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STRUCTURE AND HARDNESS OF SELECTED MUSCLES IN YOUNG BULLS

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Key words: bulls, muscles, structure, hardness

The diversification of structure and hardness of 18 muscles was investigated in bulls of the black-and-white breed in the age between 20–22 months. The histological specimens were prepared by means of the paraffin method. The structure (fibre surface, perimysium thickness and the contents of intramuscular fat) was evaluated by means of a computer analysis of a MultiScan picture. The hardness was tested by an Instron 1140 apparatus at 80% distortion. The sequencing of the obtained results according to multiplicity was completed by means of an ascending muscle series as well as with an analysis of the divergence significance. However, contrary to the formerly applied methods, the muscles were grouped in series assuming their statistical homogeneity or significant divergences in relation to the muscle having the closest value to the mean of the given indicator as the basic criteria of grouping.

The majority of muscles indicated a tendency towards increased hardness, an increase in the fibre surface and perimysium thickness and a decrease in fat content.

INTRODUCTION

The texture constitutes the basic criterion for the evaluation of the meat quality. It refers, above all, to its mechanical properties. These, in turn, are closely related to the characteristics of the structural components of meat [Lepetit & Culioli, 1994; Tornberg *et al.*, 1994]. The texture, according to Tyszkiewicz [1995], "... is the external sign of the internal structure of the product, and all the changes in the structure are immediately manifested in the alterations of the texture characteristics."

Bouton *et al.* [1978] claimed that the mechanical properties of meat are a function of the mechanical properties of myofibrils and connective tissue, therefore the changes in the hardness result from the variable participation of these components in its formation, as well as the alterations within the interactions among these components.

A direct correlation between the structure and the crispness of the muscle was also affirmed by Kłosowska [1975], who considered the fibres thickness and their bundles size to constitute the basic effective factors. Hiner *et al.* [1953] showed that the increase in the fibre diameter is accompanied by an increase in the shear force, which was confirmed by high correlation coefficients.

In the opinion of many authors, the connective tissue and the collagen contained in it constitute one of the basic factors forming the meat texture [Sacks *et al.*, 1988; Palka, 1995; Połczyńska & Górska, 1997]. A significant positive correlation between the collagen content in the perimysium and endomysium and the shear force, was achieved by Johnson *et al.* [1988], who stated simultaneously, that for the individual muscles there existed considerable variations in the values of the correlation coefficients. Also, Shorthose and Harris [1990], having investigated 12 bovine muscles, stated that the increase in hardness probably resulted from the endurance of the connective tissue structure or its increasing quantity.

The high correlation between the perimysium thickness and the hardness of the 18 muscles of bulls was also shown by Oryl and Lachowicz [2000], who obtained significant correlation coefficients between these parameters.

The fat content constitutes another factor of the structure, the relation of which with the texture is frequently emphasized in literature. The relations between the fat content and the hardness are described by Kłosowska [1975], Hodgson *et al.* [1991], Albrecht *et al.* [1996]. Dransfield [1977] showed the negative correlation between the quality of the intramuscular fat and the compressive force in various bovine muscles.

As becomes evident from the literature, however, not each case confirmed the correlation between the structure and texture. Lewis *et al.* [1977], for instance, investigating the LD, PM, and QF in the cattle of various breeds, obtained a positive correlation between the fibre diameter and the shear force for the PM solely. Also, Whipple *et al.* [1990] did not note any significant correlations between the type and surface of the fibres and the shear force in the bovine LL. Likewise, a number of other authors, although not denying the influence of fat content on texture, stated that the general confirmation of the significance of this relationship is difficult. This opinion is shared among others by Tatum *et al.* [1990], and McKeith *et al.* [1985]. Johnson *et al.* [1988], for instance, while indicating considerable differences in the fat content in the individual muscles, obtained a very low correlation between this parameter of the structure and the shear force. It is thus evident that there exist divergences of opinions, and, what is more, the obtained results are contradictory. Although the scientific output on

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the texture and structure of the bovine meat is plentiful, very often such simultaneous research was conducted only on a few muscles, mainly the LD, PM, and ST from animals of different ages and breeds.

This work aims at the investigation of the hardness and the structure of 18 selected muscles of 20–22-month-old black-and-white bulls and the detection of a correlation between them.

TABLE 1. Significance of differences between the hardness in the examined muscles of the young bulls of the black-n-white breed.

No.	MUSCLES	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
1.	*m.triceps brachii* **TB**		x		x	x	x									x		x	x
2.	*m.infraspinatus* **INF**	x		x	x	x		x	x	x	x	x	x	x	x		x	x	x
3.	*m.longissimus dorsi* **LD 1-6**		x		x	x	x									x			
4.	*m.longissimus dorsi* **LD 7-12**	x	x	x			x	x	x	x		x	x		x	x	x		
5.	*m.longissimus dorsi* **LD 13-6**	x	x	x			x	x	x	x	x	x	x	x	x	x	x		
6.	*m.psoas major* **PM**	x		x	x	x		x	x	x	x	x	x	x	x	x	x	x	x
7.	*m.semimembranosus* **SM**		x		x	x	x				x	x	x	x				x	x
8.	*m.gracilis* **GRA**		x		x	x	x				x			x		x		x	x
9.	*m.adductor* **ADD**		x		x	x	x									x		x	x
10.	*m.pectineus* **PEC**		x			x	x	x	x						x	x			
11.	*m.semitendinosus* **ST**		x		x	x	x	x							x	x			
12.	*m.biceps femoris* **BF**		x		x	x	x	x							x	x			
13.	*m.gluteus medius* **GM**		x			x	x	x	x						x	x			
14.	*m.gluteus profundus* **GP**		x		x	x	x				x	x	x	x			x	x	x
15.	*m.gluteus accessorius* **ACC**	x		x	x	x	x		x	x	x	x	x	x			x	x	x
16.	*m.vastus medialis* **VM**		x		x	x	x								x	x			
17.	*m.rectus femoris* **RF**	x	x				x	x	x	x					x	x			
18.	*m.vastus lateralis* **VL**	x	x				x	x	x	x					x	x			

x – denotes the statistically significant differences between the individual muscles considering the investigated feature at $\alpha=0.05$
Empty space indicates the assignment of muscles to the same homogeneous groups.

TABLE 2. Significance of differences between the fibre surface in the examined muscles of the young bulls of the black-n-white breed.

No.	MUSCLES	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
1.	*m.triceps brachii* **TB**				x	x		x	x		x		x	x	x	x		x	x
2.	*m.infraspinatus* **INF**				x	x		x	x		x		x	x	x	x		x	x
3.	*m.longissimus dorsi* **LD 1-6**				x	x		x	x		x		x	x	x	x		x	x
4.	*m.longissimus dorsi* **LD 7-12**	x	x	x			x	x	x	x		x			x	x	x		
5.	*m.longissimus dorsi* **LD 13-6**	x	x	x			x	x	x	x	x	x			x	x	x		
6.	*m.psoas major* **PM**				x	x		x	x		x		x	x	x	x	x	x	x
7.	*m.semimembranosus* **SM**	x	x	x	x	x	x						x	x				x	x
8.	*m.gracilis* **GRA**	x	x	x	x	x	x						x	x				x	x
9.	*m.adductor* **ADD**				x	x					x		x	x		x		x	x
10.	*m.pectineus* **PEC**	x	x	x		x	x			x		x					x	x	
11.	*m.semitendinosus* **ST**				x	x					x		x	x	x	x		x	x
12.	*m.biceps femoris* **BF**	x	x	x			x	x	x	x		x					x	x	
13.	*m.gluteus medius* **GM**	x	x	x			x	x	x	x		x					x	x	
14.	*m.gluteus profundus* **GP**	x	x	x	x	x	x					x						x	x
15.	*m.gluteus accessorius* **ACC**	x	z	x	x	x	x			x		x						x	
16.	*m.vastus medialis* **VM**				x	x	x				x		x	x				x	x
17.	*m.rectus femoris* **RF**	x	x	x			x	x	x	x	x	x	x	x	x	x	x		x
18.	*m.vastus lateralis* **VL**	x	x	x			x	x	x	x		x			x		x	x	

x – denotes the statistically significant differences between the individual muscles considering the investigated feature at $\alpha=0.05$
Empty space indicates the assignment of muscles to the same homogeneous groups.

MATERIAL AND METHODS

The right half-carcasses of young bulls of the black-and--white breed within the 20–22 month age category constituted the material for the study. The slaughter and the carcass processing were conducted in accordance with the regulations applied in the meat industry. After 48 hours' cooling at 4°C, the meat in the form of a quarter-carcass was delivered to the MAS-AR Experimental Production Enterprise.

The study was performed on the following muscle

TABLE 3. Significance of differences between the perimysium thickness in the examined muscles of the young bulls of the black-n-white breed.

No.	MUSCLES	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
1.	*m.triceps brachii* **TB**					x	x					x		x	x				x
2.	*m.infraspinatus* **INF**			x	x	x		x		x	x	x	x	x	x				x
3.	*m.longissimus dorsi* **LD 1-6**		x			x	x		x			x			x				x
4.	*m.longissimus dorsi* **LD 7-12**		x			x	x		x			x							x
5.	*m.longissimus dorsi* **LD 13-6**	x	x	x	x		x	x	x	x	x		x	x		x	x	x	
6.	*m.psoas major* **PM**	x		x	x	x		x		x	x	x	x	x	x	x	x	x	x
7.	*m.semimembranosus* **SM**		x			x	x		x			x			x				x
8.	*m.gracilis* **GRA**			x	x	x		x			x	x	x	x	x				x
9.	*m.adductor* **ADD**		x			x	x					x		x	x				x
10.	*m.pectineus* **PEC**		x			x	x		x			x			x				x
11.	*m.semitendinosus* **ST**	x	x	x	x		x	x	x	x	x		x	x	x	x	x	x	x
12.	*m.biceps femoris* **BF**		x			x	x		x			x			x				x
13.	*m.gluteus medius* **GM**	x	x			x	x		x	x		x				x	x	x	
14.	*m.gluteus profundus* **GP**	x	x	x			x	x	x	x	x	x	x			x	x	x	
15.	*m.gluteus accessorius* **ACC**					x	x					x		x	x				x
16.	*m.vastus medialis* **VM**					x	x					x		x	x				x
17.	*m.rectus femoris* **RF**					x	x					x		x	x				x
18.	*m.vastus lateralis* **VL**	x	x	x	x		x	x	x	x	x	x	x			x	x	x	

x – denotes the statistically significant differences between the individual muscles considering the investigated feature at $\alpha=0.05$
Empty space indicates the assignment of muscles to the same homogeneous groups.

TABLE 4. Significance of differences between the content of intramuscular fat in the examined muscles of the young bulls of the black-n-white breed.

No.	MUSCLES	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
1.	*m.triceps brachii* **TB**		x	x		x	x	x				x		x	x			x	
2.	*m.infraspinatus* **INF**	x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	x
3.	*m.longissimus dorsi* **LD 1-6**	x	x		x	x		x	x	x	x	x	x	x	x	x	x	x	x
4.	*m.longissimus dorsi* **LD 7-12**		x	x		x	x	x			x	x			x			x	x
5.	*m.longissimus dorsi* **LD 13-6**	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x		x
6.	*m.psoas major* **PM**	x	x		x	x		x	x	x	x	x	x	x	x	x	x	x	x
7.	*m.semimembranosus* **SM**	x	x	x	x	x	x		x			x	x	x	x		x	x	
8.	*m.gracilis* **GRA**		x	x		x	x	x			x	x			x			x	x
9.	*m.adductor* **ADD**		x	x		x	x					x		x	x			x	
10.	*m.pectineus* **PEC**		x	x	x	x	x		x			x	x	x	x			x	
11.	*m.semitendinosus* **ST**	x	x	x	x	x	x	x	x	x	x		x		x	x	x	x	x
12.	*m.biceps femoris* **BF**		x	x		x	x	x			x	x			x		x	x	
13.	*m.gluteus medius* **GM**	x	x	x		x	x	x		x	x				x	x	x	x	x
14.	*m.gluteus profundus* **GP**	x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	x
15.	*m.gluteus accessorius* **ACC**		x	x		x	x					x		x	x			x	
16.	*m.vastus medialis* **VM**		x	x		x	x	x				x	x	x	x			x	
17.	*m.rectus femoris* **RF**	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x		x
18.	*m.vastus lateralis* **VL**		x	x	x	x	x		x			x		x	x			x	

x – denotes the statistically significant differences between the individual muscles considering the investigated feature at $\alpha=0.05$
Empty space indicates the assignment of muscles to the same homogeneous groups.

specimens: *m. triceps brachii* – TB; *m. infraspinatus* – INF; *m. longissimus dorsi*, which was divided into 3 elements: (1) cut between the last cervical / the first thoracic vertebrae and the sixth / seventh thoracic vertebrae – designated LD 1–6; (2) cut between the sixth / seventh thoracic vertebrae and the last / second-last thoracic vertebrae – designated LD 7–12; (3) cut between the twelfth / thirteenth thoracic vertebrae and the last lumbar / the first sacral vertebrae – designated LD 13–6; *m. psoas major* – PM; *m. semimembranosus* – SM; *m. gracilis* – GRA; *m. adductor* – ADD; *m. pectineus* – PEC; *m. semitendionosus* – ST; *m. biceps femoris* – BF; *m. gluteus medius* – GM; *m. gluteus profundus* – GP; *m. gluteus accessorius* – ACC; *m. vastus medialis* – VM; *m. rectus femoris* – RF; *m. vastus lateralis* – VL.

The samples for the study of the structure were taken from the central part of each muscle. The histological specimens were prepared by means of the paraffin method and tinted with the hematoxiline and eosine [Burck, 1975]. The structure was evaluated by means of a computer analysis of a MultiScan picture. The histometrical measurements were conducted on the fibre surface, perimysium thickness and the surface of the fat concretions in the entire preparation (all the mean surfaces of concretions were counted, the result is given in per mille units).

The samples for the hardness study, identical in size, were wrapped in thermal foil and heated in a microwave oven up to 75°C in the central part of the sample. After cooling off and a 12-hour storage under refrigeration they were sliced at 20±1 mm. The hardness was tested according to Bourne, [1982] by means of an Instron 1140 apparatus with the application of a 0.62 cm plunger which was forced twice to the depth of 80% of the sample. The fibres of the samples were parallel to the force action.

The results were statistically elaborated according to the monofactorial procedure of the ANOVA variance analysis [Podgórski, 1995]. The diversification of the muscles within the carcass was attempted by means of a statistical test of difference significance in which the muscles were compared by the “one-to-one” method for each indicator respectively (Tables 1–4).

RESULTS AND DISCUSSION

Four indicators were estimated for 18 muscles. Successively, the muscles were sequenced according to ascending values, the value of the arithmetic mean was calculated, and the muscle of the closest characteristics to the mean was determined for each indicator. Afterwards, the muscles were grouped within each series according to the basic criterion, *i.e.* their statistical homogeneity or significant differences in relation to the muscle which was the closest to the mean value of the respective indicator (Figures 1 and 2). In this way, the series of muscles of the lowest and highest values were indicated – all of them differed significantly from the muscle of the closest value to the mean. Moreover, each muscle in the series of the values considerably higher than the mean differed significantly from the others with values lower than the respective mean. Conversely, each muscle in the series of values statistically lower than the mean differed considerably from the other muscles of the values higher than the mean.

As becomes evident from the study, the mean hardness of all the investigated muscles equalled 36.1 N. The softest were: PM<INF<ACC, whereas the hardest were: LD13-6>LD7-12>VL>RF. The hardness of the remaining muscles, *i.e.* GP<SM<GRA<TB<ADD<LD1-6<VM<<ST<BF<PEC<GM did not differ statistically from the mean, which enabled their grouping within the series of the medium hardness (Figure 1).

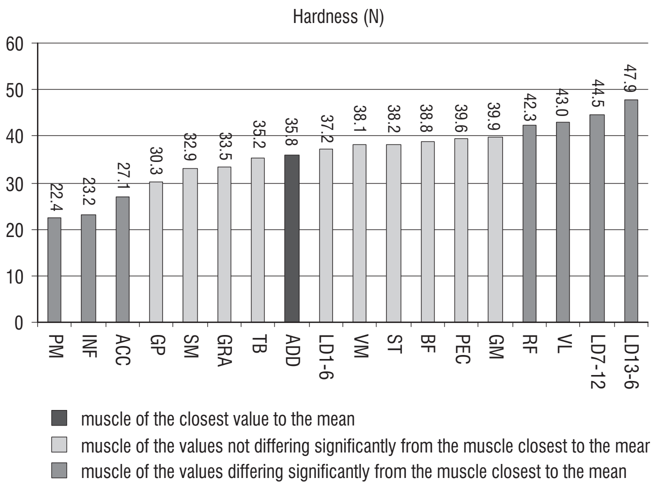

FIGURE 1. Hardness. Muscle series according to ascending values.

While sequencing the muscles according to the fibre surface and the perimysium thickness, a similar sequence was noted with respect to their hardness (Figure 2). The smallest surface of fibres was noted in: PM<INF<TB<LD1-6<ST, whereas the largest was noted in: RF>LD13-6>LD 7–12>VL. The fibre surface of ADD<VM<SM<GRA<<GP<ACC<PEC<BF<GM did not differ significantly from the mean which equalled 927.4 μm^2. The thinnest perimysia were noted in: PM<INF<GRA, whereas the thickest were noted in: ST>LD13-6>VL. The mean thickness of perimysium equalled 21.4 μm and the muscles: VM<ACC<TB<RF<ADD<LD1–6<SM<BF<PEC<<LD7–12<GM<GP constituted a group of statistical values homogeneous with the mean (Figure 2).

The lowest content of intramuscular fat was observed in: RF<LD13-6<SM<PEC<VL, while the higest was in: PM>LD1-6>INF>GP>ST. The following: ACC<ADD<<TB<VM<BF<LD7-12<GRA<GM did not significantly differ from the mean content of fat which equalled 10.84. (Figure 2).

In comparing the series of muscles in an ascending sequence according to the hardness, fibre surface and the perimysium thickness, there are noticeable differences in certain muscle locations, despite obvious similarities. For instance, the ST, which had a small surface and the thickest perimysium, was characterized by the mean hardness. In addition, the RF, with the largest surface of fibre and the medium thickness of perimysium, was grouped with the hard muscles. After considering certain exclusive cases, it can be assumed that the majority of muscles indicate a tendency towards hardness increase along with an increase in the fibre surface and the perimysium thickness. This is supported by highly significant positive correlations: for the hardness and the fibre surface (r = 0.69) and for the hardness and the perimysium thickness (r = 0.61) (Table 5).

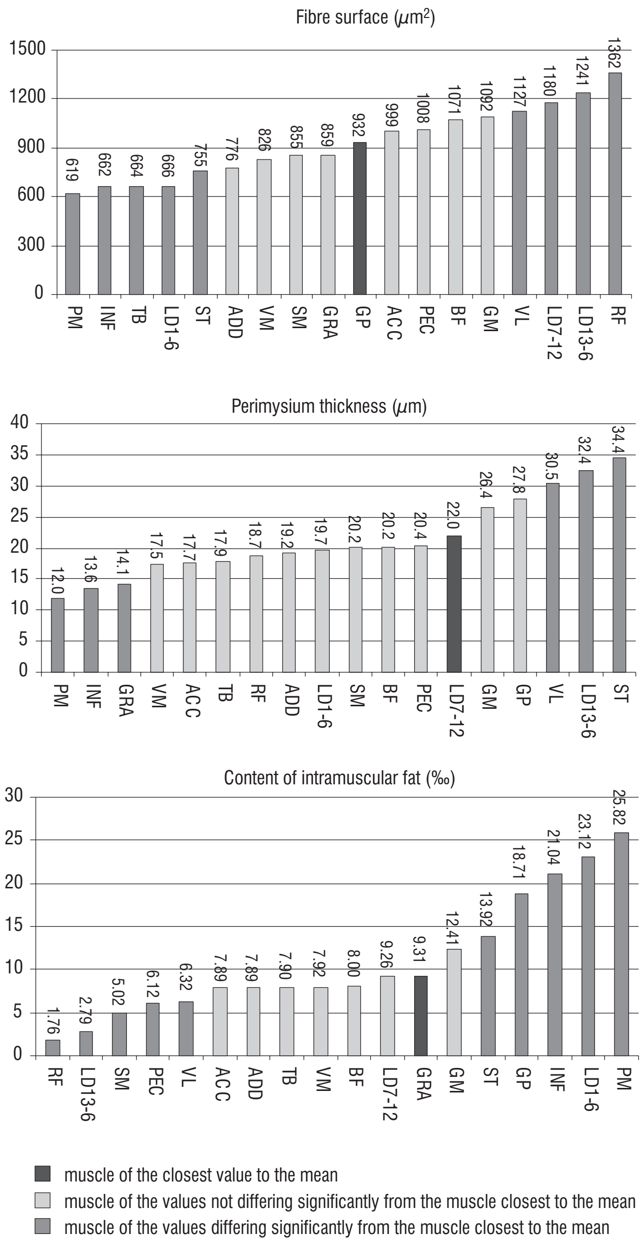

FIGURE 2. Structure. Muscle series according to ascending values.

TABLE 5. Coefficients of simple correlation between hardness and structure of bulls muscles.

Feature	Fibre surface (μm²)	Perimysium thickness (μm)	Content of intramuscular fat (‰)
Hardness (N)	0.689**	0.608**	-0.642**

* – significant correlation at the level of $\alpha \leq 0.05$; ** – significant at the level of $\alpha \leq 0.01$

The relation between the fibre surface and the hardness was described by Herring *et al.* [1966], Cooper *et al.* [1968] and Berry *et al.* [1974]. It is contrary to the results obtained by Melton *et al.* [1974], who did not achieve a correlation between the diameter of the fibres of the investigated muscles and the shear force.

The significant effect of the perimysium tissue on meat hardness as observed in this study corresponds with the results of Kołczak *et al.* [1992]. In additon, the indicated relations between the hardness and the perimysium thickness and the collagen contained in it are confirmed by the results obtained by Hill [1967], Pełczyńska [1979], Sadowska [1987], and Liu *et al.* [1996], who investigated the relationship between the shear force and the perimysium diameter in the chicken muscles. On the contrary, Prost [1975] did not indicate any significant relations between the tenderness and the perimysium tissue content in the muscles of sheep. Light *et al.* [1985], conversely, confirmed the existence of the relation of the parameters in question, but did not obtain any significant correlations between the hardness and the collagen content in the perimysium and endomysium, stating that it is the nature, and not the quantity, of the collagen contained in the muscles that influences the hardness positively.

The analysis of the muscle series according to the ascending hardness and the contents of the intramuscular fat indicates that, with a few exceptions (*e.g.* ACC, SM, and ST), the muscles characterized by the lowest hardness contained the most fat and, conversely, the hardest muscles contained the least. This indicates a dependence between the hardness and the fat content in the majority of the examined muscles to be confirmed by the correlation of a highly significant coefficient (r=-0.64) (Table 5).

The relationship between the increase in the fat content and the decrease in the meat hardness indicated above agrees with the results obtained by: Wismer-Pedersen *et al.* [1973], Berry *et al.* [1974], Dransfield [1977], Sink *et al.* [1983], Wheeler *et al.* [1996] and Essen-Gustavson *et al.* [1992] who evaluated the fat content in the muscles of pigs.

However, Mc Keith *et al.* [1985] while examining 13 beef muscles stated that the most tender 3 of them (INF>LD dorsal part>LD lumbar part>PM) contained the most fat, but they did not obtain any significant correlations between both factors. Hill [1967], Melton *et al.* [1974], and Eikelenboom *et al.* [1996] did not note any significant correlations between these factors either.

The variability of the results of the above-mentioned authors, as opposed to the present study, could result from the application of different methods. For instance, Mc Keith *et al.* [1985] estimated the content of fat by means of a chemical method, whereas Essen-Gustavson *et al.* [1992] applied the histochemical method in the estimation of fat content in the muscles of pigs and obtained, similar to the results in this study, a negative significant correlation between the shear force and the content of the intramuscular fat.

In considering the successive cause of the discrepancy, it should be stated clearly that a beef carcass consists of about 300 muscles [Kołczak, 2000]. The most frequently examined muscles were: LD, PM, SM, ST and the complex QF. The material for this study were the 18 main muscles encountered in the culinary meat which should constitute a more representative attempt at the evaluation of the structure and hardness of beef meat. Moreover, the above-mentioned authors examined muscles of animals of various ages and breeds, at a various *post mortem* times, and heated them at the various parameters of the heating process. Due to Wajda, [2001] the quality of meat is greatly dependent upon the carcass treatment after the slaughter.

CONCLUSIONS

The majority of the examined muscles indicated a tendency to increase hardness along with an increase in the fibre surface and the perimysium thickness. A relation between the hardness increase and a decrease in the fat content was observed in the majority of the examined muscles. Even the highly significant correlations obtained between the individual indicators do not match the ideal accord in the successive sequence of the individual muscles in relation to these indicators. It becomes obvious that the texture can also be formed by other factors than those discussed in this study. This confirms the opinion of Marchello *et al.* [1970] that "... the quality of meat is affected by a number of various features of the muscular fibres and the perimysium in many ways. Therefore, it is very complicated to determine the relative effect of each feature".

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STRUKTURA I TWARDOŚĆ WYBRANYCH MIĘŚNI BUHAJKÓW

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Badano zróżnicowanie struktury i twardości 18 mięśni buhajków rasy czarno-białej w kategorii wiekowej 20–22 miesiące. Preparaty histologiczne sporządzano metodą parafinową. Oceny struktury (powierzchni włókna, grubości perimysium i zawartości tłuszczu śródmięśniowego) dokonano przy pomocy komputerowej analizy obrazu MultiScan. Twardość badano na aparacie Instron 1140 przy odkształceniu 80%.

Z uwagi na wielość otrzymanych wyników dla ich uporządkowania posłużono się rosnącym szeregowaniem mięśni, a także analizą istotności różnic (tab. 1-4). Jednak, w odróżnieniu od dotychczas stosowanych metod, w otrzymanych sekwencjach grupowano mięśnie przyjmując jako podstawowe kryteria grupowania ich statystyczną jednorodność, lub istotne różnice w stosunku do mięśnia najbliższego średniej danego wskaźnika (rys. 1 i 2). Większość mięśni przejawiała tendencję wzrostu twardości ze wzrostem powierzchni włókna i grubości perimysium oraz spadkiem zawartości tłuszczu (tab. 5).