3:8 Histological traits of two muscles of lambs as affected by age in comparison with some muscles of Buffaloes

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Introduction

According to many investigators the hiostological structure of muscle tissues markedly affected the tenderness of meat, specially with regard to the muscle fibre diameter, sarcomere length as well as the amount and firmness of connective tissues. The structural traits of muscle tissues undergo considerable changes after slaughter which also changes the physical properties of meat. (Satorius and child, 1938; Ramsbottom et al., Hiner et al., 1955; Biro, 1969; Herring et al., 1965, Eino and Stanley, 1973; Berry et al., 1974 and El-Dashlouty, 1978).

The aim of this investigation was to study the structural changes of muscle tissues obtained from the longissimus dorsi and Biceps Femoris muscles of lambs as affected by age (8, 10 and 12 months of age) as well as of buffalo males (18 months of lage). Histological changes were also studied during autolysis at 4°c and frozen storage at - 10°c.

Materials and Methods

A number of 9 Ossimi lambs and 3 buffaloe males were used in this study. These animals were raised at the farm of the Faculty of Agriculture of Moshtohor.

Lamb and buffaloe calves were weaned at 4 months of age. Then fattening was carried out on a pelleted ration composed of cotton seed meal, 65%; wheat bran, 12%; rice bran, 20%; calcium carbonate, 2%; and sodium chloride, 1%.

The daily intake for lambs and calves from weaning to slaughtering; the energy and protein content of each ingredient were as reported in the Ministry of Agriculture Bull. (1968). Lambs were slaughtered at 8,10 and 12 months of ages (3 lambs at each age) while three calves were slaughtered at 18 months of age. Samples were taken from thelongissimus dorsi (LD) and biceps femoris (BF) muscles after about 1.5 hours of slaughter (fresh), then after one and six days of cold storage at 4°c as well as after one and two months of frozen storage at - 10°c.

Samples of fresh and cold-stored tissues were fixed at room temperature, while frozen stored samples were fixed at 4°c using 10% solution of neutral formalin. After fixation samples were dehydrated in alcohol solutions, then blocked in collodion. Section of about 5 microns thickness were prepared and stained by hematoxilin-iosin and Van-Geison's methods (Kisili, 1962). Sections were investigated using light microscope and photographed. The sarcomere lengths and muscle fibre diameters were measured and average values for three animals are given.

Results and Discuss ion

A) General appearance:
1. Antemorten variations:
The muscle fibres of lambs are held together in bundles or fasciculi by the endomysium connective tissue. A number of these bundles in turn are held together by perimysium which is more thick and firmly build as compared with the endomysium. Inside the connective tissues the blood vessels and fat tissues could be observed. Also the thick and firm perimysium could be easily defferentiated from the relatively fine endomysium (Fig. 1-a). In the longitudinal sections the muscle fibres were straight or slightly waved, being incased in a thin membrane-the sarcolemma. The muscle fibres are cross-striated due to the persence of sarcomeres. Nuclei are arranged at the perifery of the muscle fibres. The general appearance of muscle tissues was the same for mutton and buffaloes meat with slight differences. Buffaloes meat, however, showed more thick fibres, more distinct faciculi and more dense and firm endomysium and perimysium (Fig. 1-a,c).

With advancing of age the connective tissues became more firm and increased in the amount (Fig. 1-a). Visually, the general appearance may also show the increase in muscle fiber ticknesses with age (Fig. 1-a).

As indicated by the visual examination of muscle tissue sections one may observe the increase of the amount and firmness of connective tissues in biceps fe moris than in the longissimus dorsi muscle. The former muscle showed also more thick muscle fibres (Fig. 1-a, b, c).

The aforementioned observations indicated that some histological changes could be observed due to antemortem factors as the species, age and part (muscle).

Fig. (1-a) Fig. (1-b) Fig. (1-c)

Fig. 1 Effect of age, species and part, 1.5 hour of slaughter, (5 x 40).
Fig. 1-a Longissimus dorsi, lambs, 8 months of age, small amount of connective tissues, small fibre diameters.
Fig, 1-b Biceps femoris, lambs, 8 months of age, more thick muscle fibres.
Fig. 1-c Longissimus dorsi, buffaloes, 18 months of age, large amounts of connective tissues, thick muscle fibres.

2. Postmortem variations:
a) Cold-storage:
After slaughter the muscle fibres of fresh meat were straight or slightly waved. Interstitial clear areas were either slight or not found. The crossstriation was relatively wide. Fresh samples of buffaloes showed more undulations which may be ascribed to the tension due to more struggle at agony when combared with lamb.

After one day storage at 4°c the muscle fibres became undulated taking the wavy and zigzag forms; cross striation became narrow. Due to the undulations interstitial clear areas were more wide. No changes occured in the appearance of connective tissues and nuclei. The effect of species, part and age was not marked at rigor mortis, i.e. due to muscle contraction.

After six days storage the muscle fibres of mutton were straight, thin and with wide cross striations. Interstitial clear areas were narrow as compared with one day storage but relatively wide when compared with the fresh samples. Muscle fibre breaks with granulated substances were numerous indicating the aging stage and increase of tenderness. Similar changes were reported by Paul et al., 1944; El-Dashlouty et al., 1967 and El-Dashlouty and El-Ashri, 1970).

With regard to buffaloes meat, after six days storage some undulations still existed and no fibre breaks with granulated substances appeared which indicated that the tissues were far away from the stage of aging and requires for more time of cold-storage. Only small number of fibre breaks with no granulated substances were found for buffalo meat at the 6th day of storage.

b) Frozen-storage:
After one month storage at -10°c the muscle fibres of lamb and buffaloe meat were thin compared to fresh samples (Fig. 2-a), which could be due the loss of water which diffused from the fibres outside forming ice crystals between them, and causing the pressing of muscle cells with the appearance of wide clear interstitial areas (Fig. 2-a and b). The wide clear interstitial areas, thereby appeared due to presing of fibres into groups by the effect of ice crystals. The fibres became undulated due to pressing of the hard outer layers of meat samples in as much as the outer parts may be rapidly frozen when compared with the inner parts. The formation of the ice crystal caused also the appearance of the muscle fibre breaks (Fig. 2-a,b,c).

After two months storage (compared to one month) the muscle fibres became more thin, the intersitial clear areas were more wide (Fig. 2-a,b) and the fibre breaks increased. Further grouping of pressed fibres was observed. This could be explained on the basis that fluctuation of temperature lead to the less growth of ice crystals. Due to the water loss from fibres the adding of ice crystals between the bundles was noticed while the crossstriation became more narrow. At this period, i.e after two months storage at -10°c the fat tissue cells which were compacted showed some breakage and connective tissue were broken and loose at some parts of the sections which may be also due to the effect of big ice-crystals.

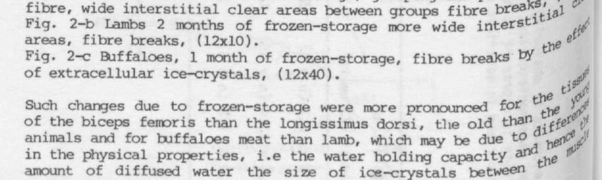

Fig. (2-a) Fig. (2-b) Fig. (2-c)

Fig. 2 The effect of frozen-storage, biceps femoris.
Fig. 2-a Lambs 1 month of forzen-storage, grouping and pressing of muscle fibre, wide interstitial clear areas between groups fibre breaks, (12x10).
Fig. 2-b Lambs 2 months of frozen-storage more wide interstitial clear areas, fibre breaks, (12x10).
Fig. 2-c Buffaloes, 1 month of frozen-storage, fibre breaks by the effect of extracellular ice-crystals, (12x40).

Such changes due to frozen-storage were more pronounced for the tissues of the biceps femoris than the longissimus dorsi, the old than the young animals and for buffaloes meat than lamb, which may be due to differences in the physical properties, i.e the water holding capacity and hence the amount of diffused water the size of ice-crystals between the muscle cells and bundles.

B) Muscle fibre diameters and sarcomere lengths:
The variations of the muscle fibre diameters and sacromere lengths for lamb and buffaloes meat are given in tables 1 and 2.

1. Antemortem variations:
a- Effect of age: From tables 1 and 2, it could be noticed that with advancing of age both the muscle fibre diameters and sarcomere lengths increased.

The results in table 2, show that the rate of increase in the fibre diameters and sarcomere lengths was relatively higher for the logissimus dorsi muscle and lower for the biceps femoris between 10 and 12 months of age as compared with the period between 8 and 10 months. Nevertheless the total summation of the two periods showed that the growth in the muscle fibre diameters and sarcomere lengths was less pronounced for the longissimus dorsi muscle (144.99 and 110.00% respectively) when compared with the biceps Femoris (148.23 and 111.11% respectively).

Table 1: The average muscle fibre diameters of the longissimus dors (LD) and biceps femoris (BF) muscles of lambs and buffaloes (in microns)

Muscle fibre diameters		Lambs						Buffaloes	
		8 mth.of age		10mth.of age		12mth.of age		18mth.of age	
		LD	BF	LD	BF	LD	BF	LD	BF
Fresh Samples	microns	21.83	28.74	26.25	35.64	31.71	42.60	43.96	52.30
	%	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1 day at 4°C	microns	28.77	38.55	-	-	42.96	58.46	56.89	69.30
	%	131.55	134.13	-	-	135.48	137.23	129.41	132.51
6 days at 4°C	microns	18.25	25.13	-	-	27.75	39.38	47.72	60.56
	%	85.83	87.44			87.64	92.44	108.55	115.83
1 mth at-10°C	microns	19.28	24.50	-	-	26.11	33.94	33.34	37.23
	%	88.16	85.25	-	-	82.34	79.67	75.84	71.19
2 mths at-10°C	microns	15.09	18.25	-	-	20.76	25.43	26.60	30.01
	%	69.00	63.50	-	-	65.47	59.70	60.51	75.38

Table 2: The average sarcomere longths of the longissimus dorsi (LD) and biceps femoris (BF) muscle of lambs and buffaloes (in microns).

Sarcomere Lengths		Lambs						Buffaloes	
		8 mth.of age		10mth.of age		12mth.of age		18mth.of age	
		LD	BF	LD	BF	LD	BF	LD	BF
Fresh Samples	microns	3.00	2.70	3.10	2.90	3.30	3.00	2.75	2.41
	%	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1 day at 4°C	microns	2.00	1.69	-	-	2.10	1.78	1.84	1.52
	%	66.67	65.59	-	-	63.64	59.33	66.91	63.07
6 days at 4°C	microns	2.66	2.16	-	-	2.67	2.34	2.20	1.87
	%	82.00	80.00	-	-	80.91	78.00	80.00	77.59
1 mth at-10°C	microns	2.45	2.05	-	-	2.62	2.23	2.13	1.85
	%	81.67	75.93	-	-	79.39	74.33	77.46	76.76
2 mths at-10°C	microns	1.82	1.55	-	-	1.95	1.71	1.57	1.35
	%	60.67	57.41	-	-	59.09	57.00	57.09	56.02

Table 3: The effect of age on the muscle fibre diameters and sarcomere lengths of the longissimus dorsi (LD) and biceps femoris (BF) muscle of lambs (%).

Age / Muscle	8-10 months		10-12 months		8-12 months	
	LD	BF	LD	BF	LD	BF
Fibre diameters	120.03	124.01	120.80	119.53	114.99	148.23
Sarcomere lengths	103.33	107.41	106.45	103.45	110.00	111.11

From table 3, it could be also observed that the increase in muscle fibre diameters was more pronounced than the increase in the sarcomere lengths. The small increase in the sarcomere lengths is sufficient to cause marked longitudinal growth of the muscle in as much as very large number of these sarcomeres is found in each myofibril, while the number of myofibrils is not so large as the number of sarcomeres, hence marked increase in the myofibril thickness (and fibre diameters) should occur to cause the cross growth of the muscle, which may be observed from the results of table 3.

b- Effect of species: From tables 1 and 2, it could be notieced that the muscle fibre diameters were larger and sarcomeres were smaller in buffalo muscles than the lambs, taking into consideration that the buffalo animals were also older than the lambs. The muscle fibre diameters of buffaloes (18 months of age) were 138.63% and 122.77% of the lambs fibres at 12 months of age for the longissimus dorsi and biceps femoris muscles respectively; sarcomere were 83.33% and 80.33% respectively.

c- Effect of part (muscle): The results in tables 1 and 2, show that the biceps femoris muscle showed more fibre diameters and smaller sarcomeres regardless age, species (and storage conditions). Such results go in parallel with the results of Herring et al, (1965) and El-Dashlouty et al, (1967). From table 4, it could be noticed that in fresh samples the muscle fibre diameters of the biceps femoris were 131.65-134.34% that of the longissimus dorsi muscle, the value was 118.97% for fuffaloes. Sarcomere lengths of the biceps femoris muscle of lambs were 90.00-93.55% that of the longissimus dorsi; the value for buffaloes was 87.64%. Hence the differences between the muscle fibre diameters of the biceps femoris and longissimus dorsi muscle were more pronounced for lambs than buffaloes. The opposite was found for the sarcomere lengths.

The aforementioned results and discussions may indicate that the meat of buffaloes is less tender than lamb meat, biceps femoris is also less tender and with advancing of age the meat tenderness decreased. Herring et al, 1965 and Sakolov, 1965, reported th at the increase of fibre diameters and decrease of sarcomere lengths are associated with the low tenderness of meat.

Table 4: The muscle fibre diameters (FD) and sarcomere lengths (SL) of the biceps femoris muscle (BF) as percentages of that for the longissimus dorsi (LD).

Storage	Lambs						Buffaloes	
	8 mth.of age		10mth.of age		12mth.of age		18mth.of age	
	LD	BF	LD	BF	LD	BF	LD	BF
Fresh samples	131.65	90.00	135.77	93.55	134.34	90.91	118.97	87.64
1 day at4°C	133.65	84.50	-	-	136.08	84.76	121.81	82.61
6 days at4°C	137.70	87.81	-	-	141.71	87.64	126.95	85.00
1mth at-10°C	127.08	83.67	-	-	129.99	85.12	111.67	86.86
2mth at-10°C	120.94	85.17	-	-	122.50	87.69	112.82	85.99

2. Postmortem variations:

a) Cold-storage: From tables 1 and 2, it could be noticed that after one day of cold storage the muscle fibre diameters increased while the sarcomere lengths decreased indicating the attack of rigor mortis. From tables 1,2 and 4, it could be noticed that contraction is more pronounced for the biceps femoris than the longissimus dorsi muscle, for the old animals than the young ones, while was lower for buffaloes muscles as compared with lambs meat. The lower rate of contraction in buffaloes meat could be ascribed to that meat was still far away from the top of rigor mortis; being 3 days according to El-Dashlouty and El-Ashri (1970); while mutton was at full rigor, being after 24 hours of cold storage (El-Dashoulty et al., 1967).

After 6 days of cold-storage the muscle fibre diameters of lambs meat decreased below the level charaterizing the fresh meat, while the sarcomere lengths increased indicating relaxation of tissues and aging (Tables 1 and 2). For buffalo meat muscle fibre diameters after 6 days storage were still thick indicating that relaxation was not completed and aging requires for more period of storage time. With regard to age of lambs it could be concluded from the changes of muscle fibre diameter and sarcomere lengths that the meat of old animals and biceps femoris muscle showed lower rates of aging when compared with the meat of young animals and longissimus dorsi muscle. From table 4, it could be noticed that the differences, in the histological measurements, between the biceps femoris and longissimus dorsi muscle increased with cold-storage which may also indicate the more rate of contraction at rigor mortis and the slow rate of aging and relaxation characterizing the biceps femoris muscle.

b. Frozen-storage: From tables 1 and 2 it could be noticed that as the time of frozen-storage increased the muscle fibre diameters decreased and the sarcomere lengths also decreased, indicating the diffusion of water outside the cells to form extracellular ice crystals (shrinkage). This decrease was more pronounced for the biceps femoris than the longissimus dorsi muscle, for the tissues of old lambs than the young ones and for the buffaloes meat than the mutton, which may be ascribed to the different water holding capacities. By frozen storage the differences between the biceps femoris and longissimus dorsi muscle in the fibre diameters and sarcomere length decreased (Table 4) indicating that the biceps femoris muscle lost much more water which diffused from the cells. Such difference mostly decreased as the time of frozen-storage increased which may show that the deterioration of the water binding ability and quality of the biceps femoris muscle may be more pronounced as compared with the longissimus dorsi case.

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