

REGULAR ARTICLE

Meat quality of Limousin young bulls slaughtered at 6, 9 and 12 months of age

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ABSTRACT

The aim of this study was to compare the quality of meat of the young Limousin bulls slaughtered at the age of 6, 9 and 12 months, with particular regard to the residual glycogen content in the meat and the value of the glycolytic potential. The study was conducted on bovine *longissimus lumborum* muscle. The residual glycogen content, glycolytic potential value (96 h *post-mortem*), pH value (45 min, 24 h, 48 h and 96 h *post-mortem*), IMP/ATP index (45 min *post-mortem*), colour parameters (L*, a* and b*), natural and cooking losses, free water content, the chemical composition, sensory parameters (aroma, flavour, juiciness and tenderness) as well as instrumental tenderness based on cutting test (96 h *post-mortem*) were analysed. The slaughter age of bulls had significant ($p < 0.05$) effect on following meat parameters: concentration of glycogen, glycolytic potential value, lightness (L*) and redness (a*), shear force value, intramuscular fat content and sensory evaluation of aroma, flavour, juiciness and tenderness. The *longissimus lumborum* muscle from young bulls slaughtered at the age of 6 months had significantly ($p < 0.05$) lower values for glycogen concentration, glycolytic potential and intramuscular fat content when compared to animals slaughtered at the age of 12 months. Moreover, the colour of the examined muscle from the youngest bulls was characterised by the highest L* value and the lowest a* value as well as the lowest shear force value when compared to the meat of older bulls slaughtered at the age of 9 and 12 months.

Keywords: Young bulls; Age; Meat quality; Glycolytic potential; Residual glycogen

INTRODUCTION

Limousin is a popular and valued cattle breed used for the meat production. Recently, consumers prefer to buy beef meat from young, lean animals, which is characterised by an adequate tenderness, juiciness, flavour and easiness to prepare (Frank et al., 2016). The technological and sensory quality of meat depends mostly on the genetic and environmental factors (Vestergaard et al., 2000; Correia et al., 2016; Iida et al., 2016); however, glycolytic changes that occur *post-mortem* are also of significant importance (Immonen et al., 2000; Koćwin-Podsiadła et al., 2006; Przybylski et al., 2016). The insufficient glycogen content at slaughter results in the production of meat with an abnormally high final muscle pH (> 6.0), known as a dark-cutting or dark, firm and dry (DFD) meat. It is the most

frequently observed beef quality defect that occurs mostly in the meat of young bulls (Węglarz 2010). In many studies the effects of different slaughter ages on beef meat quality has been examined (Ahnström et al., 2012; Lucero-Borja et al., 2014); however, most of them focused mainly on animals slaughtered at the age of more than 12 months (Bureš & Bartoň 2012; Girard et al., 2012; Humada et al., 2014). Only few publications concerned the meat quality of younger cattle (Prado et al., 2015; Araujo et al., 2016). Moreover, to the best of our knowledge, available literature sources lack information on glycogen content of beef meat as the main indicator of its quality; most of the published results concern pork meat (Przybylski et al., 2016). Therefore, this study aimed to compare the quality of meat of the young Limousin bulls slaughtered at the age of 6, 9 and 12 months, with particular regard to the

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residual glycogen content in the meat and the value of the glycolytic potential.

MATERIALS AND METHODS

Materials

The experimental material comprised of 18 Limousin young bulls, slaughtered at the age of 6, 9 and 12 months. Each group consisted of six animals. All animals originated from the same herd and were the progeny of one sire. Before transportation to the experimental farm, bulls were weaned at four months of age. During the fattening period bulls were kept in the free-stall system on a deep-litter in an open barn. They were fed *ad libitum* with maize silage, supplemented with concentrates and hay. At the completion of the fattening, the animals fasted for 24 h with constant access to the water. Subsequently, they were transported to an abattoir, located 12 km away from the farm. The average weight of the slaughtered animals in the group of 180 ± 4 days old bulls was 191 kg; in the group of 270 ± 5 days old bulls it was 288 kg, while in the group of 360 ± 7 days old bulls it was 351 kg. The slaughter was performed according to the current veterinary and technological regulations.

Methods

Following parameters of *longissimus lumborum* muscle were determined: pH value, glycolytic potential, glycogen, lactic acid, index of energy changes (IMP/ATP), colour, shear force, natural drip, cooking loss, free water and chemical composition. Moreover, in order to complement the quality assessment of meat, sensory evaluation was also conducted.

pH measurements

The measurements of pH values were taken at 45 min (pH_{45v}), 24 h (pH_{24h}), 48 h (pH_{48h}) and 96 h (pH_{96h}) *post-mortem*, directly in the tissue of the *longissimus dorsi* muscle (*mlD*), in the *longissimus lumborum* section (*mlL*), behind the last rib. A portable Handylab 2 (Schott Geräte, Mainz, Germany) apparatus with a glass-calomel electrode (Schott L68880) was used.

Glycolytic potential (GP), residual glycogen (RG) and lactic acid

The GP was determined in the samples collected from *mlL* 96 h *post-mortem*. For the analyses, approximately 1 g of *mlL* was homogenised with 10 ml 0.6 M perchloric acid (PCA). In order to determine the content of RG in the muscle, an enzymatic method by Dalrymple and Hamm (1973) was applied to 0.5 ml homogenate. The remaining homogenate was centrifuged for 15 min at $1,118 \times g$. The obtained supernatant was examined for the content of lactic acid according to the method described by Bergmeyer (1974). GP was presented as the sum of two main compounds participating in glycolytic changes in the muscle tissue and

was calculated based on the formula developed by Monin and Sellier (1985): $GP = [2 \times \text{residual glycogen}] + \text{lactic acid}$. It was expressed as μmol of lactic acid equivalent per g of fresh muscle ($\mu\text{mol/g}$).

The index of energy changes (R₁)

R₁ was determined as the ratio of the inosine monophosphate to adenosine triphosphate (IMP/ATP) at 45 min after slaughter according to Honikel and Fischer (1977). This index has been proposed as an indicator for the distinction between normal and faulty meat by Koćwin-Podsiadła et al., (2006).

Meat colour

The meat colour was determined according to CIE L*a*b* system at 96 h *post-mortem* using a Minolta 508i spectrophotometer with the D 65 light source (Osaka, Japan). Colour of raw meat was measured on a freshly cut surface of slices (20 mm of thickness), after being exposed to atmospheric oxygen for 30 minutes (Avilés et al., 2015). Samples were measured by scanning 5 different locations. Each result corresponds to an average of 5 measurements collected per sample. The instrument was calibrated with a white standard plate before measurements.

Meat tenderness (shear force)

Instrumental measurements of steaks tenderness (by shear force values) were conducted at 96 h *post-mortem* using an Instron type 1140 apparatus with a Warner-Bratzler attachment, after previous thermal treatment of the samples. Steak samples (25-30 mm of thickness) were heated up in a water bath to a core temperature of 72 °C. Subsequently, samples were kept in water bath at 80-81 °C for another 90 min. After cooling steaks were cut into cuboids with the cross-section of 10 mm x 10 mm and length of approx. 40 mm. The cutting procedure was done along the parallel arrangement of muscle fibres. Samples were subjected to the action of shear force so that the arrangement of fibres was perpendicular to the shear plane. For each sample, 8 replicates were performed and the average of all replicates was used for statistical analysis (Grześ et al., 2007).

Sensory evaluation of meat

For sensory evaluation meat samples (25-30 mm thick) were subjected to the thermal treatment which is described in the meat tenderness section. After cooling the samples were cut in parallel to the longitudinal orientation of the muscle fibres into thinner slices of 2-3mm. Coded samples were placed on plastic disposable plates. The panel consisted of six panellists with a long experience in sensory profiling in meat and meat products. Sensory evaluation was carried out in accordance with the method described by Barylko-Pikielna & Matuszewska (2009). Evaluation of aroma,

flavour, juiciness and tenderness was conducted based on the linear scaling method (1-10 points) where 1- denoted the least desirable score and 10 - the most desirable one.

Natural drip, cooking loss, water holding capacity (WHC)

The natural drip was determined according to Prange et al., (1997), while the percentage volume of cooking loss was determined from the difference in weight of meat slices before and after cooking. Water holding capacity, measured as free water content (%) was determined following Grau and Hamm's method (1952) modified by Pohija and Ninivaara (1957).

Chemical composition

The chemical composition (water contents, crude protein and intramuscular fat) were determined by AOAC (1996) procedures.

Statistical analysis

Recorded results were analysed statistically using the SAS v.9.2 programme (2014), applying the analysis of covariance. The Duncan test was performed to provide a detailed comparison of object means. Moreover, Spearman's phenotypic correlation coefficients between the slaughter age and selected traits of meat quality were also calculated.

RESULTS AND DISCUSSION

Effect of age on GP and RG content

GP is an important factor affecting *post-mortem* muscle metabolism and is an estimator of the glycogen content in the muscle at the time of slaughter. According to the reports indicating that GP does not change with the time *post-mortem* (Maribo et al., 1999), in this study, it was measured only 96 h after slaughter. Its values ranged from 115 $\mu\text{mol/g}$ to 136 $\mu\text{mol/g}$ depending on the age group of slaughtered animals (Table 1).

According to Wulf et al., (2002), GP below the threshold of approx. 100 $\mu\text{mol/g}$, is associated with a higher ultimate muscle pH and a higher risk of dark-cutting beef. When GP is greater than 100 $\mu\text{mol/g}$, low ultimate pH prevents the complete conversion of glycogen to lactic acid leaving behind a certain amount of so-called residual glycogen (RG). Glycogen reserve in muscles is rarely totally depleted (Immonen & Puolanne 2000). The presence of residual glycogen is the result of slowing and stopping the process of anaerobic glycogenolysis. In our experiment, level of RG was low and varied from 10.65 $\mu\text{mol/g}$ to 17.55 $\mu\text{mol/g}$ (Table 1). The performed analyses revealed the significant positive correlations between the slaughter age and both glycolytic potential and residual glycogen (Table 3). The

young bulls slaughtered 12 months after birth were characterised by higher values of both parameters than two other groups of younger bulls (Table 1). Some studies showed that the presence of RG has a negative impact on technological quality of meat (Przybylski et al., 2012). In our study the RG did not affect the technological quality (the natural drip, cooking loss and free water content) of meat from this age group when compared to the groups of 6 and 9-month-old bulls; however, significant ($p < 0.05$) deterioration of tenderness (in both instrumental and sensory evaluation) and juiciness was observed in the meat of 12-month-old bulls (Table 1-2). Little is known about the association between the age at slaughter and GP, as well as possible mechanisms underlying this relationship. Gardner et al., (2009) found the link between the increased age and a greater adrenaline response in 15 and 36-month-old cattle. It may be caused by the changes in a muscle fibre type composition (Gardner et al., 2014). Several reports indicate that muscle fibre type proportions undergo considerable variation during the growth of cattle, although the results are not completely consistent (Jurie et al., 1995).

Effect of age on muscle acidification and water holding capacity

No significant differences were found between slaughter age and meat acidification in all groups (Table 1). This agrees with the results of Wulf et al., (2002). The mentioned authors showed, that approx. 100 $\mu\text{mol/g}$ GP is enough to ensure a normal ultimate muscle pH, and a higher GP values have no further effect on meat acidification. The lack of association between slaughter age and the final muscle pH was also reported in other studies (Ahnström et al., 2012; Marti et al., 2013). However, in the study of Yim et al., (2015), pH values of meat from 5-month-old Holstein calves were higher than of 8-month-old group. On the other hand, in the experiment of Bureš & Bartoň (2012), meat samples from older bulls were characterised by a higher pH measures. The acidification of meat affects its quality and can be influenced by different stressors such as high temperature, transportation, starvation as well as proceedings before and during the slaughter (Terlouw et al., 2008; Węglarz 2010). Moreover, it was shown that type of diet could also influence meat acidity (Immonen et al., 2000).

The values of pH recorded in this study indicate a good quality of meat in all analysed groups of young bulls. Moreover, a small volume of water loss from meat and the rate of ATP breakdown and IMP formation, expressed as R1, confirmed the absence of meat quality defects in the examined samples (Table 1).

There is a close association between *post-mortem* biochemical changes and water holding capacity of meat (Huff-

Table 1: Physicochemical indicators of *m. longissimus lumborum* quality depending on slaughter age

Traits	Influence of slaughter age	Slaughter age (months)					
		6		9		12	
		\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
RG $\mu\text{mol/g}$	*	10.65 ^a	1.05	10.79 ^a	1.75	17.55 ^b	4.15
GP $\mu\text{mol/g}$	*	115 ^a	2.08	124 ^a	7.97	136 ^b	9.58
pH _{45'}	ns	6.79 ^a	0.04	7.03 ^a	0.15	6.99 ^a	0.17
pH _{24h}	ns	5.92 ^a	0.27	5.86 ^a	0.08	5.83 ^a	0.10
pH _{48h}	ns	5.53 ^a	0.10	5.49 ^a	0.03	5.59 ^a	0.10
pH _{96h}	ns	5.51 ^a	0.10	5.47 ^a	0.06	5.45 ^a	0.03
R1 (IMP/ATP)	ns	0.91 ^a	0.03	0.93 ^a	0.03	0.92 ^a	0.02
Colour: L*	*	44.19 ^a	3.51	41.39 ^{ab}	0.84	39.32 ^b	1.80
a*	*	10.08 ^a	2.78	11.33 ^{ab}	1.68	13.48 ^b	1.61
b*	ns	9.07 ^a	1.35	8.31 ^a	0.76	8.54 ^a	0.74
Shear force (N/cm ²)	*	109.41 ^a	21.39	143.93 ^b	5.12	141.67 ^b	16.00
Water content (%)	ns	76.85 ^a	0.31	76.83 ^a	0.45	76.97 ^a	0.42
IMF content (%)	*	0.27 ^a	0.15	0.44 ^{ab}	0.28	0.59 ^b	0.22
Protein content (%)	ns	22.03 ^a	0.46	21.61 ^a	0.41	21.69 ^a	0.28
Natural loss (%)	ns	2.10 ^a	1.05	1.37 ^a	0.75	1.62 ^a	0.58
Cooking loss (%)	ns	42.78 ^a	1.01	43.99 ^a	1.44	43.70 ^a	1.23
WHC, Free water (%)	ns	36.63 ^a	0.79	36.87 ^a	1.43	37.56 ^a	1.54

RG: Residual Glycogen, GP: Glycolytic Potential, R1 (IMP/ATP) - the index of energy changes - ratio of inosine monophosphate to adenosine triphosphate, L* - Lightness, a* - Redness; b* - Yellowness; IMF: Intramuscular fat, WHC: Water Holding Capacity, means designated with different letters differ significantly at $p < 0.05$, *-influence statistically significant ($p < 0.05$); ns – influence statistically non-significant

Table 2: Sensory indicators of *m. longissimus lumborum* quality depending on slaughter age

Selected traits (scores, 1-10)	Influence of slaughter age	Slaughter age (months)					
		6		9		12	
		\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
Aroma	*	8.83 ^a	0.67	9.32 ^{ab}	0.06	9.38 ^b	0.50
Flavour	*	7.97 ^a	0.33	8.67 ^b	0.38	8.12 ^{ab}	0.54
Juiciness	*	6.28 ^a	0.64	4.66 ^b	0.72	4.83 ^b	0.85
Tenderness	*	7.58 ^a	0.42	4.98 ^b	1.04	5.21 ^b	0.99

Means designated with different letters differ significantly $p < 0.05$, *-influence statistically significant ($p < 0.05$); ns – influence statistically non-significant

Table 3. The Spearman's phenotypic correlation coefficients between slaughter age and selected traits of meat quality

Traits	r
Residual glycogen muscle $\mu\text{mol/g}$	0.66**
Glycolytic potential $\mu\text{mol/g}$	0.76**
L*(Lightness)	-0.64**
a*(Redness)	0.60*
Intramuscular fat content (%)	0.58*
Shear force (N/cm ²)	0.68**
Tenderness, scores	-0.64**
Juiciness, scores	-0.54*
Aroma, scores	0.49*

**Values significant at $p < 0.01$; *values significant at $p < 0.05$

Lonergan & Lonergan 2005). As the pH decreases the protein denaturation, including proteins involved in binding water occurs. In this study, the proper pattern of the acidification was observed in the meat of all animals, regardless of their slaughter age. Therefore, there were no statistically significant differences in levels of water holding measures between different age groups of young bulls (Table 1). Immonen et al., (2000) found that water losses from beef were affected by the RG concentrations.

Several authors analysed the effect of the slaughter age on the water holding capacity changes in beef cattle meat, yet the results are not consistent (Bureš & Bartoň 2012; Yim et al., 2015).

Effect of age on meat chemical composition

The basic chemical composition of meat derived from analysed animals is reported in Table 1. Meat from the 12-month-old bulls was characterized by a significantly higher content of intramuscular fat (IMF) than meat from young bulls slaughtered at the age of 6 months. In this study, the IMF content increased with the age of young bulls what is in agreement with previously reported results (Ahnström et al., 2012; Marti et al., 2013). In this study, we analysed meat from very young animals, thus to obtain a greater muscle marbling, the longer fattening period would have been required to slaughter animals at the heavier body weight (Sañudo et al., 2004). Interestingly, requirements of the consumers concerning the content of intramuscular fat are different. Meat with slight marbling is desirable in Western and Central Europe, while consumers from Asian countries (Korea, Japan and Taiwan) generally prefer meat

with a moderate amount of marbling (Hwang et al., 2010; Lida et al., 2016).

Effect of age on colour, tenderness and sensory parameters of meat

The colour is one of the most important quality parameters of beef. It indicates its freshness and suitability for consumption. It is the first characteristic factor in meat selection by the consumers. This study revealed that the *mll* of the animals slaughtered at the age of 6 months was significantly lighter (L^*) and had a lower redness (a^*) in comparison to the muscles of animals slaughtered at the age of 12 months (Table 1). Moreover, a significant negative correlation was observed between the slaughter age and lightness of colour (L^*) and a positive correlation between slaughter age and a^* parameter (Table 3). Similar observations were reported by Preziuso and Russo (2004) and Cho et al., (2015). The colour of meat depends on concentration and chemical form of myoglobin which is the main hem pigment of the muscle tissue (Bak et al., 2012). Its concentration in skeletal muscles depends on age, breed, physical activity of animals during the lifespan, feeding and environment that beef is reared in, season of slaughter as well as on conditions of storage of carcasses and meat packing (Węglarz 2010; Bak et al., 2012; Bureš & Bartoň 2012; Avilés et al., 2015).

The tenderness of the meat is a major quality characteristic highly related to the overall consumers' acceptability of beef. It is highly variable and can be affected by the numerous factors. The tenderness develops during meat ageing, which is a long-term process that changes muscle fibres' structure. In this study, regardless of the slaughter age of bulls the obtained shear force values were high, indicating the tough meat (Table 1). Significantly lower shear force values (meat more tender) were found in the samples of meat obtained from 6-month-old young bulls in comparison to the oldest one (Table 1). Usually, after longer storage of meat, an improvement of its tenderness is observed (Brewer & Novakofski 2008). According to Koohmaraie & Geesink (2006), satisfactory tenderness is achieved after at least 14 days of beef storage.

Similarly, significant ($p < 0.05$) differences were shown in the tenderness of the *mll* evaluated by the sensory method; the meat of 6-month-old young bulls received the highest notes (Table 2). The similar tendency was observed for meat juiciness, whereas the aroma and flavour of the meat from the 6-month-old beef had the lowest scores ($p < 0.05$), Table 2). The correlation results between slaughter age and beef tenderness, juiciness and aroma (Table 3) correspond to the results obtained by other authors (Lucero-Borja et al., 2014; Girard et al., 2012).

The lower tenderness observed in the meat from older animals can be a result of the higher collagen content, especially insoluble cross-linked collagen in the muscles of older animals (Ngapo et al., 2002). The other explanation may involve the calpain/calpastatin system, related to the changes of *post-mortem* muscle fibres proteolysis. According to Cruzen et al., (2014) the ratio of calpain to calpastatin is lower in the old animals compared to the calves, indicating the reduced potential for proteolysis in the mature cattle.

The meat of older young bulls is also characterised by a higher intramuscular fat content which is positively correlated with meat tenderness (Fiems et al., 2000). This could explain the results of Yim et al., (2015) who received higher shear force values for meat from 5-month-old Holstein calves than for the meat of the 8-month-old group. At the same time, fat content in the *longissimus dorsi* muscle of 5-month-old animals was significantly ($p < 0.05$) lower (0.55 %) when compared to the meat of 8-month-old animals (1.52 %). Three times higher content of intramuscular fat (IMF) in the meat of older Holstein bulls could be a crucial factor for the lower shear force values. In the current study meat of highly muscled Limousin beef cattle was examined. This type of meat is characterised by a low content of IFM regardless of slaughter age when compared to the meat of dairy Holstein cattle. This factor could have affected the lack of improvement in meat tenderness in older animals. Immonen et al., (2000) hypothesised that beef tenderness classified by shear force was positively associated with residual glycogen. That observation was confirmed in this study, as the lowest hardness (the best tenderness) was found in the meat of 6-month-old animals, which were also characterized by the lowest RG content (Table 1).

CONCLUSIONS

The quality of meat from young Limousin bulls slaughter at different age (6, 9 and 12 months) differed among the groups. The results of tenderness and juiciness evaluation indicate that meat of 6-month-old bulls received the highest scores for these parameters what can suggest that this type of meat will be more required by the consumers than the meat of 9 and 12-month-old bulls.

The content of residual glycogen (RG) in the meat of young bulls increased with their slaughter age, without affecting the technological properties of meat (the natural drip, cooking loss and free water content). However, the increase of RG content caused the deterioration of the tenderness (both in instrumental and sensory evaluation) and the juiciness of the meat. Due to the small amount of scientific reports on the effect of residual glycogen on technological and sensory characteristics of beef cattle, further studies are encouraged.

Compliance with ethics requirements

This article does not contain any studies with animal subjects.

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