

Nutrition During the Early Rearing Period Affects the Incidence of Wooden Breasts in Broilers

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This study aimed to evaluate the relationship between early nutrition and the incidence of wooden breasts (WB) in broilers. Sixteen male and twenty female neonatal ROSS 308 broiler chicks were divided equally into four flocks. From 0–12 days of age, starter diet H, composed of 22.4% crude protein (CP), 6.6% crude fat (CF), 1.25% lysine, 0.48% methionine, and $\geq 3,070$ kcal/kg metabolizable energy (ME), was fed to two flocks, and starter diet L, composed of 19.9% CP, 2.5% CF, 1.04% lysine, 0.38% methionine, and $\geq 2,930$ kcal/kg ME, was fed to the remaining two flocks. All the flocks were fed the same commercial finisher diet, composed of 20.3% CP, 7.5% CF, 1.18% lysine, 0.44% methionine, and $\geq 3,300$ kcal/kg ME, from 12–47 days of age. The birds were weighed every 2–5 days, subjected to a wing-lift test, and histology was conducted on the pectoralis major muscle tissue samples from all the birds necropsied at 47 days of age. Significant differences in the mean body weight between groups H and L were observed during 6–16 days and 24–26 days of age in males and during 6–26 days of age in females. Regarding the score evaluation of the individual lesions reflecting wooden breast, the birds in which back-to-back wing contact was not possible had higher lesion scores than those in which back-to-back wing contact was possible. The absence of back-to-back wing contact appeared more frequently in flocks fed the starter diet L, particularly in males. These results indicate that inappropriate nutrition levels in the starter diet increase the incidence of WB. Therefore, avoiding early nutrition deficits is a cost-effective feeding strategy.

Key words: broiler, lesion score, nutrition, starter diet, wing contact, wooden breast

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Introduction

In the broiler industry, rapid weight gain and higher yields in chickens are associated with an improved meat production efficiency. However, on occasion, rapid weight gain can lead to an increased incidence of various health disorders during the broiler rearing period (Julian, 1998). In particular, a fast initial growth rate can lead to increases in issues concerning rearing management, such as the incidence of metabolic disorders (Afsharmanesh *et al.*, 2016).

Currently, the major high-yielding broiler lines grow rapidly

and are capable of reaching over 3 kg in weight by 45 days of age. Broiler growth rates have increased by over 400%, with a concurrent 50% reduction in the feed conversion ratio (FCR) from 1957 to 2005 (Zuidhof *et al.*, 2014). In recent years, the incidence of a remarkably hardened pectoralis major muscle, which has a rubber-like texture and is commonly called “wooden breast,” has occurred frequently in broilers with rapid growth performance (Sihvo *et al.*, 2014; Kuttappan *et al.*, 2016; Petracci *et al.*, 2019), and it has routinely been detected during the process of cutting and trimming breast fillets in the poultry slaughterhouse. This pectoralis major muscle abnormality causes chronic economic attrition in the broiler industry worldwide, and it is being studied from a variety of angles. Wooden breast syndrome starts as swollen, discolored lesions, which first appear in the most distal region on the cranial aspect and expand to the caudal aspect of the pectoralis major muscles (Abasht *et al.*, 2019). These changes are closely linked to an inability to sufficiently lift wings due to an increase in breast stiffness (Kawasaki *et al.*, 2016). Histologically, observations have

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been made of myofibers of variable diameter, which are devoid of their characteristic polygonality in cross-section, degenerated, or necrotic myofibers, loss of striation, infiltration of inflammatory cells, regenerated myofibers, diffuse thickening of the interstitium with variable amounts of loose connective tissue, fibrosis, and on occasion, perivascular infiltration around veins and splitting myofibers (Sihvo *et al.*, 2014).

In recent years, several studies have focused on restricted feeding to control broiler growth rates properly. Trocino *et al.* (2015) showed that the incidence of wooden breasts was significantly affected by gender: the incidence in males was two-fold higher than in females. Furthermore, under the same experimental conditions, it has been reported that muscular tissue lesions indicating wooden breasts were observed from around 14 days of age (Radaelli *et al.*, 2017). Meanwhile, Livingston *et al.* (2019) showed that restricted feeding from 8 to 47 days of age significantly reduced broiler weight. They also indicated that the signs of wooden breasts were less severe in the birds that were subjected to restricted feeding. These studies suggest that the incidence of wooden breasts is affected by the diet restriction strategy when using the same nutritionally balanced diet and that appropriate diet restriction could reduce the incidence of wooden breasts.

As previous studies have shown that wooden breast is expressed during the early stage of rearing (Radaelli *et al.*, 2017; Kawasaki *et al.*, 2018), it was hypothesized that an improvement in feeding during the initial stage of rearing could reduce the incidence of wooden breast syndrome. Therefore, this study aimed to examine the influence of feeding two different starter diets of different nutritional composition on the incidence of wooden breast syndrome in broiler chickens. The findings of this study are important in informing the poultry industry with regard to maximizing the benefits and minimizing the costs of starter diets of different nutritional composition during the early stage of rearing.

Materials and Methods

Ethics Statement

All protocols and procedures in animal handling and sampling were approved by the Rakuno Gakuen University Institutional Animal Care and Use Committee (No. DH19A4) in accordance with the Japan Veterinary Medical Association guidelines for industrial animal medicine and the Act on

Welfare and Management of Animals of the Japanese government. Birds were observed and cared for by animal-stock technicians daily, and clinical conditions were checked by a poultry veterinarian, as necessary, during the rearing period. Euthanasia was performed gently under anesthesia and the direction of veterinarians.

Flocks, Feeds, and Rearing

ROSS 308 is a broiler strain that accounts for the majority of broilers produced in Japan in recent years, and the rearing manual for broiler nutrition is supplied by its breeding company, Aviagen. According to the latest nutritional manual for ROSS 308, 3000 kcal ME and 23% CP are recommended in the starter diet. In rearing ROSS 308 broilers, it is generally recommended to feed a starter diet up to 10 days of age, a grower diet from 11–24 days of age and then 3 steps finisher feed to slaughter age as needed (Aviagen, 2019). In this study, the timing of switching the starter diet to the finisher diet was determined to be 12 days old, referring to the current situation in commercial broiler rearing in recent Japan and the nutrition manual.

Thirty-six ROSS 308 day-old chicks (20 males and 16 females) were used in this study. When the broiler chicks hatched in the poultry house at Rakuno Gakuen University, they were removed from the hatching incubator, individual identification numbers were written on each chick's head and foot with a marker pen, and they were weighed. They were divided into four flocks (V, W, X, Y in order of adjacent rearing compartments) of nine birds, each with an equal weight distribution and sex ratio of four males and five females (Table 1). Flocks V and X (group H) were fed a corn based commercial starter mash diet (H), which was composed of 22.4% crude protein, 6.6% crude fat, 1.25% lysine, 0.48% methionine, and $\geq 3,070$ kcal metabolizable energy (ME)/kg, from 0 to 12 days of age. Flocks W and Y (group L) were fed a corn based commercial starter mash diet (L), which was composed of 19.9% crude protein, 2.5% crude fat, 1.04% lysine, 0.38% methionine, and $\geq 2,930$ kcal ME/kg, from 0 to 12 days of age. All the flocks were fed the same commercial finisher mash diet, which was composed of 20.3% crude protein, 7.5% crude fat, 1.18% lysine, 0.44% methionine, and $\geq 3,300$ kcal ME/kg from 12 to 47 days of age (Table 2).

Each flock was reared in brooder boxes, with 0.55 m² of floor space, from 0 to 12 days of age, and in a clean 0.55 m²

Table 1. Mean body weight and the coefficient of variation in body weight (CV%) of each the neonatal chick flock

Group (by starter diet)		H		L	
		V	X	W	Y
Flock					
Number of chicks		9	9	9	9
Mean body weight (g)	Male	39.5	40.5	39.8	41.5
	Female	41.8	40.6	41.0	40.4
	All of the flock	40.8	40.6	40.4	40.9
CV% of body weight in the flock		6.4	5.7	5.8	6.8

No significant difference between all groups.

concrete-floored pen covered with clean, soft sawdust litter from 12 to 47 days of age. The brooder boxes were permanently lit with light bulbs, and natural lighting was used for the pens. Diet and water were offered *ad libitum* throughout the rearing period. At 0, 3, 6, 9, 11, 16, 19, 24, 26, 30, 33, 37, 41, 45, and 47 days of age, all the birds were individually weighed and subjected to a wing-lift test to determine their ability to achieve back-to-back wing contact (Fig. 1).

None of the birds were vaccinated with any vaccine. Using serum collected at 41 days of age, antibodies against infection with the Newcastle disease virus, infectious bronchitis virus, and infectious bursal disease virus were measured using an appropriate enzyme-linked immunosorbent assay (ELISA).

Muscle Tissue Sampling

At 47 days of age, all birds were injected with 20–30 mg/kg sodium pentobarbital via the wing vein and euthanized by exsanguination from the cervical artery under full anesthesia before post-mortem investigation. Sodium pentobarbital was injected using a sterilized disposable 2.5 ml syringe with a 23-gauge needle. Muscle tissue samples were cut out from the cranial region of the pectoralis major muscles along the

myofibers and fixed in 10% formalin. These muscle samples were collected from all necropsied birds.

Histological Examinations

The formalin-fixed muscle tissue samples were trimmed transversally and longitudinally to approximately 5 mm thickness and embedded in paraffin following routine procedures.

The paraffin-embedded muscle tissues were sliced to a thickness of 3 μ m, dried sufficiently after mounting on glass slides, the paraffin was removed, and then AZAN staining was performed on the muscle tissues. The AZAN-stained muscle tissues were observed using optical microscopy (BX-41, Olympus, Tokyo, Japan). The individual lesions in muscle tissues revealed in previous reports by Sihvo *et al.* (2014) and Kawasaki *et al.* (2016) were evaluated using a five score index of severity considering the following features in the muscle: myofibers of variable diameter, myofibers devoid of polygonality, splitting fibers, degenerated or necrotic myofibers, loss of striation, infiltration of inflammatory cells in the interstitium, diffuse thickening of the interstitium with loose connective tissues, fibrosis, myofiber degeneration, perivascular inflammatory cell infiltration, and vacuolar myofiber degeneration (Fig. 2).

The evaluation criteria for each lesion were the scores according to the apparent ratio of each lesion to the visual area of the tissue section.

Score 0: No lesion.

Score 1: The lesion occupied less than 10% of the tissue area.

Score 2: The lesion occupied more than 10% and less than 40% of the tissue area.

Score 3: The lesion occupied more than 40% and less than 80% of the tissue area.

Score 4: The lesion occupied more than 80% of the tissue area.

To eliminate the influence of prejudice on the evaluation, the histological observation was performed repeatedly in ascending sample number order until the evaluation was

Table 2. The composition of ingredients on the feed labels

	Starter diet		Finisher diet
	H	L	
Cereals	54	65	64
Vegetable oil residue	34	31	24
Animal feed	5	—	6
chaff and bran	1	—	—
Other	6	4	6

Animal fats are included in other items in the starter diet H and the finisher diet.

Numerical unit is percent.



Fig. 1. Wing-lift test to confirm the ability of achieving back-to-back wing contact. The left bird is unable to achieve back-to-back wing contact when the wings are lifted, and the right bird can achieve back-to-back wing contact.

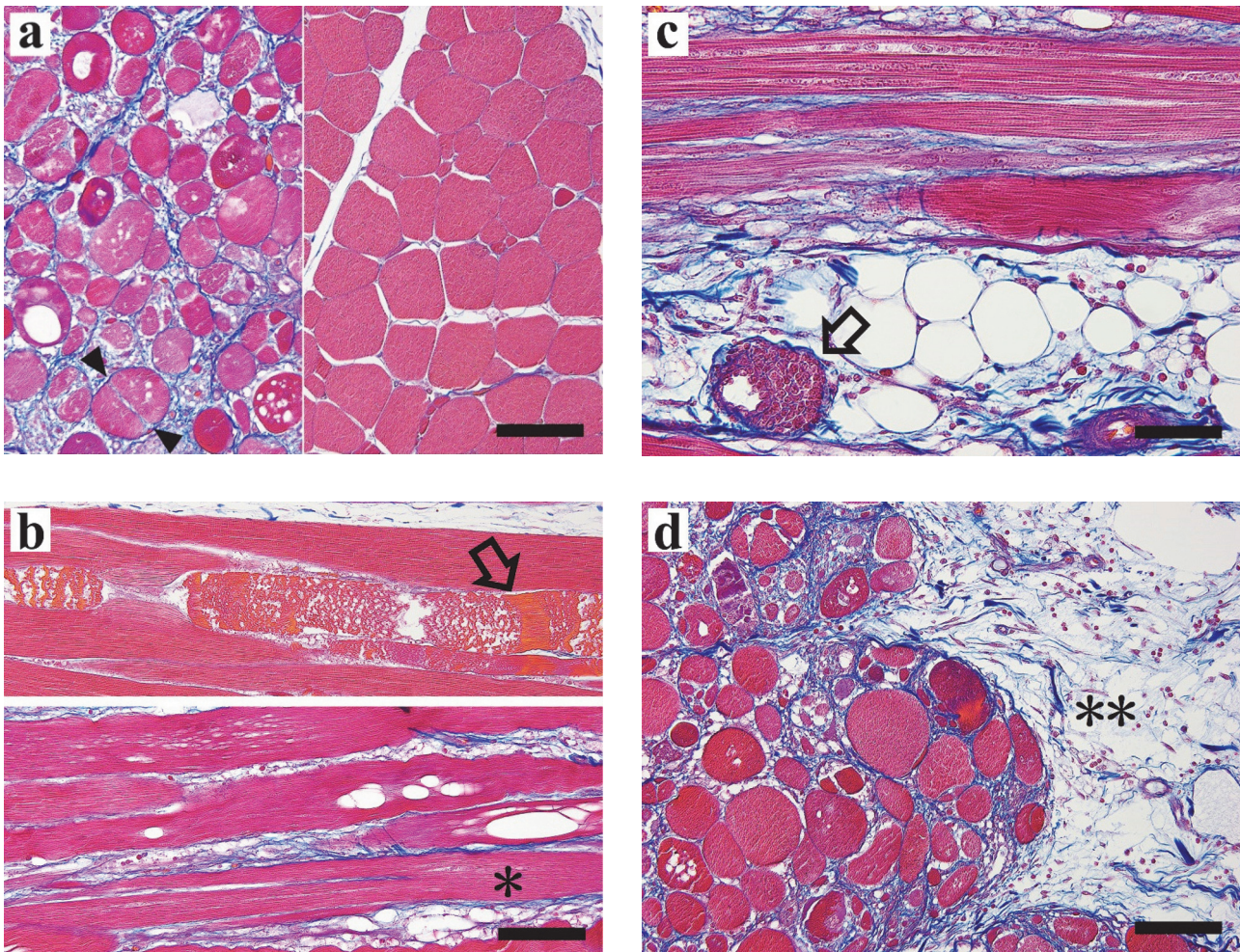


Fig. 2. Representative images of the individual lesions evaluated in muscle tissues.

(a) Cross-section of myofibers of pectoralis major muscles. Left: Most myofibers have a round shape due to being devoid of polygonality, and the diameter of myofibers is variable. A splitting fiber is pointed to by two arrowheads. Vacuolar myofibers and some other degenerated fibers are also observed. The interstitium is expanded with fibrosis. Right: Most of the myofibers are polygonal. Small caliber myofibers are observed sporadically in normal muscles. Bar=100 μm . (b) Longitudinal section of pectoralis major muscles. Upper: A collapsing hyalinized myofiber that lost the striations in the region pointed to with an arrow. Lower: A splitting fiber (*) and some vacuolar myofibers. Bar=100 μm . (c) Muscle regeneration: multinuclear chain myofibers and fusion of myoblasts are observed in the upper half region. The arrow indicates a perivascular inflammatory cell infiltration. Diffuse inflammatory cell infiltration is seen slightly in the interstitium. Bar=50 μm . (d) Diffuse thickening with loose connective tissues in the perimysium region (**). The endomysium region has progressive fibrosis. Diffuse inflammatory cell infiltration is seen in the loose connective tissues. Bar=100 μm .

complete, after which evaluation scores were sorted according to differences in diet and gender.

Statistical Analysis

The differences in the daily variations in body weight distribution between flocks was analyzed using the Tukey-Kramer test. The differences in the scores for each histologic lesion between sex and groups (H and L) were analyzed

using the Steel-Dwass test. To analyze the relationship between wing contact ability and distribution of each lesion score, the Wilcoxon rank sum test was performed. All statistical analyses were performed with R version 3.6.3 (R foundation, 2020).

Table 3. The mean body weight and the coefficient of variation in body weight (CV%) of H group and L group at each days of age

days of age	male				female			
	H		L		H		L	
	mean (g)	CV%	mean (g)	CV%	mean (g)	CV%	mean (g)	CV%
0	40	4.5	41	7.2	41	6.8	41	5.6
3	67	7.3	65	11.0	72	15.1	69	6.8
6	127 ^a	8.7	103 ^b	14.6	126 ^a	19.6	101 ^b	11.3
9	234 ^a	8.1	184 ^{bc}	17.7	225 ^{ab}	18.6	173 ^c	13.1
11	303 ^a	8.5	231 ^{bc}	16.9	284 ^{ab}	18.6	217 ^c	14.2
16	558 ^a	8.2	405 ^{bc}	18.8	489 ^{ab}	18.1	368 ^c	14.3
19	763 ^a	9.3	611	26.1	658 ^a	17.1	507 ^b	14.4
24	1,240 ^a	9.7	1,000 ^{bc}	14.0	1,043 ^b	14.4	849 ^c	12.1
26	1,440 ^a	9.8	1,184 ^{bc}	13.2	1,215 ^b	13.2	996 ^c	11.6
30	1,893 ^a	9.6	1,625 ^a	12.4	1,595 ^b	12.2	1,339 ^b	10.7
33	2,264 ^a	9.8	2,013 ^{ab}	10.8	1,873 ^{bc}	12.0	1,630 ^c	10.1
37	2,601 ^a	9.2	2,536 ^a	8.9	2,181 ^b	11.7	2,033 ^b	9.1
41	3,085 ^a	7.6	2,985 ^a	7.5	2,552 ^b	10.1	2,372 ^b	8.2
45	3,456 ^a	7.0	3,319 ^a	7.3	2,820 ^b	10.1	2,636 ^b	7.6
47	3,721 ^a	6.5	3,603 ^a	6.6	3,011 ^b	10.6	2,868 ^b	6.6

H, L; group distinguished by starter feed

^{a,b,c} Means with different alphabet superscripts within a row differ significantly ($p < 0.05$).**Table 4. The broiler rearing performance of each flocks**

Flock	H		L		H	L
	V	X	W	Y	V + X	W + Y
Mean body weight (kg) *	3.32	3.33	3.24	3.15	3.33	3.19
Number of survived birds at 47 days of age	9	9	9	9	18	18
Total consumption of starter diet (kg)	2.90	2.90	2.71	2.67	0.32	0.30
Total consumption of finisher feed (kg)	42.50	42.10	41.36	40.30	4.70	4.54
FCR	1.52	1.50	1.51	1.52	1.51	1.51
Age in days	47	47	47	47	47	47
Livability (%)	100	100	100	100	100	100
PEF	466	472	457	441	469	449

H, L; group distinguished by starter feed

PEF = (Livability × Mean body weight) / (FCR × Age in days) × 100

* No significant difference between mean body weight of each flock

Results

Broiler Rearing Performance

No significant difference existed between the flocks in their mean body weight and the coefficient of variation percentage (CV%) at 0 days of age. From 24–47 days in group H and 30–47 days in group L, the mean body weight of males was significantly heavier than that of females (Table 3). Significant differences in mean body weight between groups H and L were seen from 6–16 days and 24–26 days of age in males and from 6–26 days of age in female (Table 3). For other ages, there was no significant difference in the mean body weight between groups H and L per gender. The CV% of body weight between groups generally increased until the middle of rearing period and decreased toward slaughter age, but there was no significant difference be-

tween group H and L per gender (Table 3).

The broiler rearing performance of each flock is shown in Table 4. In groups H and L, the livability rate at the age of 47 days was 100%. In the two flocks of the H group, 2.90 kg of the starter diet was consumed by each flock in total (converted to 0.32 kg per bird) and 42.50 kg of the finisher diet was consumed in total by flock V and 42.10 kg in total by flock X (converted to 4.72 and 4.68 kg per bird, respectively). In the two flocks of the L group, 2.71 kg of the starter diet was consumed in total by flock W and 2.67 kg in total by flock Y (converted to 0.30 kg per bird) and 41.36 kg of the finisher diet was consumed in total by flock W and 40.30 kg in total by flock Y (converted to 4.60 and 4.48 kg per bird, respectively). The mean body weight at 47 days of age was 3.33 kg in group H (3.32 kg in flock V and 3.33 kg in flock X) and 3.19 kg in group L (3.24 kg in flock W and 3.15

kg in flock Y). The feed conversion ratio (FCR) calculated on the total feed consumption, the number of survived birds, and the total body weight of each group at the age of 47 days was 1.52 in flock V, 1.50 in flock X, 1.51 in flock W, and 1.52 in flock Y. The production efficiency factor (PEF) was 469 in group H (466 in flock V and 472 in flock X) and 449 in group L (457 in flock W and 441 in flock Y).

The antibody tests performed using ELISA at 41 days of age showed that the antibodies against infection with Newcastle's disease virus, the infectious bronchitis virus, and

infectious bursal disease virus were under the detected level.

Wing-lift Test

Except that the inability to achieve back-to-back wing contact (wing contact (-)) was seen in some birds, no significant defects were found in the general health condition of all birds in the rearing period. Wing contact (-) began to appear at 26 days of age (one bird of group H males) and increased at 30 days of age. At 30 days of age, of the eight males and 10 females in each group, the frequency of occurrence of wing contact (-) was three male birds in group H,

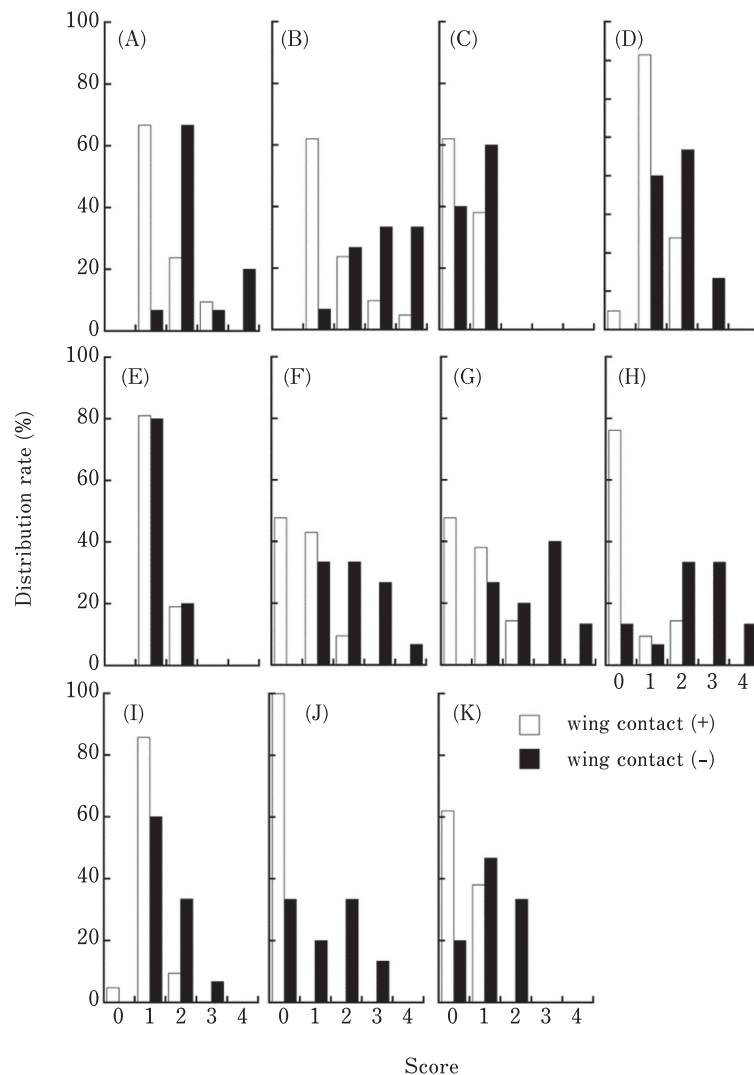


Fig. 3. The distribution of the results of wing-lift test for each histologic lesion score in 47 day old birds. (A) Myofibers of variable diameter, (B) Devoid of myofiber polygonality, (C) Splitting myofibers, (D) Degenerated myofibers, (E) Loss of striation, (F) Infiltration of inflammatory cells in the interstitium, (G) Diffuse thickening of the interstitium with loose connective tissues, (H) Fibrosis, (I) Myofiber regeneration, (J) Perivascular inflammatory cell infiltration, (K) Vacuoles of myofibers.

Score 0: No lesion, Score 1: The lesion occupied less than 10% of the tissue area, Score 2: more than 10% and less than 40% of the tissue area, Score 3: more than 40% and less than 80% of the tissue area, Score 4: more than 80% of the tissue area. Total of wing contact (-) birds: $n=15$, and of wing contact (+) birds: $n=21$.

Table 5. **Histological evaluation of lesion scores in broilers fed a high and low nutrient density starter diet**

Lesions	male		female	
	H	L	H	L
Myofibers of variable diameter (Small caliber fibers increased)	1.9±0.6	2.6±1.1	1.4±0.7	1.6±0.7
Devoid of myofibers polygonality	2.8±1.0 ^a	2.9±1.1 ^a	1.4±0.7 ^b	1.8±1.0
Splitting fibers	0.4±0.5	0.6±0.5	0.6±0.5	0.3±0.5
Degenerated myofibers	1.4±0.5	1.9±0.8	1.3±0.5	1.2±0.6
Loss of striation	1.1±0.3	1.1±0.3	1.3±0.5	1.1±0.5
Infiltration of inflammatory cells in the interstitium	1.1±0.9	2.0±1.2	0.7±0.6	1.2±1.0
Diffuse thickening of the interstitium with loose connective tissues	1.6±1.0	2.5±1.3 ^a	0.6±0.7 ^b	1.1±0.9
Fibrosis	1.6±1.1	2.4±1.5 ^a	0.3±0.6 ^b	0.7±0.9
Myofiber regeneration	1.3±0.4	1.8±0.7 ^a	1.1±0.3	0.9±0.3 ^b
Perivascular inflammatory cell infiltration	Vein	0.5±1.0	1.5±1.0 ^a	0.0±0.0 ^b
	Artery	0.0±0.0	0.0±0.0	0.0±0.0
Vacuolar myofiber degeneration	0.9±0.6	1.4±0.7 ^a	0.4±0.5	0.3±0.5 ^b

Each score value is mean±standard deviation.

H, L; group distinguished by starter feed.

^{a, b} Means with different alphabet superscripts within a row differ significantly ($p < 0.05$).

seven male birds in group L, no female birds in group H, and one female bird in group L. At 47 days of age, these numbers were five male birds in group H, seven male birds in group L, no female birds in group H, and three female birds in group L.

Evaluation of Histological Lesions

The histologic evaluations with lesion scores of muscles are shown in Table 5. The myofiber-devoid polygonality (Fig. 3B) tended to be more frequent in males. Diffuse thickening of the interstitium with loose connective tissues (Fig. 3G), myofiber regeneration (Fig. 3I), perivascular inflammatory cell infiltration (Fig. 3J), and vacuolar myofiber degeneration (Fig. 3K) were significantly more severe in L-group males compared to females in H or L group. Fibrosis (Fig. 3H) was significantly more severe in H-group males compared to females in H group. The inflammatory cells that infiltrated the interstitium were mainly composed of heterophils, macrophages, and lymphoid cells. Perivascular infiltrations were lymphocytic, limited around the vein, and were not found around the artery. Lesion scores between same genders showed no significant differences statistically, but in the comparison of the mean scores, L group of the same gender tended to be higher than H group of same gender. In all samples, splitting fibers were not frequently observed, so the difference in expression rate was not apparent. Degenerated myofibers (Fig. 3D) and infiltration of inflammatory cells in the interstitium (Fig. 3F) were different between samples, albeit not significantly so between groups.

Relationship Between Histologic Lesions and the Results of the Wing-Lift Test

The distribution of the wing-lift test against each histologic lesion score in 47-day-old chicks is shown in Fig. 3. No individual was assigned a score of zero for the three lesions; myofibers of variable diameter (Fig. 3A), myofiber-devoid polygonality (Fig. 3B), and loss of striation (Fig. 3E). In all the index lesions, the birds which exhibited back-to-back wing contact (wing contact (+)) had lower lesion

scores, while the wing contact (−) birds had higher lesion scores.

The results of the Wilcoxon rank sum test ($p < 0.05$) supported that all lesion score distributions (except splitting fiber and loss of striation) were significantly more severe in wing contact (−) birds than in wing contact (+) birds.

Discussion

In the histologic evaluation, every wing contact (−) bird exhibited lesions reflecting acute or subacute inflammation (Fig. 3F, G, and J). Fibrosis resulting in chronic injury was observed in the majority of wing contact (−) birds, but some of them were only mildly affected (Fig. 3H). These results indicate that the inability to perform back-to-back wing contact is associated with acute or subacute inflammation of the pectoralis major muscles, suggesting that lesions in wooden breasts are essentially acute or subacute myositis. Additionally, perivascular inflammatory cell infiltration was not observed in every wing contact (+) bird but was observed in one-third of the wing contact (−) birds, suggesting that these lesions are a manifestation of the inflammatory changes associated with wooden breasts. Myofiber polygonality, diffuse thickening of the interstitium with loose connective tissues, fibrosis, myofiber regeneration, perivascular inflammatory cell infiltration, and vacuolar myofiber degeneration tended to more severe in males than females. These results indicate that male birds tended to be affected by WB more than female birds, which is consistent with the result in a previous report (Trocino *et al.*, 2015).

Wing contact (−) was first expressed at 26–30 days of age. It then slightly increased to 47 days of age. This clinical symptom was commonly expressed in males, and was especially high in group L males, and most affected birds had developed the symptom by approximately 30 days of age. Previous studies have shown a close relationship between the development of wooden breasts and clinical signs of wing contact (−) (Kawasaki *et al.*, 2016; 2018). In the present

study, wing contact (–) birds had higher lesion scores than wing contact (+) birds (Fig. 3). These results support that wooden breasts begin to develop at approximately 30 days of age.

The two starter diets used in this experiment significantly differed in their provision of metabolizable energy. Diet L had a lower amount of crude protein and fat than diet H. These differences in nutritional value caused significant weight differences per gender between the group L and group H chickens during the rearing period (Table 3). Differences in weight between groups L and H during the rearing period may also have been affected by the fact that starter diet consumption in group L was slightly lower than that of group H (Table 4). Any weight difference during this period was counterbalanced by feeding chickens with a finisher diet after the starter diet (Table 4). Weight at slaughter age was similar, even if a slight weight loss did occur due to nutritional deficiency in the early stages of feeding on the starter diet.

Siegel *et al.* (1997) revealed that chicks tend to favor high-protein diets in the early rearing period, and with age, favor high-energy diets. This report also showed that feed preference influences the time that chicks spend drinking water and eating. Thus, differences in protein concentration and metabolizable energy between starter diets H and L may affect diet preference in chicks, resulting in a slight difference in starter feed consumption. Furthermore, in commercial broiler rearing, infections such as *Aviadenovirus* (Cowen *et al.*, 1978; Hess, 2013) and *Escherichia coli* (Kumar *et al.*, 2003), feed contamination by mycotoxins such as Ochratoxin A (Kumar *et al.*, 2003), and excessive heat exposure (Cooper and Washburn, 1998) in the early stages of rearing reduce starter diet consumption. Together with feeding an inadequate starter diet, the factors leading to reduced feed intake during the early stages of feeding can exacerbate broiler wooden breasts. Regarding the relationship between the several factors causing suppression of starter diet consumption and the occurrence of wooden breast, it is necessary to study various conditions in the future. In addition, further research is needed on the optimal nutritional composition and feed morphology of the starter diet.

In conclusion, the findings of this study show that the lack of nutrition in the early stages of rearing can increase the incidence of wooden breasts in broiler chickens. Feed is the largest financial investment in broiler production (Yu *et al.*, 1990), representing 60%–70% of the production cost for most poultry products (Chambers *et al.*, 1983; Leeson and Summers, 2005). Therefore, feeding a nutritious starter diet to avoid inadequate early nutrition is a cost-effective method to reduce the occurrence of wooden breasts.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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