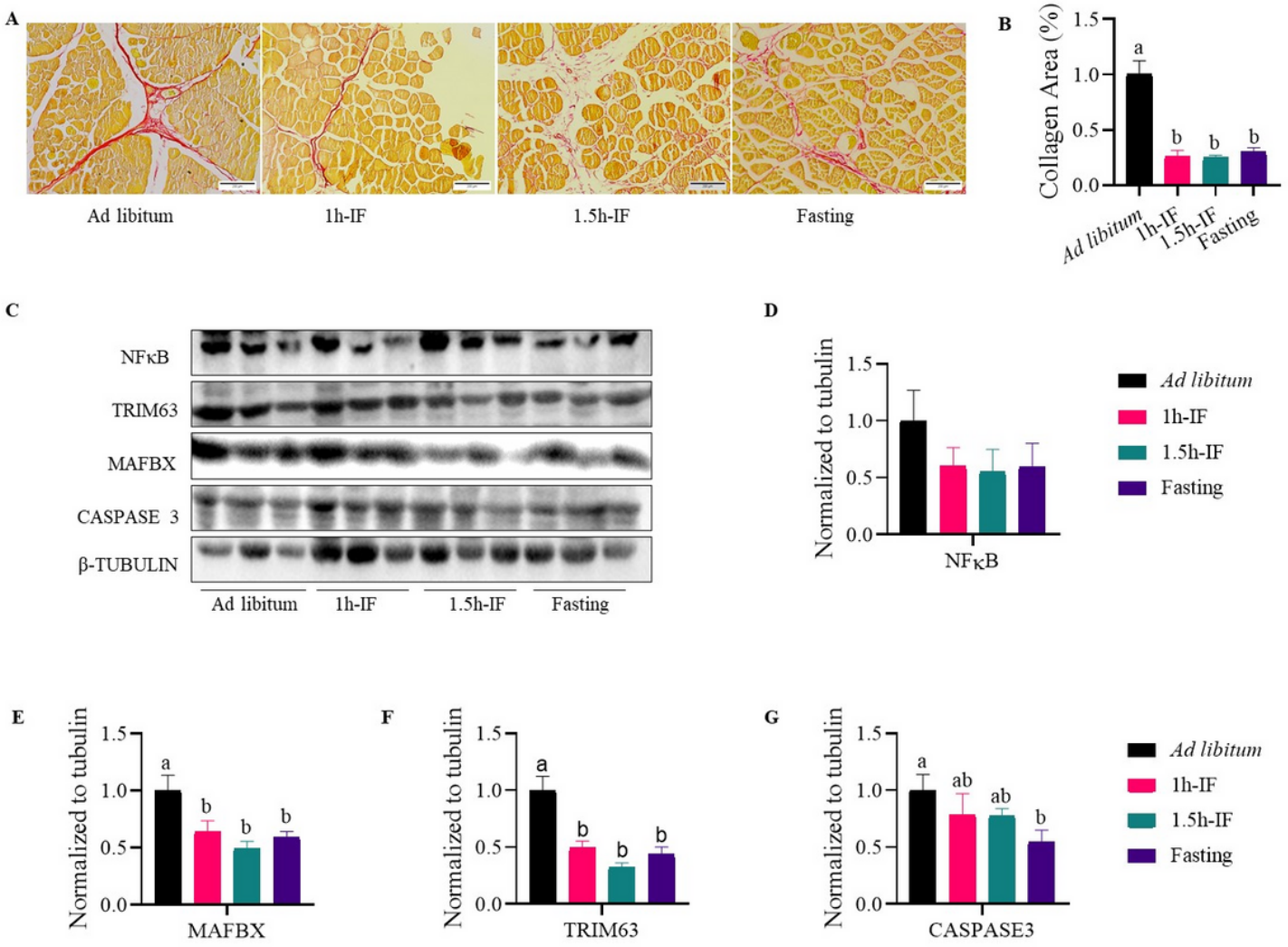

Figure 5

Restricted feeding regimens alleviate collagen accumulation in pectoralis major of broiler chickens.

(A) Picro-Sirius red staining of collagen in broiler breast muscles (scale bar = 1**00 µm)** (B) is the quantifications of % area of collagen fiber using ImageJ (C) represents different protein bands in broiler breast muscles using Western blot analysis (D-G) quantification data results of NFκB, MAFBX, TRIM63, and CASPASE-3 protein band sizes. (* $p < 0.05$, $n = 6$, data representatives are means ± SEM)