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Influence of Enclosure Size on Growth of Breast and Leg Muscle Fibers in Domestic Fowl

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Abstract: This experiment evaluated the growth of breast and leg muscle fibers of domestic fowl raised in two enclosure sizes (SE: Small Enclosure, 1.125 m^2/10 birds; LE: Large Enclosure, 5.25 m^2/10 birds). In breast muscles, the number of fibers per area decreased over time and higher values were observed in broilers housed in SE compared to LE. The fiber size increased with age and was greater in LE than SE at 56 days of age, suggesting greater hypertrophic growth of fibers in breast muscle for broilers maintained in LE. In leg muscles, the muscle cross-sectional area was greater for broilers raised in LE than SE at 56 days of age and decreased from 42 to 56 days of age in broilers raised in SE, suggesting leg muscle atrophy in these birds. The Fast Glycolytic (FG), Fast Oxidative-Glycolytic (FOG) and Slow Oxidative (SO) fibers grew until 42 days of age in both enclosure sizes. The area of FOG fibers was greater in broilers raised in LE than those in SE at 28 and 56 days of age; in LE-raised broilers, the SO area was greater at 28, 42 and 56 days of age, suggesting that the muscles of broilers housed in LE are more oxidative. The BW gain was greater for broilers raised in LE than SE, whereas BW, feed intake and feed conversion were not influenced by enclosure size. Thus, the enclosure space affected hypertrophic growth and metabolic characteristics of breast and leg muscle fibers.

Key words: Broiler, enclosure space, fiber size, pectoralis major, sartorius

INTRODUCTION

Skeletal muscles consist of Fast-contracting Glycolytic (FG), Fast-contracting Oxidative-Glycolytic (FOG) and Slow-contracting Oxidative (SO) fibers (Peter *et al*., 1972; Scheuermann, 2004). These fiber types respond to altered functional demands by undergoing conversion from one fiber type to another by adjusting their phenotypic properties (Gollnick *et al*., 1983; Pette and Staron, 2000; D'Angelis, 2004) and becoming different muscles that adapt to specific contractile and metabolic activities required for performance. A positive correlation has been reported between the percentage of slow-type fibers and the use of leg muscles in mammals (Monster *et al*., 1978; Kernell and Hernsbergen, 1998; D'Angelis, 2004). Muscle inactivity is followed by an increase in fast-type fibers (Templeton *et al*., 1988) and results in muscle atrophy with prolonged absence of use (Greenleaf and Kozlowski, 1982). In birds, many species suffer annual cycles of atrophy and reconstitution of muscles during periods of egg hatching and molting or when they stop flying (Cheral *et al*., 1988; Gaunt *et al*., 1990). In such cases, the pectoralis muscles are catabolized to produce energy and undergo atrophy as a consequence of inactivity.

Enclosure size, group size and density are critical factors for confined animals, including domestic fowl, because these features can restrict their movement and space use as well as the distance among them (Jensen *et al*., 2003; Estevez *et al*., 2007; Leone *et al*., 2007), causing discomfort, stress, health problems and physiological and behavioral alterations (Cheral *et al*., 1988; Chaplin *et al*., 1997; Christman and Leone, 2007). There are few studies examining how enclosure size and group density affect skeletal muscle growth of domestic avian. Such studies are particularly relevant in poultry production systems, in which is necessary to ensure avian welfare and health. Here, we analyzed the skeletal muscle fiber size and growth of domestic fowl housed in two different enclosure space conditions.

MATERIALS AND METHODS

Animals and experimental design: The experimental protocol followed in this study was approved by the Faculty of Agricultural and Veterinary Sciences, Committee of Ethics in Animal Use (CEUA -Protocol Number 023304/09).Two testing enclosure sizes were used: 1.13 m^2 (small enclosure, SE) and 5.25 m^2 (large enclosure, LE). For this, the pens were decreased in size on only one side to give the same width (1.5 m) for all enclosures and therefore, the LE was 4.7 times longer than the SE. The floors of the testing enclosures were covered with 5 cm of rice husk litter. The enclosure

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dimensions and potential parameters of interest are listed in Table 1. A total of 260 1-day-old male broilers chicks (Cobb) were obtained from a commercial hatchery. The birds were homogeneously distributed by mean BW into 20 enclosures (10 SE and 10 LE) that each contained 10 birds (25 to 36 kg m^{-2} pen^{-1} from 42 to 56 d of age). Sixty extra birds were used to adjust the number of birds per enclosure after sampling or in case of mortality. The broilers were vaccinated at 7 and 20 days of age against infectious bursal disease using intraocular instillation (1 drop) and at 26 days of age against Newcastle disease by oral administration. Water and feed were provided *ad libitum* throughout the experiment. The feeding program consisted of two commercial diets: the starter diet (21% crude protein and 2,950 kcal kg^{-1} of metabolizable energy) that was provided from 1 to 21 days of age and the finisher diet (18% crude protein and 2,800 kcal kg^{-1} of metabolizable energy) from 22 to 42 days of age, with both diets based on corn, soybean meal and wheat meal. The lighting program was set to 24 h light until 14 days of age and 22 h light:2 h dark from 15 to 56 days of age. The temperature and relative humidity in the pens were measured daily. The following mean temperature and relative humidity values were measured from the first to the eighth week, respectively: 32.3°C, 28.8°C, 26.7°C, 27.1°C, 25.6°C, 26.3°C, 24.1°C and 23.0°C; 44.1%, 48.3%, 43.4%, 50.0%, 62.6%, 63.4%, 74.0% and 78.3%.

Body weight, weight gain, feed intake and feed conversion: Body Weights (BW) of broilers housed in the small and large enclosure spaces were analyzed at different ages (14, 28, 42 and 56 days). Effects of enclosure size on BW gain, feed intake and feed conversion from 1 to 56 d of age were also evaluated.

Muscle sampling and analysis: Analyses were performed using five broilers each at 14, 28, 42 and 56 days of age that were randomly selected from each enclosure size. After cervical dislocation followed by decapitation, the pectoralis major and sartorius muscles were quickly dissected. It known that pectoralis major is a white muscle constituted by Fast Glycolytic Fibers (FG) and sartorius is a mixed muscle composed by Slow Oxidative (SO), Fast Oxidative-Glycolytic (FOG) and FG fibers. Thus, we used routine histological processing to analyze the fiber size and number of the breast muscles and histochemistry to identify the fiber types in the leg muscles.

Pectoralis major muscles: Segments were obtained from pars sternobranchialis of the left breast muscles and rapidly fixed in Bouin solution for 24 h at room temperature. Tissues were washed in distilled water (3 x 5 min), dehydrated in ethanol, cleared in xylene and embedded in paraplast. Five semi-serial cross-sections (6 µm thickness) were stained with hematoxylin-eosin, dehydrated, cleared and embedded in Entellan. The fiber size was evaluated by measuring the cross-sectional area (μm^2) of 150 fibers per bird, by tracing their outline. The mean number of fibers (FF) per area was obtained by counting the Total Number of Fibers (TNF) in five area (each area: 892,967 μm^2) per bird. Data were obtained using an image analysis system (digital image processing and analysis software for professional microscopy: Qwin V3 Leica).

Sartorius muscles: Right sartorius muscles were isolated from each bird, rapidly frozen in N-hexane, cooled in liquid nitrogen and stored at -70°C until further processing. Semi-serial cross sections (12 µm) at the midpoint along the sartorius muscles length were cut in a cryostat at -25°C, mounted on glass slides, air-dried and processed histochemically for nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) to identify SO, FOG and FG fibers. NADH-TR activity was observed according to the technique developed by Peter *et al.* (1972) and modified by Dubowitz and Brooke (1984). Cryosections were incubated for 5 min at room temperature and then for 40 min at 39°C in a fresh solution of 0.08% NADH (reduced form) and 0.1% nitroblue tetrazolium in 0.2 M Tris-HCl buffer, pH 7.4. The sections were washed in distilled water, fixed for 5 min at room temperature in 5% formaldehyde in 0.1 M phosphate buffer, pH 7.4, washed in distilled water, dehydrated in ethanol, cleared in xylene and embedded in Entellan. Oxidative fibers showed the darkest staining for nicotinamide adenine dinucleotide tetrazolium reductase, whereas oxidative-glycolytic and glycolytic fibers showed moderate staining and no staining, respectively (Fig. 1). For each sartorius muscle, we used five muscle cross-sections at the midpoint along the muscle length. The mean cross-sectional area of the muscle was evaluated by tracing the outline of each cross-section using digital image processing and analysis software for professional microscopy (Qwin V3, Leica) linked to an estereomicroscope. The total number of fibers within the each muscle cross-section was counted using the same system of image analysis. Mean Cross-Sectional Area (CSA) of each fiber type was measured by tracing the outline of 150 fibers of each type using a similar image analysis system, linked to an microscope.

Statistical analysis: Body weight gain, feed intake and feed conversion were compared between enclosure sizes. Body weight, muscle cross-sectional area and muscle fiber data were analyzed using a 4 x 2 factorial design (ages: 14, 28, 42 and 56 days; enclosure sizes: small and large). Cross-sectional area of the fiber types in the sartorius muscle were compared within each age

Table 1: Parameters for each enclosure size

Enclosure size	Group size	Total area (m^2)	Area (m^2)/bird	SD (birds/m^2)	Width (m)	Length (m)	W:L ratio	TP (m)	Perimeter (m/bird)
Small enclosure	10	1.13	0.11	8.9	1.5	0.75	2:1	4.5	0.45
Large enclosure	10	5.25	0.50	1.9	1.5	3.5	1:2.3	10	1

W = Width; L = Length; SD = Stocking Density (birds/m^2); TP = Total Perimeter (m)

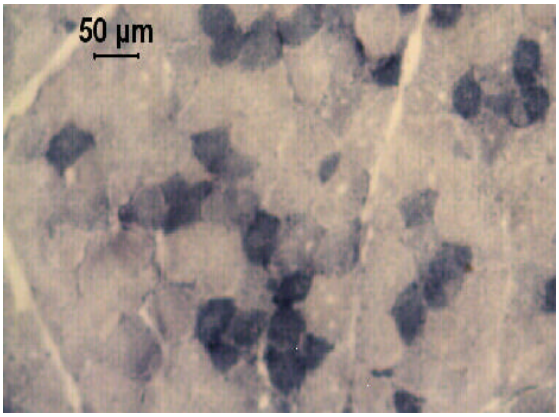

Fig. 1: Cross-sections of the sartorius muscles from broiler chicken at 28 days of age and raised in small enclosure, submitted to nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR). 1 = slow oxidative fiber, 2 = fast oxidative-glycolytic fiber, 3 = fast glycolytic fiber

in broilers housed in SE and LE. The presence of outliers was also verified. The normality presuppositions of studentized errors (Cramér-von-Misses test) and the equality of variances (Levene's test) were also tested. After confirming that these presuppositions were not violated, data were subjected to analysis of variance using the GLM procedure of SAS® software (2004). The means were compared with Tukey's test (5%) when significant differences were detected.

RESULTS

The body weight of the broilers was not affected ($p>0.05$) by the enclosure size or by an interaction between enclosure size and age. However, there was a significant effect of the age ($p\leq0.05$), indicating that the BW increased with age in both enclosure sizes (Table 2).

The Cross-Sectional Area of the Fibers (CSAF) from pectoralis major muscles was influenced ($p\leq0.05$) by the enclosure size, age and the interaction between both (Table 2). The interaction (Table 4) showed that the CSAF increased with age in both enclosure sizes and this area was greater for broilers housed in LE than SE at 56 days of age. The Fiber Number per area (FN) was influenced by the treatment and age ($p\leq0.05$), but no interaction occurred between these variables (Table 2). The FN decreased over time independent of enclosure size and at 56 days of age, it was greater for broilers housed in SE than in LE (Table 2).

Table 2: Effects of enclosure size and age on Body Weight (BW), Cross-Sectional Area (CSAFP) and number of fast glycolytic fibers (NFP) from pectoralis major muscle

	BW (g)	CSAFP (µm^2)	NFP
Enclosure Size (ES)			
SE	1783.21	44294	34.50^a
LE	1920.84	50563	29.44^b
Age (A)			
14	416.20^d	8981	76.75^a
28	1246.90^c	37123	25.00^b
42	2360.00^b	58912	14.63^{bc}
56	3385.00^a	84698	11.50^c
Probability			
ES	0.0991	0.0003	0.0465
A	<0.0001	<0.0001	<0.0001
ES x A	0.1321	0.0044	0.6400
CV_{ES}	12.60	5.12	19.81
CV_A	11.63	11.12	16.54

$^{a-d}$Mean with different superscripts (columns) differ significantly ($p\leq0.05$). LE = Large Enclosure; SE = Small Enclosure

In sartorius muscles, the TNF did not differ ($p>0.05$) with enclosure size or with age (Table 3). There was a significant effect ($p\leq0.05$) of the age and of the interaction between treatment and age for cross-sectional area of sartorius muscles (CSAS; Table 3). The interaction (Table 4) showed that the CSAS increased from 14 to 42 days of age and the values at 56 days of age were similar to those at 28 days for broilers housed in SE. However, muscle growth occurred from 28 to 56 days of age in broilers housed in LE. In addition, the CSAS was lower at 56 days of age for birds housed in SE than those in LE.

Significant effects ($p\leq0.05$) of age on the FG fiber area, of age and the interaction between enclosure size and age for FOG fibers and of the enclosure size, age and the interaction between enclosure size and age for SO fibers (Table 3) were detected in the sartorius muscles. Independent of the enclosure size, the FG fiber area increased until 42 days of age (Table 3). The FOG fiber area (Table 4) increased until 42 days of age and remained unchanged from 42 to 56 days of age for broilers housed in SE and LE. In addition, this area was lower in broilers in SE than for LE at 28 and 56 days of age. The SO fiber area (Table 4) also increased until 42 days of age and values were maintained from 42 to 56 days. The SO area was lower in broilers housed in SE than LE at 28, 42 and 56 days of age.

Figure 2 shows the results of comparison of areas of the three types of sartorius muscle fibers for the different ages and enclosure spaces. The FOG and FG fiber areas were similar ($p>0.05$), but they were greater ($p\leq0.05$) than the SO fiber area for all ages and for both types of enclosure spaces.

Table 3: Effects of enclosure size and age on Cross-Sectional Area (CSAS) and the Total Number of Fibers (TNF) of the medial region of the sartorius muscle; cross-sectional area of Fast Glycolytic (FG), Fast Oxidative-Glycolytic (FOG) and Slow Oxidative (SO) fibers from the sartorius muscle of broiler chickens

	CSAS (mm^2)	TNF	FG* (μm^2)	FOG (μm^2)	SO (μm^2)
Enclosure Size (ES)					
SE	1445.20	667.55	44685.56	43135.00	15138.70
LE	1733.00	661.21	46483.83	45486.00	17148.70
Age (A, days)					
14	813.00	803.50	19424.27^{c}	18008.00	7293.00
28	1330.00	617.98	44701.36^{b}	42462.00	15830.00
42	1981.00	597.97	63089.78^{a}	61889.00	20834.50
56	2233.00	637.66	59339.82^{a}	58272.00	21634.80
Probability					
ES	0.0510	0.9312	0.4883	0.6347	<0.0001
A	<0.0001	0.0906	<0.0001	<0.0001	<0.0001
ES x A	<0.0001	0.1474	0.0937	0.0390	<0.0001
CV_{ES}	24.94	32.83	5.33	14.16	36.98
CV_{A}	19.54	28.81	3.48	7.97	8.03

$^{a-c}$Mean with different superscripts (columns) differ significantly ($p \leq 0.05$). SE = Small Enclosure; LE = Large Enclosure. *Processed date ($y^{0.4183}$)

Table 4: Interaction between age and enclosure size for the Cross-Sectional Area of Fast-glycolytic Fibers (CSAFP) from pectoralis major muscles and Cross-Sectional Area of the medial region (CSAS), Fast Oxidative-Glycolytic (FOG) and Slow Oxidative (SO) fibers from sartorius muscles

	Age (days)	SE	LE	P
CSAFP (μm^2)	14	8487.60^{Ad}	9474.90^{Ad}	0.7943
	28	37097.09^{Ac}	37148.40^{Ac}	0.9892
	42	56885.52^{Ab}	60937.74^{Ab}	0.2918
	56	74707.45^{Ba}	94689.13^{Aa}	<0.0001
	P	<0.0001	<0.0001	-
CSAS (mm^2)	14	868^{Ab}	758^{Ac}	0.5806
	28	1416^{Aab}	1244^{Ac}	0.3899
	42	1996^{Aa}	1966^{Ab}	0.8799
	56	1502^{Bab}	2964^{Aa}	<0.0001
	P	<0.0001	<0.0001	-
FOG (μm^2)	14	16961.82^{Ac}	19054.56^{Ac}	0.3570
	28	40551.93^{Bb}	44372.89^{Ab}	0.0379
	42	60869.01^{Aa}	62705.86^{Aa}	0.4449
	56	55811.14^{Ba}	61348.08^{Aa}	0.0265
	P	<0.0001	<0.0001	-
SO (μm^2)	14	7239.10^{Ac}	7346.99^{Ac}	0.8962
	28	13311.23^{Bb}	18349.88^{Ab}	<.0.0001
	42	19161.11^{Ba}	24727.00^{Aa}	<.0.0001
	56	19686.57^{Ba}	22269.11^{Aa}	0.0058
	P	<0.0001	<0.0001	-

$^{A-B, a-d}$Means with different superscripts (lines and columns, respectively) differ significantly ($p \leq 0.05$). ES = Small Enclosure; LE = Large Enclosure

Table 5: Effect of enclosure size on Feed Intake (FI), Body Weight Gain (BWG) and Feed Conversion (FC) of broilers from 1 to 56 days of age

Enclosure sizes	FI (kg)	BWG (kg)	FC (FI:BWG)
SE	5.596	3.318^{b}	1.69
LE	5.693	3.411^{a}	1.67
P	0.2235	0.0345	0.1928
CV (%)	3.06	2.69	1.75

$^{a-b}$Mean with different superscripts (columns) differ significantly ($p \leq 0.05$). SE = Small Enclosure; LE = Large Enclosure

Table 5 shows the results of total Feed Intake (FI), Weight Gain (WG) and Feed Conversion (FC) during the experimental period (until 56 days of age). There were no significant differences ($p>0.05$) for FI and FC when comparing the two types of enclosure spaces, but the WG was greater for broilers in LE than SE. A moderate positive correlation ($r^2 = 0.6513$, $p = 0.0414$) was detected between the FI and WG for broilers housed in SE and a high positive correlation ($r^2 = 0.9463$, $p = 0.0001$) was detected in broilers bred in LE.

DISCUSSION

In this study, muscle fiber growth in the pectoralis muscle was investigated by morphometric analysis of the Cross-Sectional Area (CSA) of the fibers and by Fiber Number per Area (FNA), which gave us information about hypertrophyc muscle fiber growth. The results demonstrated that the CSAF increased and the FNA

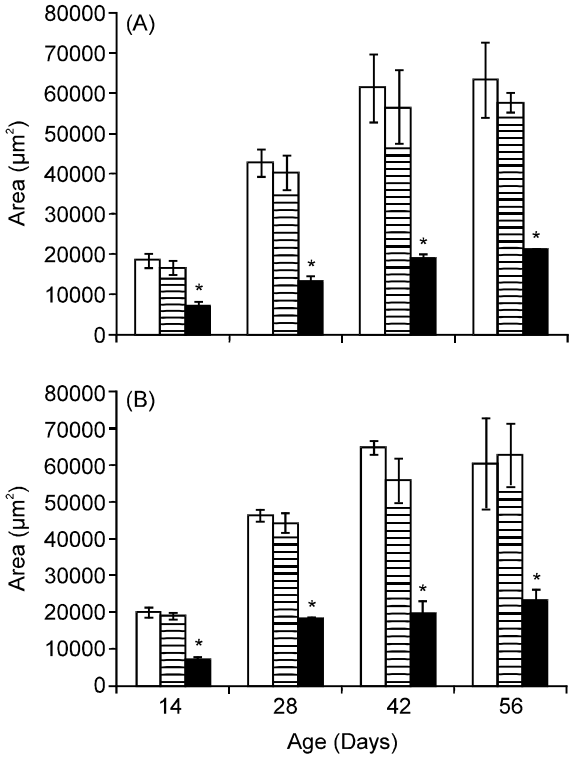

Fig. 2: Cross-sectional area of the fast glycolytic (white bars), fast oxidative-glycolytic (dashed bars) and slow oxidative (black bars) fibers in the sartorius muscles of broilers housed in small (A) and large (B) enclosures, according to age (14, 28, 42 and 56 d). *Indicate significant differences with other fiber types within each age ($p \leq 0.05$)

decreased with age in both enclosure sizes. However, at 56 days of age, this area was greater for broilers housed in LE than SE, while the FNA was greater for broilers housed in SE than in LE. These results clearly showed that hypertrophy occurred in the pectoralis major muscle independent of enclosure sizes, but hypertrophy was higher in broilers housed in LE between 42 and 56 days of age, resulting in greater fiber size in LE than in SE at the end of the experiment. The reduction observed in FN over time in the pectoralis muscle was a consequence of hypertrophy. Hypertrophy of muscle fibers promoted by physical activity has been reported in mammals (D'Angelis, 2004) and birds (Cheral *et al*., 1988; Chaplin *et al*., 1997). During the experimental period, broilers housed in LE presented more intense activity compared with the broilers from SE (data not showed). Therefore, it is probable that the higher hypertrophy seen in broilers housed in LE were caused by the more intense activity of the broilers in LE. Although the broiler line that was used in this experiment was selected for its greater and faster growth of the breast, the results showed that the enclosure size influences the phenotypic expression of its genetic potential for the growth of breast muscles. Analysis of the total fiber number in the breast muscle giving us information about hyperplasic muscle fiber growth were not possible because of the highly irregular fiber alignment and distribution in the muscle.

Concerning to growth of the sartorius muscle fiber types, we used histochemical morphometric analysis of the CSA of each fiber type to evaluate the fiber hypertrophy. In addition, morphometric analysis of the CSA of sartorius muscles at the midpoint along the muscle length and counting of the total fiber number within muscle cross-section were realized and interpreted as indicators of muscle growth and muscle fiber hyperplasy, respectively. Our results showed that enclosure size affected the growth of the FOG and SO fibers, which had lower CSA in broilers housed in SE than LE at 28 and 56 days of age and at 28, 42 and 56 days of age, respectively. Increases in the SO and FOG fiber area in physically active individuals have been reported in mammals (D'Angelis, 2004) and birds (Moss, 1968; Cheral *et al*., 1988). Thus, the increased hypertrophy of the FOG and SO fibers from 28 days of age in the leg muscles of in broilers housed in LE compared to broilers housed in SE broilers may be related to higher muscle activity, once that in the LE during the experiment, the broilers interacted more with each other, such as running behind each other (personal observations). In addition, our results showed increasing in the CSA of the FG, FOG and SO fibers only until 42 days of age in both SE and LE. This absence of hypertrophy from 42 to 56 days of age for the three types of fibers from sartorius muscles appears to indicate at the first view that leg muscle fiber reached their maximum growth at 42 days. However, considering that broilers are still growing at this same time (Havenstein *et al*., 1994), why the hypertrophic growth of the leg muscle fiber stopped at 42 days of age remains to be understood.

We have found here that the CSA of the sartorius muscles of broilers housed in LE increased from 28 to 56 days of age, while in broilers housed in SE, the muscle CSA increased until 42 days of age and decreased from 42 to 56 days of age reaching the values encountered at 28 days. These data clearly demonstrated that broilers housed in SE presented sartorius muscle atrophy. According to Urso *et al*. (2006), lack of muscle activity cause decrease in the protein content of the muscle extracellular matrix. Therefore, the absence of hypertrophy from 42 to 56 days of age for the three types sartorius muscle fibers and the simultaneous reduction in the cross-sectional area of the muscle reinforce the idea that the atrophy in the leg muscle of broilers housed in SE involved reduction extracellular connective tissue. Our data indicate that absence of fiber hypertrophy and extracellular matrix reduction are the first step in leg

muscle atrophy of broilers. Considering that the slaughter age for the broiler line used in this experiment is 42 days of age, the results showed that SE influenced the performance if the broilers were raised for a longer period. During the experiment, mainly from 35 days of age, we observed the broilers housed in SE spent a greater part of the time laying down. Considering that lack of exercise can lead to muscle atrophy and vice versa (Rosser *et al*., 1987; Cheral *et al*., 1988; Gaunt *et al*., 1990; Chaplin *et al*., 1997), the results in our current experiment suggest that the atrophy seen in broilers housed in SE is a result of the low physical activity that was a consequence of the small space available for locomotion. Growth continues after 42 days (6 weeks) of age for broilers with fast development (Havenstein *et al*., 1994) and BW is a factor that can lead to leg problems from 7 to 12 weeks of age (Kerstin *et al*., 2001). Thus, results of our current experiment suggest the possibility of leg problems are related to lower development of the leg muscles to support high body weight, once that hypertrophy of the leg muscle fiber occurred only until 42 days of age in both SE and LE.

Our research did not detected changes in the TNF of sartorius muscle with the age in broilers housed in SE and LE and nor differences in this parameter between these two groups of birds. These results reinforce the findings of several authors (Moss, 1968; Kang *et al*., 1985; Rehfeldt *et al*., 2000), who reported that hyperplasic growth of fibers does not occur during posthatch muscle growth of the birds, because the number of fibers is determined during embryonic myogenesis. In addition, the absence of difference in TNF between SE and LE reinforced our view that the lower CSAS seen at 56 days of age in broilers housed in SE may have resulted from atrophy of the connective tissue of muscle extracellular matrix.

We also compared here the areas of the three types of sartorius muscle fibers for the different ages and enclosure spaces. The FOG and FG fiber areas did not differ one another and were greater than the SO fiber area for all ages and for both SE and LE. These results are in agreement with Peter *et al*. (1972), who concluded that SO fibers are the smallest ones. However, they differed from results of these authors describing that FOG fibers have sizes between those of SO and FG fibers. Our results differed from those obtained for broilers raised at a thermoneutral temperature (Sartori *et al*., 2001) in which SO and FOG fibers are the same size and are smaller than FG fibers.

In the present study, the Body Weight (BW), total Feed Intake (FI), Weight Gain (WG) and Feed Conversion (FC) of the broilers housed in SE and LE were also analyzed. The BW of the broilers increased with age in both enclosure sizes and there was no differences between the body weight of the birds housed in SE and LE at the ages in which the muscle fiber growth was analyzed. Thus, this data show that differences in the muscle fiber growth between broilers housed in SE and LE are not related with difference in body weights. Broilers housed in LE presented greater weight gain although no difference was detected comparing the total feed intake and feed conversion in broilers housed in SE and LE. However, a high positive correlation between feed intake and weight gain was detected for broilers housed in LE, while a moderate positive correlation was detected for broilers housed in SE. These results demonstrate that lower weight gain shown by broilers in SE may be related with the lower muscle fiber growth in the pectoralis and sartorius muscles after 42 days of age, well as with a atrophy in the leg muscles. Broilers have more intense activity when housed in lower stocking densities (Estevez *et al*., 2007). In our current experiment, as already mentioned, we observed that broilers raised in LE had higher locomotor activity than those birds housed in SE during all the experimental period. Therefore, the greater WG of the broilers housed LE, by its time, may be a result of their more intense activity, given that the values of TFI and FC in broilers housed in SE and LE did not differ.

Our current experiment showed that the enclosure size influenced the breast and leg muscle fiber growth in broilers, resulting in atrophy of the leg muscles from birds raised in a small enclosure after 42 days of age. In practice, the results indicated that confinement did not affect muscle development until slaughter (42 days of age), but more research about muscle growth should be performed as a tool to measure animal welfare during long period of confinement.

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