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DIMMENSIONAL FEATURES OF THE SKELETAL MYOCYTES OF THE LAYING HENS REARED WITHIN SEVERAL HUSBANDRY SYSTEMS

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ABSTRACT

The EU regulations in poultry welfare impose modification of the classical housing systems applied in laying hens exploitation. The study goal was to assess the dynamics of the somatic myocytes thickness at the hens reared in certain versions of accommodation systems. 1731 "Lohmann Brown" hens were used, divided in four groups: control L_c-conventional cages, L_1exp.-enlarged cages (600 cm^2/hen), L_2exp.-enlarged cages (1000 cm^2/hen) and L_3exp.-conventional cages with opened front panels (summarized 1000 cm^2/hen in nesting+resting cage and feeding+watering cage) + free movement on the floor. 15 individuals/group were selected to sample tissue from four muscles: *Pectoralis superficialis*, *Biceps brachialis*, *Quadriceps femoris* and *Gastrocnemius lateralis*. The histological smears were examined by photonic digital microscopy, measuring the small and large diameters of the myocytes. The average thickness of these cells was calculated. Statistical analysis has been run by the ANOVA single factor method. Histometry revealed differences between the hens reared within classical and alternative housing systems. The myocytes average diameter in *P. superficialis* muscles varied between 39.24µ-L_c and 44.81µ-L_3exp. The L_2exp. had the thickest fibers in wings muscles-31.47µ, while the thinnest ones were noticed in L_c group-29.73µ. In rear limbs muscles the L_3exp. hens had the thickest myocytes (40.62µ-*Q. femoris*, respectively 36.21µ-*G. lateralis*), while the thinnest myocytes in thighs and shanks have been measured at Lc group. Very significant differences occurred for *P. superficialis* myocytes diameter and distinguished significant ones for rear limbs muscles cells, between the fowl accommodated in classical cages and the L_3exp group. The results suggest the existence of a relation between the freedom of movement and the hypertrophy of the skeletal musculature.

Key words: laying hens, cages, husbandry systems, myocytes, texture

INTRODUCTION

It is well known that in accordance with the European Union regulations concerning poultry welfare, the classical exploitation systems of the laying hens should be changed till 2012, in order to be able to keep producers licenses and to send eggs on market. Therefore, the traditional accommodation system which uses pyramidal type batteries, should be either furnished with supplemental cage endorsements, either totally eliminated and replaced by a free-range system, in order to provide to the fowl those conditions for better exteriorise their yield potential in quite similar environmental conditions to the natural ones. Certain approved alternative exploitation systems are a mixture between the superintensive system (traditional cages), the intensive system (rearing on permanent layer - eg minced hay) and the extensive one (totally free range). Consequently, the laying hens could benefit from a wider range of freedom, being accommodated cages without front panels, allowing them to have access on the hall floor [15]. The aim of our

study was to evaluate the dynamics of the skeletal myocytes histometric features at the hens accommodated and exploited in four versions of husbandry systems.

MATERIALS AND METHODS

The whole flock size reached 1.731 capitis of "Lohmann Brown" laying hens, studied during production period (fowl age 20-80 weeks). The hens were randomly allocated in four groups, at the experiment onset, as it follows: control L_c-conventional cages, L_1exp.-enlarged cages (600 cm^2/hen), L_2exp.-enlarged cages (1000 cm^2/hen) and L_3exp.-conventional cages with opened front panels (summarized 1000 cm^2/hen in nesting+resting cage and feeding+watering cage) + free movement on the floor.

When fowl reached the age of 80 weeks, 60 specimens (15 from each group) have been selected, in order to run muscular tissue sampling. The experimental design, including the husbandry conditions are revealed in table 1, corresponding to the classical and alternative systems we tested, in accordance with the technological specifications of the hybrid management guide [12] or with the EU regulation related to laying hens welfare [15].

Table 1

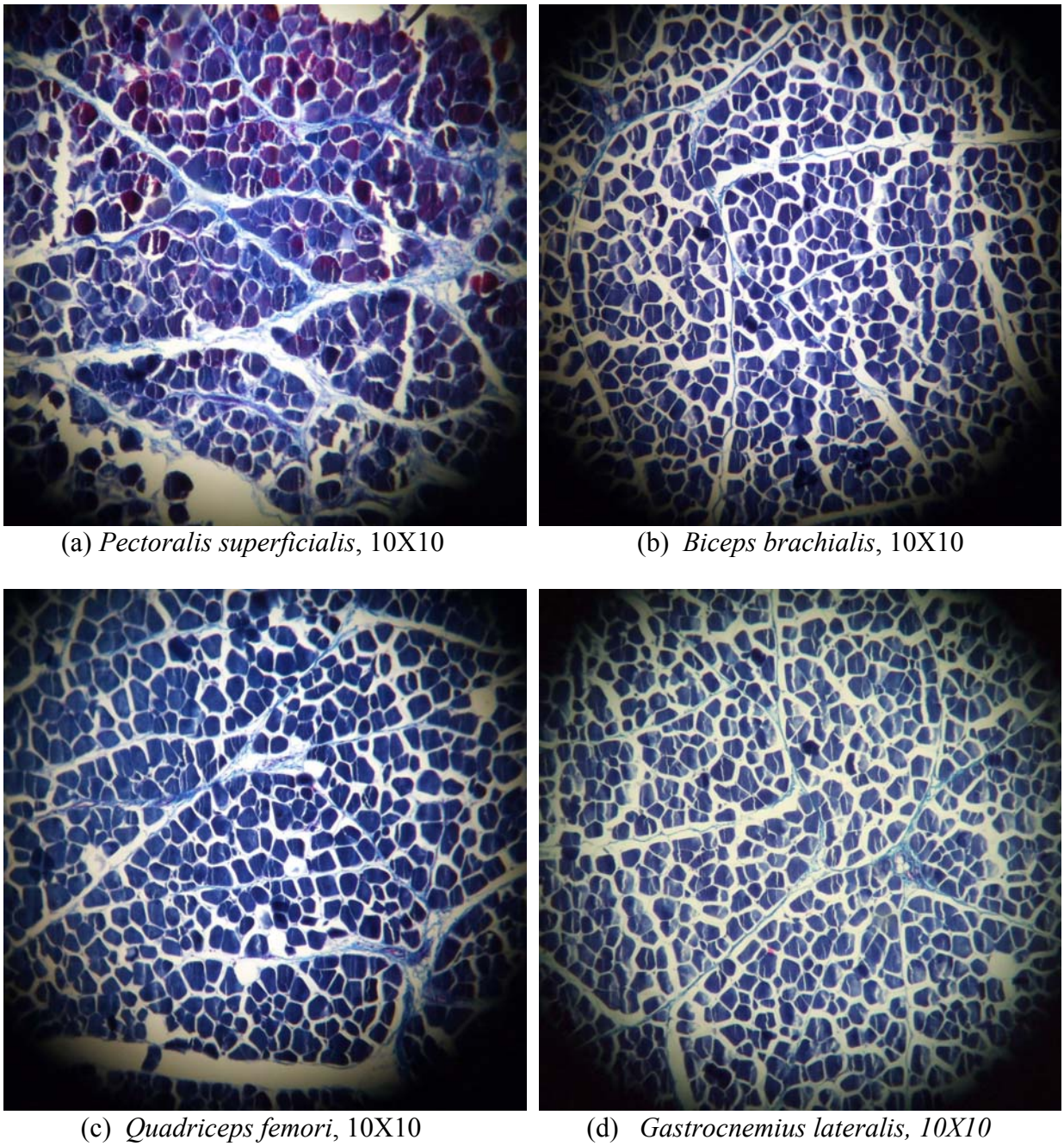
Experimental design

Notice	Experimental groups			
	Lc	**L_1exp**	**L_2exp**	**L_3exp**
Husbandry system	superintensive	superintensive	superintensive	intensive
Accommodation density	4 hens/coop of 2000 cm^2	5 hens/coop of 3000 cm^2	6 hens/coop of 6000 cm^2	4 hens/coop of 2000 cm^2
Cage type	Standard	Size modified	Size modified	Modified-improved (2 types): feeding+watering coops and laying+resting coops
Cage floor surface/hen (cm^2)	500	600	1000	500 cm^2 in laying + resting coop and 500 cm^2 in feeding + watering coop
Cage size (cm)	length=40; width= 50	length=60; width= 50	length=120; width= 50	length=40; width= 50
Access on the pen floor	NO	NO	NO	YES

The samples of muscular tissue have been taken from breast (*Pectoralis superficialis* muscles), wings (*Biceps brachialis* muscles), thighs (*Quadriceps femoris* muscles) and shanks (*Gastrocnemius lateralis*). Each sample was toileted then fixed in 10% formalin, dehydrated in ethylic alcohol of progressive concentrations (60° till 96°), immersed in amyl alcohol, in order to be prepared for impregnation in paraffin at +56°C. After this stage, the pieces have been cut at microthome, then clarified and mounted on glass blades and coloured with acid fuchsine and Evans blue, resulting histological smears. A trinocular photonic digital microscope, Motic DMB1-30 has been used to study the smears. The microscope was endorsed with objective micrometer, ocular micrometer, micrometric grid, Image Plus 2.0 – imaging data analyzing software, Motic M230 and FujiFilm Finepix A800 digital cameras. The dimensional features (large and small diameters) of the sectioned myocytes were analogically measured then the captured pictures were digitally processed and check the first measurement accuracy. These data served to calculate the mean thickness, the ratio between diameters and the cross-section area of the studied muscular fibres. 100 readings and/or computations have been carried on for each analyzed feature. The achieved values have been statistically processed running the ANOVA single factor algorithm.

RESULTS AND DISCUSSIONS

Certain shots from the microscopic field are presented in Fig. 1, at an 10X10 magnification power. The numeric data issued from the histometric assessments and from statistical analysis are presented in tables 2 (main features – myocites diameters) and 3 (myocytes cross-section area). The results are better illustrated in figures 2 and 3.



(a) *Pectoralis superficialis*, 10X10 (b) *Biceps brachialis*, 10X10

(c) *Quadriceps femori*, 10X10 (d) *Gastrocnemius lateralis, 10X10*

Fig. 1 – Captures from the microscopic field, revealing aspects of the myocytes in the four skeletal muscles issued from Lohman Brown laying hybrids, exploited within classical and alternative technological systems

In the *Pectoralis superficialis* (breast fillet component) fibers, the small diameters values oscillated between 37.36±0.57μ (Lc group) and 42.47±1.51μ (L3exp group), while the homogeneity for the studied character was considered as good (v=3.25…3.81%). The mean values for the large diameters were calculated within the 41.13±1.23μ (Lc group) – 47.15±1.82μ (L3exp group). The variation coefficient had wider limits (v=3.93…5.61%). Consequently the amplitude of myocytes mean thickness values was quite high, oscillating between 39.24±0.70μ (Lc) and 44.81±1.60μ (L3exp) (fig. 2a). The values assessed for the other groups with hens accommodated in the enlarged cages, were closer to the values measured in the LC samples. The ANOVA testing resulted in very significant differences ($\hat{F} > F_{\alpha}(0.001)$ at 1; 198 LD) for the large and average diameters, between groups Lc, L1exp, L2exp on the one hand and group L3exp (free movement on the pen floor) on the other hand. The same comparison revealed significant differences ($\hat{F} > F_{\alpha}(0.05)$ at 1;198 LD) for the small diameter values.

Table 2

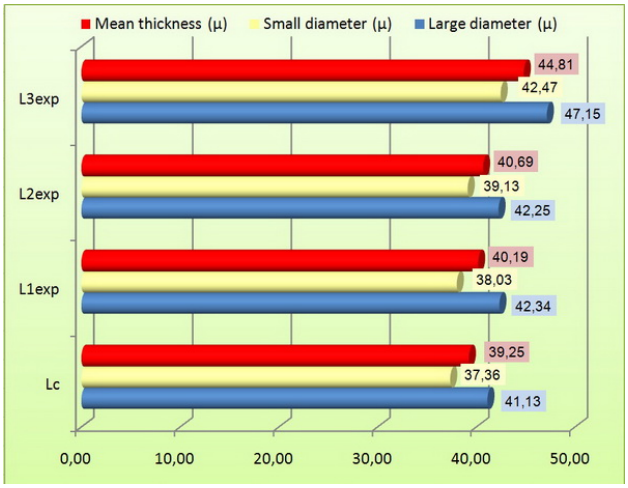
Histometric features of the myocytes (assessed on cross section) in some skeletal muscles of Lohman Brown laying hybrids, exploited within different types of technological systems (n=100)

Feature	Studied muscle	Experimental groups							
		Lc		L1exp		L2exp		L3exp	
		$\overline{X} \pm s_{\bar{x}}$ (μ²)	v %	$\overline{X} \pm s_{\bar{x}}$ (μ²)	v %	$\overline{X} \pm s_{\bar{x}}$ (μ²)	v %	$\overline{X} \pm s_{\bar{x}}$ (μ²)	v %
Large diameter (LD) (μ)	*Pectoralis superficialis*	41.13^{a}±1.23	3.93	42.34^{a}±1.14	3.25	42.25^{a}±1.37	4.12	47.15^{d}±1.82	5.61
	Biceps brachialis	32.30±0.77	6.72	33.48±1.14	8.76	33.14±1.09	8.85	32.57±1.04	7.36
	Quadriceps femoris	38.97^{a}±1.23	5.31	39.34^{a}±1.42	7.02	39.02^{a}±1.35	6.23	42.13^{c}±1.51	8.62
	Gastrocnemius lateralis	35.19^{a}±1.09	6.14	36.27^{a}±1.21	7.25	35.97^{a}±1.14	7.25	38.92^{c}±1.22	7.66
Small diameter (sd) (μ)	*Pectoralis superficialis*	37.36^{a}±0.57	3.25	38.03^{a}±0.82	3.31	39.13^{a}±0.98	3.53	42.47^{d}±1.51	3.81
	Biceps brachialis	27.16^{a}±0.42	7.25	28.14^{a}±0.97	7.48	29.80^{b}±0.72	7.54	29.47^{b}±0.87	5.89
	Quadriceps femoris	35.61^{a}±1.08	4.92	36.80^{a}±1.15	5.49	36.72^{a}±1.18	6.82	39.11^{c}±1.22	7.19
	Gastrocnemius lateralis	30.15^{a}±0.85	5.89	29.77^{a}±0.79	5.57	31.51^{a}±1.08	6.02	33.50^{c}±0.71	7.14
Mean thickness (μ)	*Pectoralis superficialis*	39.24^{a}±0.70	3.00	40.19^{a}±0.94	3.06	40.69^{a}±1.12	4.19	44.81^{d}±1.60	4.32
	Biceps brachialis	29.73±0.50	6.97	30.81±1.10	8.21	31.47±0.80	7.89	31.02±0.98	6.61
	Quadriceps femoris	37.29^{a}±1.19	5.16	38.07^{a}±1.31	6.87	37.87±1.22	5.91	40.62^{c}±1.30	7.44
	Gastrocnemius lateralis	32.67^{a}±1.05	6.03	33.02^{a}±1.07	6.42	33.74^{a}±1.11	6.83	36.21^{c}±0.90	7.28
LD/sd ratio	*P. superficialis*	1.10		1.11		1.08		1.11	
	B. brachialis	1.19		1.19		1.11		1.11	
	Q. femoris	1.09		1.07		1.06		1.08	
	G. lateralis	1.17		1.22		1.14		1.16	

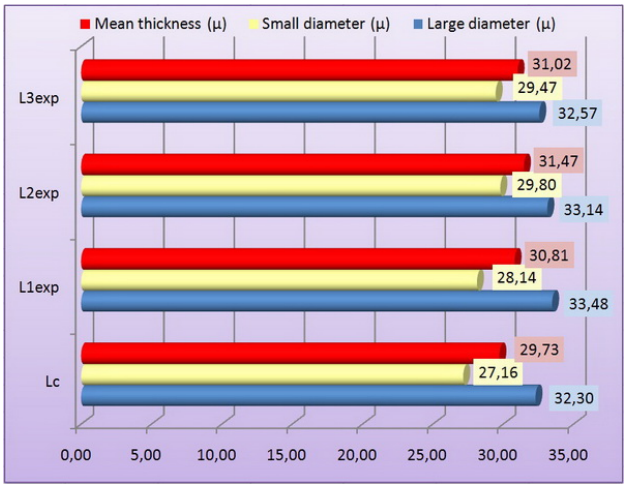
ANOVA significance, superscripts within the same row, between experimental groups:
no superscript = no statistical significance for the differences between means;
^{ab} = significant differences between means, $\hat{F} > F_{\alpha}(0.05)$ at 1;198 LD;
^{ac} = distinguished significant differences between means, $\hat{F} > F_{\alpha}(0.01)$ at 1; 198 LD;
^{ad} = very significant differences between means, $\hat{F} > F_{\alpha}(0.001)$ at 1; 198 LD.

The values for the average diameters were found higher than in other researches focusing on other fowl categories texture, such as chicken broilers (26.6µ - large diameter) [2, 9, 10, 14], knowing that meat-type hybrids have better developed breast fillet but thinner myocytes, resulting a better tenderness [1, 2, 6].

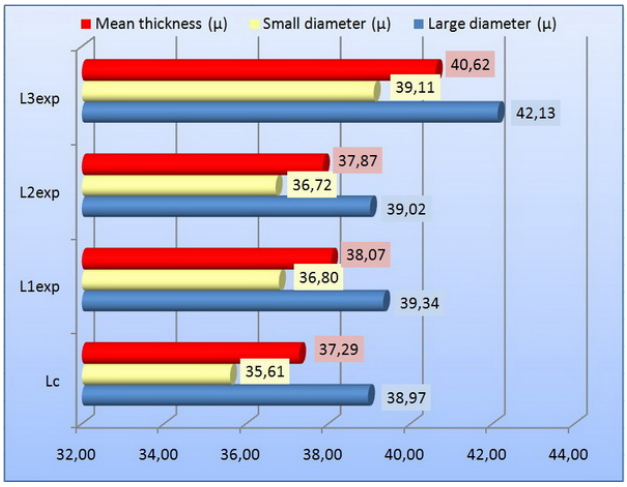
In the wing muscles (*Biceps brachialis*) the differences existing between the four studied technological versions were closer and not statistically significant. However, the situation was different than that observed in breast muscles, meaning the higher values were found in the musculature of the hens belonging to the L2exp group (enlarged cages – 1000 cm^2/hen), while the lower ones in the control group musculature. Thus, the small diameter varied between 27.16±0.72µ and 29.80±0.72µ, the large one between 32.30±0.77µ and 33.48±1.14µ, while the highest mean thicknesses were found within the 29.73±0.50µ - 31.47±0.8µ (tab. 2, fig. 2 b). Despite the closer values, the variability was higher, the coefficient of variation oscillating between 6.72% - 8.85%. These dimensions were found, once again, higher than those of the chicken broilers (thinner fibers) but slightly similar to those existing in waterfowl females (31.09µ in goose and 29.71µ in duck) [4, 7, 8, 10, 14].



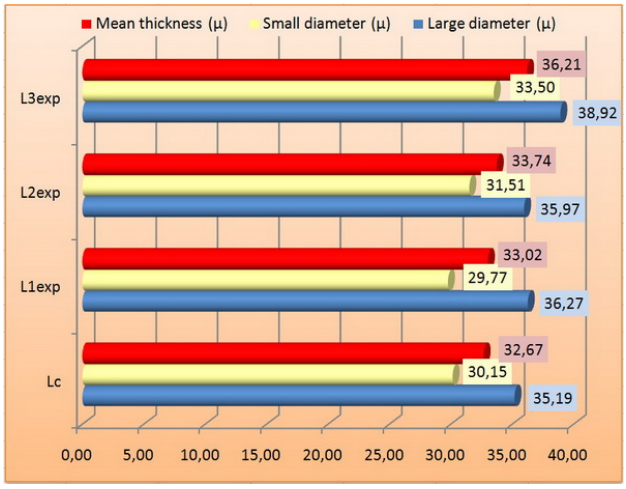
(a) *Pectoralis superficialis* muscles



(b) *Biceps brachialis* muscles



(c) *Quadriceps femoris* muscles



(d) *Gastrocnemius lateralis* muscles

Fig. 2 – Diameters of the skeletal muscles fibres at the Lohmann Brown laying hens, exploited within different husbandry systems

The rearlimbs musculature presented thicker fibres than that of the wings but thinner than the white fibres in the pectoral muscles. The myocytes in the *Quadriceps femoris* muscles (thighs) presented values of the mean diameters between the 37.29±1.19μ (Lc) and the 40.62±1.47μ (L3exp) limits, with values oscillating from 1089.91±9.62μ^2 till 1294.10±12.43 μ^2, at the former and later specified populations. The mean thickness value reached by the hens having access to free movement was closer to that observed in duck females reared within semiintensive conditions (pen + paddock) (40.73 μ) [8]. The shanks analyzed myocytes, issued from the *Gastrocnemius lateralis* muscles sampling, proved to be even thinner than those in the thighs meat, varying within the 32.67±1.05μ - 36.21±0.9μ range (Lc – L3exp), respectively from 833.29±7.54μ^2 till 1024.02±8.76μ^2. These values were also quite similar to those found for the domestic duck but lower than those observed in domestic goose (females) [8].

After the ANOVA algorithm processing, the differences were found distinguished significant ($\hat{F} > F_{\alpha}(0.01)$ at 1; 198 LD) for the diameters of the Quadriceps femoris and Gastrocnemius lateralis muscles, between the means of groups control, L1exp and L2exp and those of the L3exp group, in most of the cases. The exception was given by the comparison for the small diameters of the shank muscles, whose differences were considered as highly significant ($\hat{F} > F_{\alpha}(0.001)$ at 1; 198 LD).

The results related to the cross surface area presented the same dynamics to that observed in myocytes thickness assessments. Thus, for the breast and rearlimbs muscles, the extreme values were found in control group (minimal), respectively in the L3exp one (maximal) (tab. 3).

The highest values were found in breast muscles (1208.68±8.36 μ^2 -1574.12±10.47 μ^2), while the lowest ones in the shanks muscles (833.29±7.54 μ^2 - 1024.02±8.76μ^2). The differences were found highly significant (*P. superficialis*) or distinguished significant (*Q. femoris* and *G. lateralis*) (fig. 3).

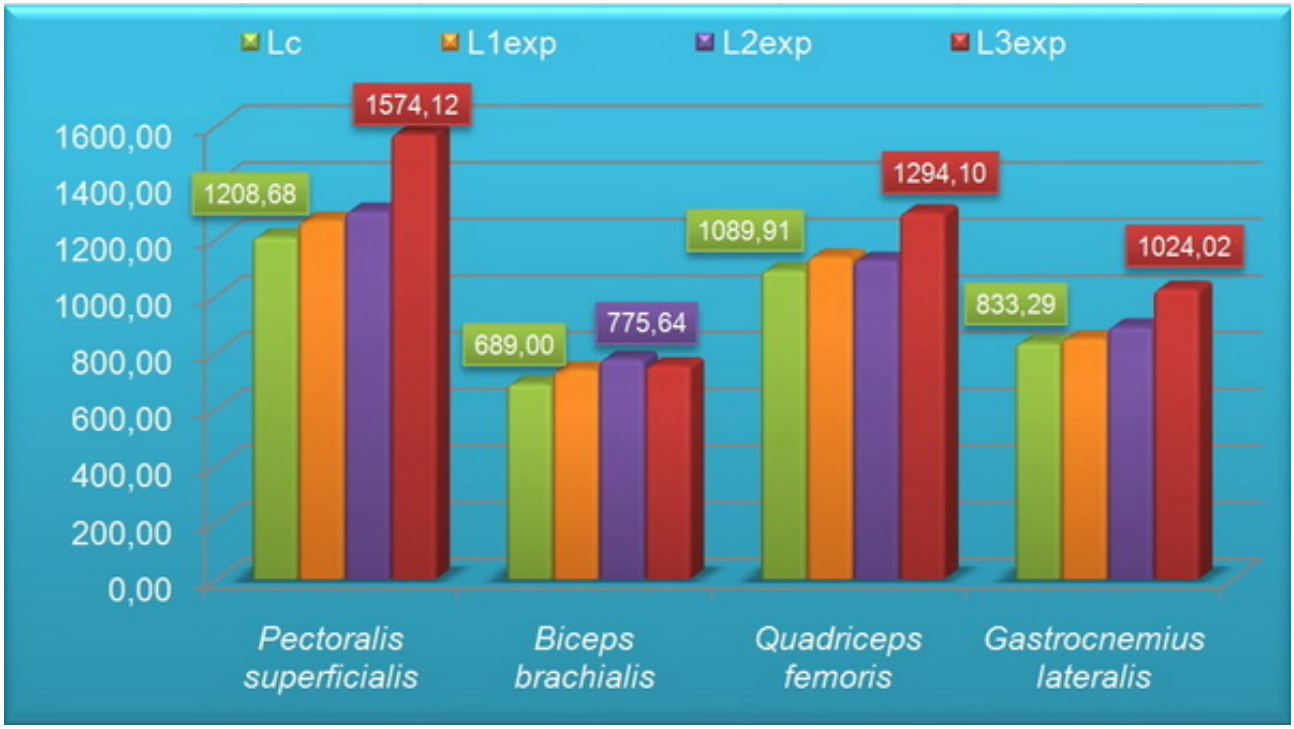


Fig. 3 – Cross section areas (μ^2) of the skeletal muscles fibers at the Lohmann Brown laying hens, exploited within different husbandry systems

Table 3

Cross section area of the myocytes in some skeletal muscles of Lohman Brown laying hybrids, exploited within different types of technological systems (n=100)

Studied muscle	Experimental groups							
	Lc		L1exp		L2exp		L3exp	
	$\overline{X} \pm s_{\bar{x}}$ (μ^2)	v %	$\overline{X} \pm s_{\bar{x}}$ (μ^2)	v %	$\overline{X} \pm s_{\bar{x}}$ (μ^2)	v %	$\overline{X} \pm s_{\bar{x}}$ (μ^2)	v %
Pectoralis superficialis	1208.68^{a}±8.36	6.09	1266.71^{a}±8.71	7.23	1299.34^{a}±9.62	8.14	1574.12^{d}±10.47	8.65
Biceps brachialis	689.00±6.72	6.72	739.94±7.04	7.28	775.64±8.94	8.92	753.85±7.69	7.84
Quadriceps femoris	1089.91^{a}±9.62	7.64	1137.03^{a}±10.97	8.02	1125.33^{a}±10.81	7.92	1294.10^{c}±12.43	8.84
Gastrocnemius lateralis	833.29^{a}±7.54	7.21	848.04^{a}±7.84	7.92	890.18^{a}±8.27	8.19	1024.02^{c}±8.76	8.53

ANOVA significance, superscripts within the same row, between experimental groups:
no superscript = no statistical significance for the differences between means;
^{ab} = significant differences between means, $\hat{F} > F_{\alpha}(0.05)$ at 1;198 LD;
^{ac} = distinguished significant differences between means, $\hat{F} > F_{\alpha}(0.01)$ at 1; 198 LD;
^{ad} = very significant differences between means, $\hat{F} > F_{\alpha}(0.001)$ at 1; 198 LD.

For the wing muscles, the values oscillated between 689.00±6.72 μ^2 (Lc) and 775.64±8.94 (L2exp.), but the differences had not statistical significance.

The variation coefficient values were found within the 6.09% - 8.84% limits, indicated thus good homogeneity.

CONCLUSIONS

Several differences have been revealed for the skeletal myocytes dimensions, consequently for the striated muscles texture, between the females reared within classical cages and alternative

Very significant differences occurred for *Pectoralis superficialis* myocytes diameter values and mostly distinguished significant ones for rear limbs muscles cells, between the groups accommodated in classical cage system and the L_3exp group.

The histometric assessments could enforce the hypothesis stating that fowl having access to free movement were predisposed to myocytes hypertrophy, generating, at the slaughtering moment, carcasses with better developed musculature. Those carcasses should include higher proportion of breast fillet, thighs and shanks. However, the data must be correlated with the eggs yield efficiency and with its hygienic and quality status.

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