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Slaughter and carcass characteristics, chemical composition and physical properties of *longissimus lumborum* muscle of heifers as related to marbling class

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Abstract. The experiment involved 68 heifers obtained from Polish Black-and-White Lowland dams (with no more than 50% Holstein Friesian blood) sired by Limousin bulls. The study was arranged to determine the slaughter and carcass traits, chemical composition and physical properties of *musculus longissimus lumborum* (MLL) as related to the level of marbling, and to examine the correlation between some of the traits mentioned above and the culinary characteristics of meat. The results of the study show that marbling significantly affected the dressing percentage and carcass traits such as subcutaneous fat content, fat of valuable cuts and MLL area. Most of the physico-chemical properties of tested muscles, with the exception of total protein and total collagen content, were related to the level of marbling. Based on the results obtained, it appears that age at slaughter and carcass weight turned out to be poor predictors of colour lightness within all marbling classes. The same conclusion applies to the amount of total collagen in large and strong marbled muscles for shear force value. In turn, soluble collagen content for shear force could be a quite good forecaster of meat tenderness but not in highly marbled muscles. Of all tested slaughter and carcass traits, only MLL fat content seems to be appropriate to assess both: the tenderness and lightness of meat.

1 Introduction

The need for a continuous improvement of slaughter and carcass traits, as well as the quality characteristics of meat demanded by consumers, are the principal objectives of beef production investigations. Beside the meat acidity and water holding capacity, the beef colour and tenderness make up the basic quality characteristics essential for both consumers and the processing industry (Wegner et al., 2000; Vestergaard et al., 2000).

Beef appearance is an essential factor at the moment of purchase and its colour and marbling are properties which consumers take into account first (Platter et al., 2005). In turn meat tenderness it is the trait determining beef quality (Warner et al., 2010). Total amount and chemical composition of collagen in the muscle as well as quantity of connective tissue is some of the factors directly affecting beef tenderness, while marbling is a factor indirectly affecting meat toughness (Wulf et al., 1996; Nishimura et al., 1999; Ueda et al., 2007). With respect to meat colour, some authors observed a significant relationship between marbling and meat lightness (Fiems et al., 2000), whereas others (Kim and Lee, 2003) reported that marbling did not affect the beef colour.

Marbling appears in raw meat as white spots or strips of fat, which describes the distribution of intramuscular fat (IMF). In turn, IMF is the chemically estimated fat content of meat; the terms marbling and IMF are commonly used alternatively. This characteristic presents considerable variability both between (Chambaz et al., 2001; Šurbt et al., 2006) and within breed populations (Węglarz, 2010a) and among muscle types depending on their anatomy and the physiological role played (Rhee et al., 2004, Searls et al., 2005). The selection of leaner beef carcasses leads to lower intramuscular fat, while IMF plays an important role in culinary and eating attributes, affecting, e.g. meat tenderness (Nishimura et al., 1999), meat colour (Fiems et al., 2000), beef juiciness (Okumura et al., 2007) and meat flavour, and a small amount of fat (about 2.0–2.5 %) is necessary for good culinary quality (Thompson, 2004).

The assumption of the present study is that differences in carcass characteristics and physico-chemical traits of heifer *longissimus lumborum* muscle are connected with intramuscular fat content, and that the relationship between tested parameters depends on the level of marbling. Therefore, the experiment was arranged to determine the slaughter and carcass traits, chemical composition and physical properties of *musculus longissimus lumborum* (MLL) as related to the amount of intramuscular fat, and to examine the correlation between some of the above traits and culinary characteristics of meat.

2 Material and methods

The study was conducted under a research protocol approved by the III Local Ethical Committee in Warsaw (No. 62/2009 from 25 September 2009).

2.1 Animals

The experiment involved 68 heifers obtained from Polish Black-and-White Lowland dams (with no more than 50% Holstein Friesian blood) sired by Limousin bulls. The animals originated from three farms with similar feeding and housing conditions. In the autumn-winter period, all the heifers were fed hay (ad libitum), corn silage and compound cereal meal, and in summer they received green fodder (ad libitum), crushed cereal grain and a small addition of hay. The diets were formulated according to the maintenance requirement of animals specified by French National Institute for Agricultural Research (INRA) feeding standards with the use of INRAtion Software for Ruminant Diet Calculation, version 2.03 (DJ Group, Krakow, Poland), drawing on the earlier chemical analysis of feeds and calculating their nutritional value. The time of cattle transport to the slaughterhouse was approximately 3 h. After a 24 h rest, heifers were slaughtered and after post-slaughter processing carcasses were cooled at 4 °C.

2.2 Sample collection

24 h after cooling the *musculus longissimus lumborum* (MLL) area and subcutaneous fat thickness were measured between the last thoracic vertebra and the first lumbar vertebra. The samples of MLL were taken from the central part of the muscle and the evaluation of marbling was performed on the same samples by three independent persons, based on a five-point marbling scale (1 point = imperceptible to 5 points = strong). Degree of marbling was presented in tables

as marbling class (class II and III with 16 individuals, class IV with 19 and V with 17 individuals). Other meat samples were packed into separate plastic bags, which were cooled at 4° for the next 24 h. Within 48 h post-mortem, the chemical and physical properties of MLL were determined.

2.3 Chemical and physical analysis

Chemical analysis of the meat (dry matter, total protein and fat content) was performed according to the standard procedures of the Association of Official Analytical Chemists (AOAC, 1995). Total collagen content was estimated in raw meat, whereas samples designed for insoluble collagen analysis were thermally treated until the internal temperature of +75 °C was reached. Collagen content was determined in fat-free dry matter, so all the meat samples were degreased by homogenization with frozen acetone (1:20), and then the hydroxyproline content was determined according to the standard procedure of the International Organization for Standardization (ISO, 1994). The absorbance was read using VARIAN CARY 300 bio spectrophotometer (Varian, Inc., Palo Alto, CA, USA). Total collagen was calculated as hydroxyproline \times 7.25 and expressed as milligrams of collagen per gram of fat-free dry matter. The amount of soluble collagen was calculated from the difference between total and insoluble collagen content and was expressed as the percentage of total collagen. The meat tenderness was measured based on shear force with the use of a Warner-Bratzler shear blade. Muscle samples (with a cross-section area of 1 cm^2 perpendicular to the muscle fibres) was analysed after heating at 75° and 30 min. The mean value of 3 replicate analyses of the same sample was expressed in $N \text{ cm}^{-2}$. The meat lightness (colour L) was estimated using Minolta Chroma Meter CR-310-HUNTER LAB system (Minolta Camera Co., Ltd., Osaka, Japan) with the 1.0 cm thick slices.

2.4 Statistical analysis

Data were statistically analysed using SAS, version 9.3 (SAS Institute, Inc., Cary, NC, USA). All the results are given in tables as least square means (LSM) with the standard error of the mean (SE) and were subjected to one-way analysis of variance to evaluate the influence of marbling on tested parameters (P value). Differences between means were tested by Tukey's method and the probability of P < 0.05 was accepted as significant. Additionally, within marbling classes, Pearson's correlation of shear force with some of the meat characteristics, and correlation of colour L parameter with some slaughter and carcass traits were estimated using the CORR procedure of SAS.

3 Results

Data presented in Table 1 show that age at slaughter and carcass weight reached almost the same values in all mar-

Table 1. Slaughter traits and carcass analysis. _

	Marbling class					
	II (<i>n</i> = 16)	III (<i>n</i> = 16)	IV (<i>n</i> = 19)	V (<i>n</i> = 17)		
	LSM	LSM	LSM	LSM	SE	P value
Age at slaughter (days)	551	546	555	562	3.54	0.256
Carcass weight (kg)	223	226	223	218	1.10	0.726
Dressing percentage	57.9 ^a	56.1 ^b	56.1 ^b	53.8 ^c	0.25	0.037
Subcutaneous fat (cm)	0.77 ^a	0.85 ^a	0.92 ^b	1.25 ^c	0.03	0.019
Fat of valuable cuts (%)	4.6 ^a	6.3 ^b	6.3 ^b	7.5 ^c	0.14	0.004
MLL area (cm ²)	61.7 ^a	64.2 ^b	65.0 ^b	65.7 ^b	0.27	0.046

LSM - least square means value

SE - standard error of the mean

MLL – musculus longissimus lumborum a,b,c, – means in the row with different letters differ significantly (P < 0.05)

Table 2. Chemical composition and physical properties of *longissimus lumborum* muscle.

	Marbling class				_	
	II (n = 16) LSM	III (n = 16) LSM	IV (<i>n</i> = 19) LSM	V (<i>n</i> = 17) LSM	SE	P value
Dry matter (%)	22.3 ^a	22.4 ^a	23.4 ^b	24.4 ^b	0.23	0.035
Total protein (%)	20.7	20.4	20.1	20.0	0.17	0.441
MLL fat (%)	2.4 ^a	3.4 ^b	3.5 ^b	4.5 ^c	0.11	0.004
Total collagen $(mg g^{-1})$	4.0	3.9	3.5	3.6	0.10	0.272
Soluble collagen (% of total collagen)	19.1 ^a	18.0 ^b	16.5 ^c	16.4 ^c	0.28	0.028
Shear force $(N \text{ cm}^{-2})$	50.1 ^a	50.8 ^a	46.5 ^b	42.6 ^c	0.63	0.003
Colour L	33.9 ^a	30.1 ^b	28.9 ^c	27.8 ^c	0.51	0.007

LSM - least square means value

SE - standard error of the mean

MLL - musculus longissimus lumborum

Colour L - lightness

a,b,c – means in the row with different letters differ significantly (P<0.05)

bling classes of longissimus lumborum muscle. The dressing percentage was highest (P < 0.05) in small marbled muscle (class II) and least significant in V marbling class. The results indicated that subcutaneous fat thickness significantly increased with increasing marbling class and the same trend concerned the percentage of fat of valuable cuts. Also the value of MLL area was higher with increasing marbling class and the result for class II was significantly lower compared with remaining classes.

The MLL of class II and III contained less dry matter (P < 0.05) relative to IV and V classes (Table 2). Total protein and total collagen content in the muscle was not influenced by marbling class. The most significant level of MLL fat was found in marbling class V and the lowest in II. No differences in this parameter occurred between medium (III) and large (IV) classes. The results showed that soluble collagen content and meat lightness decreased with rising marbling class, and only between classes IV and V were there no significant differences. In turn, in V class of marbling the value of shear force was lowest (P < 0.05) compared with remaining classes.

The correlation between shear force and the content of fat in MLL was negative in all marbling classes and the highest level of correlation (-0.897) was found in class III (Table 3). The results showed that the values in each marbling class are significant. Also, the highest but positive correlation of shear force and total collagen was found in the medium (III) marbling class, and the correlation proved to be significant only in class II and III. In turn, the correlation between shear force and soluble collagen content was negative in each marbling class. The highest correlation coefficient value was found in IV class (-0.897) and the lowest in class V (-0.377) and only in this class was the relationship not significant.

The correlation between colour L parameter and age at slaughter presented in Table 4 indicates that the relationships in all marbling classes were negative, low and not significant.

		Marbling class			
		II (<i>n</i> = 16)	III (<i>n</i> = 16)	IV (<i>n</i> = 19)	V (<i>n</i> = 17)
		Shear force (N cm ⁻²)			
MLL fat (%)		-0.636*	-0.897*	-0.643*	-0.753*
Collagen	total (mg g ⁻¹) soluble (% of total collagen)	$0.438* \\ -0.490*$	$0.474* \\ -0.703*$	$0.241 \\ -0.897*$	$0.248 \\ -0.377$

Table 3. Coefficients of Pearson's correlation of shear force with fat and collagen content.

MLL – musculus longissimus lumborum * P <0.05

 Table 4. Coefficients of Pearson's correlation of colour L with slaughter and carcass traits.

	Marbling class				
	II $(n = 16)$	III $(n = 16)$	IV $(n = 19)$	V $(n = 17)$	
	Colour L				
Age at slaughter (days)	-0.324	-0.321	-0.159	-0.208	
Carcass weight (kg)	0.011	0.163	0.347	0.365	
MLL fat (%)	0.400*	0.439*	0.424*	0.603*	

MLL – musculus longissimus lumborum

* P<0.05

The correlation was also not significant between meat lightness and carcass weight. The relationships in each marbling class were positive and low. The results of meat lightness and MLL fat correlation showed that it was positive and significant in each marbling class. The highest value of the correlation coefficient (0.603) was estimated in the strong (V) class.

4 Discussion

It is well known that animal age is one of the factors determining the IMF content (Bruns et al., 2005; Młynek et al., 2006; Webb and O'Neill, 2008; Smith et al., 2009; Węglarz, 2010c). In the present experiment, marbling class affected neither age at slaughter nor carcass weight. In turn, carcass dressing percentage, subcutaneous fat amount, fat of valuable cuts and MLL area varied depending on the content of intramuscular fat. According to May et al. (1992), marbling is closely linked to fat thickness and the percentage of kidney, pelvic and heart fat. The relationships found by these authors were very strong and correlation coefficients ranged from 0.92 to 0.96. The least significant MLL area despite the highest dressing percentage presented in this study in small marbled muscles (Table 1) may be explained by the very low MLL fat content in II marbling class (Table 2).

Numerous studies performed on various cattle breeds and disparate muscles reported a negative correlation between

muscle moisture and fat content (Savell et al., 1986; Kim and Lee, 2003; Ueda et al., 2007). The reason for this is the replacement of moisture with fat when fat content in muscle increases. In the study of Jo et al. (2013), the group of animals with low IMF in longissimus dorsi muscles (average IMF 10.71 %) had 67.69 % moisture content, while the group with high IMF (21.07%) had only 58.45%. Increasing dry matter with increasing marbling in our study is equivalent to decreasing moisture percentage with increasing fat content in the experiments mentioned before. In keeping with our observations, Savell et al. (1986) and Kim and Lee (2003) found a relatively constant level of protein regardless of fat content in bovine muscle. In contrast, Ueda et al. (2007) noted an inexplicable, significant decrease of protein content in higher marbling (up to 39% of intramuscular fat content) longissimus thoracis muscle of Japanese Black steers. Albrecht et al. (2011) explain that in highly marbled muscle, fat cells replace muscle fibres and reduce protein content in muscle.

The connective tissue is present in all muscles in the epimysium, perimysium and endomysium components encircling muscle fibres. Intramuscular fat located between muscle fibre bundles interrupted the endomysium structure, thus separating and diluting perimysial collagen fibres and disrupting the structure of intramuscular connective tissue (Hocquette et al., 2010). In accordance with Nishimura et al. (1999), this process results in an increase of meat tenderness but only in strongly marbled muscles that contain no less than 8% of fat. In our study, we found increased meat tenderness expressed as decreased shear force value of MLL in marbling class IV and V compared with II and III (the content of fat was 3.5 and 4.3 %, respectively). The values of total collagen content were very similar; however, the percentage of soluble collagen of middle (III), large (IV) and strong (V) marbled longissimus lumborum muscle was significantly reduced as compared to marbling class II. With respect to meat colour, our results do not concur either with Fiems et al. (2000) or with Kim and Lee (2003). The former authors noted that a large amount of intramuscular fat can increase meat lightness, while the latter researchers reported that marbling did not affect the meat colour.

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The significant negative correlations observed in this study between the shear force value (SF) and MLL fat content are in agreement with the results of Ueda et al. (2007), who found a significant negative correlation between SF and fat level of longissimus thoracis muscle from Japanese Black steers. Conversely, some authors observed that the shear force parameter showed no significant relationship with beef fat content or marbling score (Brooks et al., 2000; Jo et al., 2013). The correlation of the collagen and shear force value or sensory tenderness has been observed in several earlier experiments but the results differ. Nakamura et al. (2010) found a positive correlation between shear force and collagen content of the longissimus thoracis muscle of Japanese Black and Brown steers. Collagen characteristics were noticed as a poor indicator of meat toughness from young bulls of fifteen European breeds in the study by Christensen et al. (2011). In turn, in agreement with our results, Chambaz et al. (2003) observed a negative relationship between collagen solubility and tenderness in *longissimus dorsi* muscle of Charolais steers.

Meat lightness can be determined by several pre- or postslaughter factors and is often inversely correlated with level of haem iron content in muscle myoglobin which increases with animal age (Chambaz et al., 2003). Bureš and Bartoň (2012) have demonstrated that lightness of musculus longissimus lumborum was affected by the interaction between sex and slaughter age of animals, and the meat became darker with increasing age only in heifers but not in bulls. Also in muscles of male Chianina cattle, the different age of slaughter did not influence colour L parameter (Preziuso and Russo, 2004). In contrast, the beef colour stability of *mus*culus psoas, m. major, m. semitendinosus and m. longissimus thoracis from Japanese Black steers decreased with age (Muramoto et al., 2003). The suitable age at slaughter and the associated pre-slaughter weight of animals translates into carcass weight and meat quality (Bruns et al., 2004; Weglarz, 2010b). Weglarz (2010b) showed no correlation (0.01) between hot carcass weight and beef lightness of meat from young bulls. The same value was observed in the present study but only in heifers of class II. In another experiment, the same author (Węglarz, 2011) reported no relationship (coefficient of correlation = 0.00) between body weight and colour L trait of individual or group-housed cattle classified according to the EUROP system in four categories. Fiems et al. (2000) reported the negative correlation between colour L values and the fat characteristics in double-muscled and non-double-muscled Belgian Blue bulls.

The results of this study show that marbling significantly affected the dressing percentage and carcass traits such as subcutaneous fat content, fat of valuable cuts and MLL area. Most of the physico-chemical properties of heifer *musculus longissimus lumborum*, with the exception of total protein and total collagen content, were related to the level of marbling. It can be concluded that age at slaughter and carcass weight are not good predictors of colour lightness of

MLL within all marbling categories. The same conclusion applies to the amount of total collagen in large and strong marbled muscles for shear force value. In turn, soluble collagen content for shear force seems to be a quite good forecaster of meat tenderness but not in strong marbled muscles. Of all tested slaughter and carcass traits, only MLL fat content seems to be appropriate to assess both the tenderness and lightness of meat.

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