Comparison of muscle fibre structure in male and female young cattle and slaughter calves of the Polish Holstein-Friesian breed and commercial crossbreeds*

Małgorzata Ryszkowska-Siwko

University of Life Sciences in Lublin,
Department of Commodity Science and Processing of Raw Animal Materials,
ul. Akademicka 13, 20-950 Lublin

The aim of the study was to compare the muscle fibre structure of young bulls and heifers (calves and young slaughter cattle) of the Polish Holstein-Friesian breed and commercial crossbreeds. Samples were taken from the *longissimus lumborum* (MLL) muscle. The number, percentage, diameter and surface area of red, intermediate and white fibres were analysed. The age of the cattle significantly determined the histological parameters of the muscle fibres, i.e. their number, diameter and surface area. A significant effect of sex on the number of all types of fibres was noted (P≤0.01), as well as an interaction of category x breed on the percentage of white fibres (P≤0.01). Certain indicators of slaughter value, i.e. slaughter weight and hot and cold carcass weight, were found to be significantly (P≤0.001) positively correlated with the diameter (r=0.7068-0.7648) and surface area (r=0.5794-0.7804) of all types of fibres, and negatively correlated with the percentage of intermediate fibres (r=–0.5831 at –0.6023). Moreover, significant (P≤0.001) positive correlations were found between fat content and the diameter (r=0.5049-0.5763) and surface area (r=0.4498-0.5654) of all types of fibres. A slightly lower correlation (r=0.3623 at P≤0.01) indicating higher fat content in red fibres was observed.

KEY WORDS: structure muscle fibre / sex / category of cattle / breed

Meat is one of the basic foodstuffs, and its value is determined by the nutrients contained in it [24]. It is a significant source of valuable macronutrients essential to humans (including amino acids and fatty acids), as well as micronutrients (vitamins and elements) [2, 9, 15]. It is these that determine its nutritional value, appearance, flavour and aroma. The contemporary consumer expects tender meat with specific flavour and health properties. These characteristics depend mainly on the structure of the skeletal muscles, and in

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particular on the number and thickness of various types of muscle fibres and on the content of intramuscular fat and connective tissue [8].

Meat quality is the sum of all sensory, physiological, nutritional and technological/processing characteristics [22]. The range of post mortem transformations which the muscle tissue undergoes after slaughter suggests that meat quality is a complex, multifaceted system, often dependent on interrelated factors [3].

The main characteristics influencing the quality of beef during consumption are tenderness, juiciness, palatability, external appearance, and aroma.

Thus the culinary, nutritional and processing characteristics of meat quality, including palatability, aroma, tenderness, juiciness, colour, digestibility and assimilation, capacity to absorb and bind water, resistance to putrefaction, and many others, are derived from endogenous and exogenous enzymatic processes taking place in the muscle tissue after slaughter [7]. These characteristics are determined by changes in myofibril proteins (actin and myosin) and cytoskeletal proteins, which form the structure of muscle fibre (titin, nebulin, desmin, filamin, and synemin), as well as by changes in connective tissue proteins. Thus they depend on the biological structure and characteristics of muscle fibres, which determine the metabolic potential of the tissue and the efficiency of glycogenolytic transformations [5, 6, 30], which significantly influences the quality of the raw material and later the final product.

The aim of the study was to characterize and compare the microstructure of muscle fibres of cattle of different categories and breeds.

Material and methods

The study was conducted on 123 head of cattle of three age categories, i.e. young beef cattle, ‘heavy’ calves, and milk-fed calves, and two genotypes: Polish Black-and-White Holstein-Friesian and commercial crossbreeds obtained from Black-and-White cows and bulls of beef breeds.

Carcass value indicators, chemical composition, and muscle fibre structure were determined. Evaluation of the muscle fibre structure was carried out on samples from the *longissimus lumborum* muscle (MLL), distinguishing three age categories:

– young beef cattle (aged 15-22 months)
– ‘heavy’ calves (aged 5-9 months)
– milk-fed calves (aged 1-3 months)

During dissection, after the carcass had been chilled for 24 hours at 0-2°C, the *longissimus lumborum* muscle was collected for analysis. The analyses were performed at the laboratory of the Department of Commodity Science and Processing of Raw Animal Materials, University of Life Sciences in Lublin.

Evaluation of muscle fibre structure was based on photograms from an OLYMPUS CX41RF light microscope, using Multi Scan Base ver. 14 software. A histochemical colour reaction was used to evaluate muscle microstructure, according to the method described by Nachlas et al. [21] based on the enzyme activity of succinate dehydrogenase

(SDH) in different types of fibres. Depending on colour intensity three types of fibres were distinguished:

– red – (oxidative) – STO (Slow-Twitch Oxidative)
– intermediate (oxidative glycolytic) – FTO (Fast Twitch Oxidative)
– white (glycolytic) – FTG (Fast Twitch Glycolytic)

Microstructure characteristics were analysed on the basis of measurements performed in three randomly selected fascicles. The evaluation consisted of the following measurements:

– number of individual muscle fibres
– percentage of each type of muscle fibre
– diameter of each type of muscle fibre (μm)
– surface area of each type of muscle fibre (μm^2)

Statistical calculations were performed in STATISTICA 6 software from StatSoft, Inc. [28]. Muscle fibre characteristics were analysed by two-way analysis of variance with interaction, evaluating the effect of the category and breed (genotype) of cattle and of category and sex.

Pearson's linear correlation coefficients were calculated for carcass value indices, the chemical composition of the meat, and parameters characterizing muscle fibres. The significance of correlation coefficients was determined at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$.

Results and discussion

Age is a factor that significantly influences the characteristics of muscle microstructure, which largely determines meat quality. Changes in muscle microstructure are determined by factors resulting from the genotype, sex, age, and slaughter weight of animals [13, 17, 19, 23].

Tables 1 and 2 present changes taking place in muscles during their growth and development.

Table 1 shows changes in the distribution of types of fibres in the muscles of cattle in consequence of transformation of intermediate fibres (FTO) into white fibres (FTG). The transformation of intermediate fibres into white ones is confirmed by an increase in the percentage of FTG in heavy individuals. In the present study, this increase was 15.52 percentage points (p.p.) in young bulls, i.e. from 42.01% in the group of milk-fed calves to 57.53% in the young beef cattle, and 26.92 p.p. in heifers, i.e. from 29.27% in the group of milk-fed calves to 56.19% in the young beef cattle.

A study by Jurie et al. [11] on the muscles of Limousin bulls in four age groups (1, 6, 12 and 16 months) showed that the percentage of fast-twitch glycolytic (FTG) fibres increased with age, from 56% in animals at the age of one month to 70% at the age of 16 months.

Analysis of the muscle fibres taking into account the effect of category and sex (Tab. 1) revealed that sex had a significant effect at $P \leq 0.01$ only on the microstructure of the muscles, i.e. the number of red, intermediate and white fibres, and a significant

Table 1

Characteristics of muscle fibres of young bulls and heifers from the evaluated categories of cattle

Specification		Young slaughter cattle (n=33)		Heavy calves (n=27)		Milk-fed calves (n=63)		Influence		
		bulls (n=20)	heifers (n=13)	bulls (n=18)	heifers (n=9)	bulls (n=30)	heifers (n=33)	(P) sex	(K) category	(PxK) interaction
Number of red fibres	$\bar{x}$	33.17	24.49	45.50	10.75	230.25	164.17	**	**	**
	S	12.76	7.12	17.89	2.22	32.74	28.16			
Number of intermediate fibres	$\bar{x}$	28.17	24.43	29.25	17.25	214.50	138.33	**	**	**
	S	9.16	7.93	11.20	4.50	55.78	44.08			
Number of white fibres	$\bar{x}$	50.11	54.20	52.00	25.25	269.90	184.50	**	**	**
	S	9.76	7.80	22.99	10.40	58.11	46.12			
Percentage of red fibres	$\bar{x}$	17.53	19.70	22.02	11.61	25.77	21.23	ns	*	*
	S	5.90	5.11	10.86	3.21	3.16	2.80			
Percentage of intermediate fibres	$\bar{x}$	15.75	18.50	20.26	32.82	21.31	19.27	ns	**	*
	S	7.13	4.37	11.72	8.76	1.23	1.09			
Percentage of white fibres	$\bar{x}$	57.53	56.19	49.72	50.20	42.01	29.27	*	**	*
	S	5.80	7.61	3.81	7.69	6.95	6.08			
Diameter of red fibres (μm)	$\bar{x}$	54.22	50.03	40.60	40.79	19.13	17.23	ns	**	ns
	S	8.65	12.46	5.12	4.04	1.11	1.00			
Diameter of intermediate fibres (μm)	$\bar{x}$	66.60	57.13	51.06	49.64	23.07	21.26	ns	**	ns
	S	4.63	12.98	3.81	7.48	1.15	1.02			
Diameter of white fibres (μm)	$\bar{x}$	84.51	75.52	64.00	58.80	32.26	28.46	ns	**	ns
	S	6.81	14.30	14.00	3.76	2.36	1.64			
Area of red fibres (μm^2)	$\bar{x}$	3867.04	2950.72	2231.94	1820.83	446.84	397.51	ns	**	ns
	S	718.04	1512.32	900.87	320.99	34.08	26.50			
Area of intermediate fibres (μm^2)	$\bar{x}$	5512.09	4364.33	3339.52	2666.22	883.35	714.61	ns	**	ns
	S	625.31	2034.37	356.10	797.36	140.83	110.09			
Area of white fibres (μm^2)	$\bar{x}$	8265.24	7010.92	4781.90	3802.47	1584.43	1105.19	ns	**	ns
	S	641.80	2228.19	974.50	451.96	340.83	305.35			

*Significant at P≤0.05
**Significant at P≤0.01
ns – not significant

effect at P≤0.05 on the percentage of white fibres. No such effect was noted on the remaining characteristics. A significant effect of cattle category at P≤0.01 was observed on all muscle fibre characteristics analysed, with the exception of the percentage of red fibres, where the effect was significant at P≤0.05. A significant (P≤0.01) interaction of sex x category was noted for the number of red, intermediate and white fibres and for the percentage of red, intermediate and white fibres (P≤0.05). In the case of the remaining characteristics, i.e. the diameter and area of the fibres, no interaction was found.

Węglarz [31] found no significant effect of sex on the content of red and white fibres, but the meat of heifers had slightly higher content (P≤0.05) of intermediate fibres. All types of muscle fibre had a significantly greater (P≤0.01) diameter in the muscles of bulls.

Młynek [16], in a study conducted on the *semimembranosus* muscle of heifers of different ages, in the group aged 200-300 days (corresponding to heavy calves) obtained 23.6% red fibres, at, 23.5% intermediate fibres, and 52.8% white fibres. The corresponding values in the present study were 11.61%, 32.82% and 50.20%. For the group of young beef cattle the author cited obtained 19.7% red fibres, 18.8% intermediate, and 61.4% white, while the corresponding values in the present study were 19.7%, 18.5%, 56.19%.

Morita et al. [20], in a study on Mishima heifers (a native Japanese breed) slaughtered at the age of 12 months, found the following percentages and diameters for different types of fibres in the *m. longissimus*: red – 37.3% (55.8 µm), intermediate – 16.7% (49 µm), and white – 46.1% (65.2 µm). These results indicate a different range of values, particularly for the percentage of red fibres and their diameter, in comparison with the present study.

Vestergaard et al. [30], in a study conducted on the LD muscle of Holstein-Friesian bulls with a slaughter weight of 300-582 kg, reported 27.6% red fibres, 23.4% intermediate, and 47.1% white, while their surface area was 1,691 µm^2 for red fibres, 2,033 µm^2 for intermediate, and 2,770 µm^2 for white.

The analysis taking into account the category and breed of animals (Polish Black-and--White Holstein-Friesian and commercial crosses) showed no effect of the genetic group (breed) on muscle fibre parameters (Tab. 2). Category, however, had a significant effect at P≤0.01 on all characteristics, except for the percentage of red and intermediate fibres, where the effect was significant at P≤0.05. An interaction (P≤0.01) of category x breed on the percentage of white fibres was confirmed as well. No interaction was found for the remaining characteristics.

A study by Jarmuż [10] conducted on samples of the *longissimus lumborum* muscle taken from 40 young bulls of the Lowland Black-and-White breed at the ages of 2 weeks and 6, 12 and 18 months found that as the age and body weight of the animals increased, the number of red fibres with high oxidative activity decreased, while that of intermediate and white fibres with high glycolytic activity increased. An intensive increase in the diameter and percentage of intermediate and white fibres persisted until the bulls reached the age of 12 months. After this time the changes were less intensive, which may have been due to stabilization of the rate of growth after 12 months.

Table 2

Characteristics of muscle fibres of evaluated cattle with regard to category and breed

Specification		Young slaughter cattle (n=33)		Heavy calves (n=27)		Milk-fed calves (n=63)		Influence		
		PHF HO (n=13)	commercial crossbreeds (n=20)	PHF HO (n=16)	commercial crossbreeds (n=11)	PHF HO (n=42)	commercial crossbreeds (n=21)	(K) category	(R) breed	(KxR) interaction
Number of red fibres	$\bar{x}$	30.10	29.63	32.71	35.60	197.87	186.67	**	ns	ns
	S	6.93	9.14	17.82	14.20	27.29	33.24			
Number of intermediate fibres	$\bar{x}$	21.30	28.37	22.86	28.60	174.86	176.00	**	ns	ns
	S	6.12	8.53	9.48	13.10	46.41	51.32			
Number of white fibres	$\bar{x}$	55.10	49.10	37.43	51.00	226.50	225.00	**	ns	ns
	S	10.89	6.16	20.63	21.83	48.16	55.40			
Percentage of red fibres	$\bar{x}$	17.11	19.27	21.74	14.08	24.21	22.16	*	ns	ns
	S	4.28	5.64	11.21	7.43	2.76	2.93			
Percentage of intermediate fibres	$\bar{x}$	14.85	19.27	24.87	23.85	20.91	19.63	*	ns	ns
	S	3.87	5.83	13.14	8.25	0.99	1.29			
Percentage of white fibres	$\bar{x}$	59.76	54.71	46.34	54.84	35.99	34.75	**	ns	**
	S	6.43	5.04	4.72	5.46	5.94	6.89			
Diameter of red fibres (μm)	$\bar{x}$	53.99	49.62	41.69	39.22	17.54	18.84	**	ns	ns
	S	8.51	10.65	4.02	6.02	0.93	1.07			
Diameter of intermediate fibres (μm)	$\bar{x}$	66.68	60.81	51.57	49.23	22.11	21.73	**	ns	ns
	S	8.33	5.38	4.00	6.39	0.97	1.19			
Diameter of white fibres (μm)	$\bar{x}$	80.33	81.34	64.53	58.95	30.13	31.27	**	ns	ns
	S	6.86	10.08	11.63	3.91	1.80	2.13			
Area of red fibres (μm^2)	$\bar{x}$	3705.42	3364.53	2236.29	1896.07	429.20	417.41	**	ns	ns
	S	1120.02	425.10	797.41	447.51	33.72	28.74			
Area of intermediate fibres (μm^2)	$\bar{x}$	5240.30	4932.34	3248.21	2848.71	801.62	784.09	**	ns	ns
	S	1813.23	798.50	401.96	592.22	130.40	103.86			
Area of white fibres (μm^2)	$\bar{x}$	8157.45	7502.73	4496.02	4399.06	1299.89	1400.91	**	ns	ns
	S	1781.55	823.49	936.71	703.55	293.31	338.92			

*Significant at $P \leq 0.05$
**Significant at $P \leq 0.01$
ns – not significant

Młynek [17], in a study conducted on the *longissimus lumborum* muscle, found that irrespective of the beef breed used for crossbreeding, the percentage of fibres with oxidative metabolism (STO) was lower than in Black-and-White cattle. Among the crossbred animals this percentage was similar in young bulls and heifers, on average 21.3%, but in the Black-and-White breed it was higher: 24.0% in young bulls and 22.0% in heifers. The percentage of fibres with glycolytic metabolism (FTG) in the crosses was on average 57.7% in young bulls and 58.0% in heifers. Commercial crossing caused a 1.5% increase in the percentage of these fibres in young bulls and a 2.0% increase in heifers. The highest percentage of FTG fibres was observed in the muscles of young bulls from Black-and--White x Limousin and Black-and-White x Piedmontese crosses, on average 58.3%. In the case of heifers the highest percentage of these fibres was noted in the muscles of crosses obtained from Charolais and Piedmontese bulls, on average 58.8%.

Costa et al. [4], evaluating one of the most important local breeds in Portugal, Mertolenga, reported 31.39% red fibres, 35.01% intermediate fibres and 33.61% white fibres in the LD muscle, with an area of 3,069, 4,545 and 6,258 μm^2, respectively.

A study by Młynek et al. [18] assessed the effect of age at slaughter (465, 568 and 711 days) on the microstructure of the *longissimus lumborum* (LL) muscle in young bulls of Black-and-White x Limousin and Black-and-White x Charolais crosses. In the muscles of the bulls in the youngest group the percentage of red fibres was 23.5%, and the combined percentage of intermediate and white fibres was 76.5%. In the oldest animals these proportions were 30.9% and 69.1%, respectively. Greater differences in younger animals (slaughtered at 180-480 days) were also observed by Bellmann et al. [1], while Kłosowski and Kłosowska [12] reported results similar to those obtained in the present study.

Similar results for the surface area and percentages of muscle fibres in young bulls with carcass weight ranging from 157 to 322 kg and aged from 221 to 485 days were obtained by Vestergaard et al. [29].

Carcass value and quality depend, among many different factors, on the biological structure of muscles, i.e. the structure of muscle fibres. The data presented in Table 3 show that the body weight of animals before slaughter and their carcass weight before and after chilling were highly significantly (P≤0.001) positively correlated with the diameter (r=0.7068-0.7648) and area (r=0.5794-0.7804) of all types of fibres (red, intermediate and white), and negatively correlated with the percentage share of intermediate fibres (r=−0.5831 do –0.6023).

Renand et al. [25] showed positive correlations r=0.37 (at P≤0.05) between the surface area of muscle fibres and the age of young bulls slaughtered between the 64th and 82nd week in the feedlot. Similar correlations (r=0.34) between microstructure characteristics and the age of animals (slaughtered at 10 and 16 months) were obtained by Seideman and Crouse [26], Jurie et al. [11] and Bellmann et al. [1].

The data presented in Table 4 show highly significant (P≤0.001) positive correlations between fat content and the diameter (r=0.5049-0.5763) and area (r=0.4498-0.5654) of all types of fibres, and a somewhat lower correlation (r=0.3623 at P≤0.01) indicating

Table 3

Pearson correlation coefficients between indicators of carcass value and characteristics of muscle fibres

Specification	Traits of muscle fibre											
	number of red fibres	percentage of red fibres	diameter of red fibres (μm)	area of red fibres (μm²)	number of intermediate fibres	percentage of intermediate fibres	diameter of intermediate fibres (μm)	area of intermediate fibres (μm²)	number of white fibres	percentage of white fibres	diameter of white fibres (μm)	area of white fibres (μm²)
Slaughter weight (kg)	0.1432	0.3452	0.7596***	0.7251***	–0.1067	–0.6023*	0.7334***	0.5794*	0.3051	0.3116	0.7068**	0.7779***
Hot carcass weight (kg)	0.1601	0.3641	0.7621***	0.7384***	–0.0897	–0.5831*	0.7364***	0.5861*	0.2804	0.2690	0.7165***	0.7794***
Cold carcass weight (kg)	0.1629	0.3653	0.7648***	0.7398***	–0.0871	–0.5843*	0.7380***	0.5888*	0.2834	0.2683	0.7148***	0.7804***
Hot dressing percentage	0.1586	0.1780	0.0755	0.2081	0.1237	0.2167	0.0250	0.0644	–0.2578	–0.4047	0.1847	0.0508
Cold dressing percentage	0.2161	0.2305	0.1649	0.2998	0.1549	0.1281	0.1259	0.1522	–0.1783	–0.3688	0.2548	0.1474

*Significant at $P \leq 0.05$
**Significant at $P \leq 0.01$
***Significant at $P \leq 0.001$

Table 4
Pearson correlation coefficients between chemical composition of meat and characteristics of muscle fibres

Specification	Traits of muscle fibre											
	number of red fibres	percentage of red fibres	diameter of red fibres (μm)	area of red fibres (μm^2)	number of intermediate fibres	percentage of intermediate fibres	diameter of intermediate fibres (μm)	area of intermediate fibres (μm^2)	number of white fibres	percentage of white fibres	diameter of white fibres (μm)	area of white fibres (μm^2)
Protein (%)	0.0003	–0.1699	–0.1451	–0.0338	0.0528	0.2767*	–0.0516	0.0401	0.0334	–0.0730	0.0162	0.0762
Fat (%)	0.3623**	0.1803	0.5339***	0.5654***	–0.3321*	0.1238	0.5763***	0.5349***	–0.3608**	0.1878	0.5049***	0.4498***
Ash (%)	–0.1715	–0.2095	–0.0284	0.0162	–0.1784	–0.0153	0.0567	0.0841	–0.1781	0.1261	0.0927	0.1115
Water (%)	–0.1581	0.1875	0.4901**	0.4720**	–0.1466	–0.2485	0.4149**	0.3829**	–0.1265	0.2564	0.3895**	0.4033**
Collagen (%)	0.1384	0.0239	–0.0842	0.0367	0.1689	0.0189	–0.0359	0.0392	0.1509	0.0305	–0.0152	0.0625
Percentage of collagen in protein	0.1772	0.0617	–0.0884	0.0141	0.1915	–0.0256	–0.0623	-0.0054	0.1811	0.0207	–0.0516	0.0135

*Significant at P≤0.05
**Significant at P≤0.01
***Significant at P≤0.001

higher content of fat in red fibres, as reported by Skrabka-Błotnicka [27] and Litwińczuk et al. [14]. Moreover, a significant correlation ($P \leq 0.01$) was found between moisture content and the diameter ($r=0.3895-0.4901$) and area ($r=0.3829-0.4720$) of all types of fibres.

To sum up, a significant ($P \leq 0.01$) effect of sex was found on the number of all types of fibres, as well as an interaction of category x breed ($P \leq 0.01$) on the percentage of white fibres. Highly significant ($P \leq 0.001$) positive correlations were found between certain indicators of carcass value, i.e. body weight before slaughter and hot and cold carcass weight, and the diameter and area of all types of fibres, as well as negative correlations with the percentage of intermediate fibres. Moreover, highly significant ($P \leq 0.001$) positive correlations were found between fat content and the diameter and area of all types of fibres, and somewhat lower correlations indicating higher fat content in red fibres.

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