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THE EFFECT OF WATER VAPOR PRESSURE ON MUSCLE COLLAGEN SOLUBILITY AND SELECTED CHARACTERISTICS OF THE *LONGISSIMUS LUMBORUM* MUSCLE IN CROSSBRED CATTLE*

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Abstract

Most scientific studies are dedicated to the possibility of preparing beef for consumption under industrial conditions. Few publications are devoted to the issue of collagen thermohydrolysis in conditions available to the consumer. This study has analyzed the effect of small values of water vapor pressure on major culinary indices and chemical components of the *longissimus lumborum* muscle obtained from bulls with different growth rates. The experiment involved 48 animals. On the basis of the gain during the fattening time, the animals were divided into a low growth intensity group, with a daily body weight gain of ≤900 g, and a high growth intensity group with a daily gain of >900 g/day. A part of the samples of the *longissimus lumborum* muscle (control) was thermally treated in a water bath at 75°C. Another part was heat treated in a pressure-pot at 150°C, at a pressure of 0.1 MPa. The next part of samples was subjected to the same temperature, but the pressure was 0.2 MPa. The obtained results indicate that the values of the studied indices were largely affected by thermal processing parameters rather than the animals' growth rate. The highest contents of total protein and water-soluble collagen were obtained in the case of a temperature of 150°C and the highest pressure (0.2 MPa). Water vapor with increased temperature and pressure also created favorable conditions for obtaining better meat tenderness and more favorable values of the water holding capacity. The latter characteristic appeared to be strongly connected with an increasing amount of water-soluble collagen, which was confirmed by relatively high values of the correlation coefficient between these characteristics. A strong positive correlation was also shown between thermal drip and the total collagen content in meat.

Key words: bulls, growth intensity, meat characteristics, collagen solubility, eating quality

Growing consumer awareness in respect of proper nutrition makes them seek treatment methods that favor obtaining health-promoting characteristics of meat.

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However, the most often used culinary methods still include boiling and frying, which do not favor the availability of amino acids contained in hardly digestible collagen. Collagen is a protein that constitutes one of the essential components of the meat structure, and its percentage may range from 1 to 6% (Purslow, 2005; Nishimura, 2010). As evidenced by numerous studies, collagen may have a negative effect on the cooking qualities of both raw meat (Stolowski et al., 2006) and that subjected to heat treatment (Ngapo et al., 2002; Maher et al., 2004). The most common cause of a poor digestibility of this protein is low solubility in water (Velleman, 2012), which greatly deteriorates the important culinary indicator that is meat tenderness (Serra et al., 2008; Schonfeldt and Strydom, 2011). Strong cross-linking of collagen fibers during boiling of meat limits collagen thermohydrolysis and gelatinization (Torrescano et al., 2003). This largely reduces the utilization of the amino acid proline, which is commonly recommended for use in the form of preparations supplementing the human diet. An important aspect during boiling is also the large loss of meat juice, which flows from the tissue under the influence of, inter alia, the strong contraction of collagen fibers.

The factors that can significantly determine collagen solubility are: the animal age and the type of a muscle (Serra et al., 2008; Schonfeld and Strydom, 2011). Numerous studies also show that nutrition, and particularly diets with a high concentration of energy, may affect collagen solubility. This effect is associated with a higher growth rate and earlier slaughter maturity of animals fed in this way (Thenard et al., 2006; Therkildsen et al., 2008; Archile-Contreras et al., 2010). According to reports by Alberti et al. (2008) as well as Modzelewska-Kapituła and Nogalski (2014), the animal breed and sex are also of some importance.

Former scientific studies were mainly dedicated to the possibility of beef production and preparation for consumption under industrial conditions. However, few publications are dedicated to the issue of collagen thermohydrolysis, thereby, the availability of the amino acid proline in conditions available to the consumer is limited. Therefore, an experiment was conducted in which it was assumed that an increased vapor pressure used during the process of boiling will improve the absorption of hot water into the structure of meat, and, consequently, will promote processes increasing collagen solubility in water. This study has analyzed the effect of small values of water vapor pressure on major culinary indices and chemical components, including collagen solubility in the *longissimus lumborum* muscle obtained from bulls with different growth rates.

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. The experiment involved 48 bulls, commercial crossbreds obtained from Polish Black-and-White Lowland cows (with no more than 50% Holstein Friesian blood) sired by Limousin bulls. The animals were derived from two herds, and within each breed group, one sire was used. Throughout the fattening period, the bulls were tethered indoors in individual stalls, on shallow litter. They had access to fresh water and they were fed individually. The feed was administered twice every 24 h, with the same amount in the morning and in the evening. In the autumn-winter period, all the animals were fed hay (*ad libitum*), corn silage (10 kg daily) and compound cereal meal (1 kg daily), and in the summer, they received green fodder (*ad libitum*), crushed cereal grain and a small addition of hay. The diets were formulated according to INRA feeding standards with the use of INRAtion software, version 2.x.x., drawing on the earlier chemical analysis of feeds and calculating their nutritive value. The animals were fed with rations that provided their maintenance requirement. The total requirements for nutrients were determined based on the INRA system. On the basis of the gain during the fattening time, the animals were divided into a low growth intensity category, with a daily body weight gain of ≤900 g (LGI – 25 bulls) and a high growth intensity >900 g/day (HGI – 23 bulls). The average body mass of bulls on the slaughter day amounted to 598±54 kg in the LGI category and 615±39.6 kg in HGI. The *longissimus lumborum* muscle (MLL) was removed from all the animals (LGI-25 and HGI-23) between the 12th and 13th ribs, 24 hours after slaughter, and then cooled at 4°C. The electrical stimulation of the carcasses was not used. The chilling method of the carcasses was one-stage with the use of two phases (in about 24 hours). Every slice of MLL (about 300 g) removed previously was divided into four pieces (blocks about 4.0 × 4.0 × 4.0 cm) and then a physico-chemical analysis was performed. A part of the samples (control) was thermally treated in the water bath at 75°C (±0.5°C) (in thin-walled plastic bags for 30 minutes). Another part was heat treated in a pressure-pot at 150°C (±5.0°C) for 30 minutes, at a pressure of 0.1 MPa (±0.02 MPa) and the next part of samples was subjected to the same temperature, but the pressure was 0.2 MPa (±0.02 MPa). The SAPIR 2 pressure-pot (Prime Eagle International Ltd, Hong Kong, China) was equipped with a pressure manometer, a probe measuring the temperature inside the sample, a temperature regulator and a pressure control valve. The water, total protein and fat content of the meat were determined according to the standard procedures of the Association of Official Analytical Chemists (AOAC, 1995). The total collagen content was estimated in raw and thermally treated meat. The collagen content was determined in a 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). The total collagen was calculated as hydroxyproline × 7.25 and expressed as milligrams of collagen per gram of fat-free dry matter. The amount of water-soluble collagen was calculated from the difference between the total and the insoluble collagen content after homogenization with water (60°C), and was expressed as the percentage of total collagen. The water holding capacity WHC-FPPT was evaluated in raw meat according to the Pohja and Niinivaara filter pressure paper method (1957). The water holding capacity WHC-CM was determined in raw and thermally treated samples according to the Wierbicki et al. centrifuge method (1962). The meat tenderness was measured at room temperature, based on shear force, using INSTRON 5965 device (Instron®, Norwood, MA, USA) equipped with a head (type 1kN) and a Warner-Bratzler knife moving at a speed of 120 mm/min. The samples were prepared by cutting them lengthwise down to the myofibers (blocks about 5 cm long, 1 cm high and 1 cm wide) and then were sheared perpendicularly to the longitudinal axis of the muscle fibers. The mean value of 3 replicates analyses of the same sample was expressed in N cm^{-2}. The thermal drip loss was defined as the difference between the sample weight before and after thermal treatment. After 1 h, the samples were cooled in tap water (4°C) and then stored to reach room temperature.

The data were statistically analyzed using Statistica 10.0 (2016) through an analysis of variance. One-way analysis of variance was used to evaluate the influence of growth intensity on slaughter and meat quality parameters. The model of two-way analysis of variance included: growth intensity, cooking parameters (temperature and pressure) and fixed effect: growth intensity × cooking parameters. The tables give the least square means (LSM), the standard error of the mean (SEM), the level of significance of the effects and interactions. The differences between group means were tested using Tukey's method (level of significance set at 5%). Additionally, Pearson's correlations of some traits of meat with total and water-soluble collagen at different thermal cooking parameters were estimated using the ANOVA model.

Results

The analysis of data in Table 1 showed that the raw meat of bulls that were grown less intensively (LGI) was characterized by a higher protein content and a slightly higher but statistically significant percentage of fat. MLL of animals from the HGI category has a much higher percentage of water. It should be noted that this water in animals from the HGI category was more strongly bonded, which is indicated by a lower value of the indicator WHC-FPPM.

The obtained results concerning changes in the collagen content did not show a significant effect of growth intensity on the total content of this component in MLL of the studied animals. However, a marked effect of the growth intensity of animals on the proportion of water-soluble collagen was observed ($P<0.05$). This effect was noted both in respect of the amount as well as in respect of the percentage of soluble collagen relative to the total collagen. In the case of the percentage of soluble collagen, the growth rate of bulls had a stronger effect, which is indicated by the P-value, amounting to 0.003. The meat of faster growing individuals (HGI) turned out to be tenderer and had a more favorable water holding capacity (WHC-CM). In both cases, the occurrence of statistically significant differences was confirmed. The obtained values indicate that cooking parameters had a larger effect on the values of the studied indicators than the animals growth intensity (Table 2). This is illustrated by lower P-values, obtained in relation to the majority of studied indicators of chemical composition and physical traits of MLL.

Table 1. Slaughter traits and meat quality parameters of the *longissimus lumborum* muscle in bulls from different growth intensity categories

Trait	Growth intensity category			P-value
	LGI (n=25)	HGI (n=23)	SEM	
	LSM	LSM		
Age (day)	628 a	593 b	4.39	0.023
Daily body gain (g/day)	816 a	1074 b	11	0.000
Protein (%)	21.40 a	20.76 b	0.13	0.045
Fat (%)	3.03 a	2.90 b	0.07	0.045
Water (%)	73.08 a	75.03 b	0.22	0.047
Total collagen (mg g^{-1})	4.76	4.74	0.82	0.146
Water soluble collagen (mg g^{-1})	1.09 a	1.19 b	0.09	0.048
Water soluble collagen (% of total collagen)	22.89 a	25.10 b	0.54	0.003
Tenderness (N cm^{-2})	51.5 a	48.7 b	0.41	0.005
WHC-CM (%)	21.7 a	22.3 b	0.08	0.015
WHC-FPPM (%)	22.5	21.3	0.61	0.310

LSM – least square means value; SEM – standard error of the mean; a, b – means in the row with different letters differ significantly (Tukey HSD, P<0.05); LGI – low growth intensity (≤900 g/day); HGI – high growth intensity (>900 g/day); WHC-FPPM – Water Holding Capacity – Filter Pressure Paper Method; WHC-CM – Water Holding Capacity – Centrifuge Method.

Table 2. The effect of growth intensity (GI) and the thermal treatment method on the chemical composition and physical properties of the *longissimus lumborum* muscle

Trait	Growth intensity category – GI / Cooking parameter – CP [temperature (°C) and pressure (MPa)]									
	LGI			HGI			SEM	P-value		
	75°C (control)	150°C 0.1 MPa	150°C 0.2 MPa	75°C (control)	150°C 0.1 MPa	150°C 0.2 MPa		GI	CP	GI×CP
	LSM	LSM	LSM	LSM	LSM	LSM				
(n)	25	23	25	22	23	23				
Protein (%)	21.68 a	22.41 b	23.13 c	21.39 a	22.43 b	22.97 d	0.07	0.058	0.001	0.049
Fat (%)	3.75 a	4.31 c	3.95 ad	3.38 b	4.28 c	4.01 d	0.21	0.053	0.049	0.034
Water (%)	68.16 a	65.98 b	65.66 b	68.94 a	66.89 b	66.39 b	1.33	0.048	0.002	0.046
Total collagen (mg g^{-1})	4.21 a	4.51 b	4.36 a	4.52 b	4.35 a	4.66 b	0.16	0.044	0.257	0.035
Water soluble collagen (mg g^{-1})	1.23 a	1.52 b	1.57 b	1.39 c	1.50 b	1.71 d	0.02	0.000	0.004	0.019
Water soluble collagen (% of total collagen)	29.22 a	33.70 b	36.00 c	30.75 d	34.48 b	36.69 c	0.78	0.048	0.027	0.042
Tenderness (N cm^{-2})	58.4 a	52.5 c	51.4 d	54.3 b	51.0 d	50.3 e	0.41	0.007	0.003	0.034
WHC-CM (%)	20.0 a	21.9 b	22.9 c	20.3 a	22.7 bc	23.4 d	0.08	0.010	0.016	0.025
Thermal drip (%)	33.1 a	37.4 b	35.9 bc	32.8 a	36.8 b	34.4 ac	2.13	0.009	0.001	0.044

LSM – least square means value; SEM – standard error of the mean; a, b, c, d – means in the row with different letters differ significantly (Tukey HSD, P<0.05); LGI – low growth intensity (≤900 g/day); HGI – high growth intensity (>900 g/day); WHC-CM – Water Holding Capacity – Centrifuge Method.

The use of heat treatment using water vapor with a higher temperature and different pressure values affected the content of protein and water. In comparison with meat heated at 75°C, other samples contained more protein and less water (P<0.05). The analysis of fat content in meat showed the highest content of this component after the application of a temperature of 150°C and a water vapor pressure of 0.1 MPa in both growth intensity categories. The most beneficial content of water soluble collagen (P<0.05) was obtained at a temperature of 150°C and a pressure amounting to 0.2 MPa, and it should be emphasized that, in this case, the effect of growth intensity was of less importance. Further analysis of the data showed that the use of water vapor with increased pressure promoted obtaining more favorable meat tenderness (lower values in comparison with control samples) in both GI groups. The effect of the kind of treatment on meat tenderness was probably connected with obtaining a better collagen solubility, which was also confirmed by the values of the correlation coefficient (Table 3).

Table 3. Coefficients of Pearson's correlation of collagen content with the physical properties of the *longissimus lumborum* muscle

Trait	Type of collagen / Cooking parameter [temperature (°C) and pressure (MPa)]					
	Total collagen (mg g^{-1})			Water soluble collagen (% of total collagen)		
	75°C (control)	150°C 0.1 MPa	150°C 0.2 MPa	75°C (control)	150°C 0.1 MPa	150°C 0.2 MPa
(n)	25	23	25	22	23	23
Tenderness (N cm^{-2})	0.421*	0.269	0.511*	–0.260	–0.535*	–0.872*
WHC-CM (%)	–0.134	–0.363*	–0.318	0.311	0.716*	0.756*
Thermal drip (%)	0.478*	0.481*	0.708*	–0.366*	–0.475*	–0.541*

*P<0.05; WHC-CM – Water Holding Capacity – Centrifuge Method.

The use of water vapor with increased pressure resulted in obtaining significantly higher values of WHC-CM in both group LGI and HGI. This trait of meat appeared to be strongly connected with an increase in the amount of water-soluble collagen. This was confirmed by relatively high values of the correlation coefficient between these traits. The results concerning thermal drip indicate that increasing the temperature up to 150°C with a pressure of 0.1 MPa worsened this trait in the meat of bulls from both group LGI and HGI. Meanwhile, the application of treatment at a temperature of 150°C with a pressure of 0.2 MPa resulted in an improvement of this parameter. The values of the correlation coefficient showed a strong relationship between thermal drip and the amount of total collagen.

Discussion

A higher protein and fat content in the raw meat of bulls with a low growth rate in the present experiment partially coincides with the results reported by Silva et al.

(2010) concerning *m. longissimus dorsi*. In this study, an increase in the protein content was observed in bulls with a low growth rate, but the content of intramuscular fat significantly increased in bulls fed intensively. In the study by Vestergaard et al. (2000), it was indicated that the meat of Friesian bulls fed on pasture did not differ in the content of total collagen, whereas it had significantly less fat. This study confirms our results about the effect of growth intensity on the total content of collagen. Similar results were obtained by Thenard et al. (2006), who did not confirm a significant effect of feeding intensity on the content of this parameter. In the present experiment, there were observed the growth rate-dependent differences in the proportion of water-soluble collagen. Vestergaard et al. (2000) reported that the effect of feeding intensity on the proportion of soluble collagen in relation to its total amount was limited and more dependent on the kind of muscle. The authors noted significant differences concerning these parameters between the MLD (*musculus longissimus dorsi*), MST (*musculus semitendinosus*) and MSU (*musculus supraspinatus*). However, the results of this study are not fully consistent with the results obtained by Renand et al. (2001) and Purslow (2005). This may suggest that the percentage of soluble collagen is mostly related to an earlier obtainment of the slaughter weight. This effect is possibly due to higher daily gains, which is also indicated by the results of the presented study. The tendency to increase the content of fat in thermally treated MLL observed in the present experiment seems to be beneficial, because fat has a positive effect on meat juiciness. At the same time, it is notable that the proportion of this component should not be large enough to worsen nutritional qualities of meat. From the point of view of nutritional values related to the improvement of collagen water solubility, in our study, the application of water vapor at a temperature of 150°C operating under increased pressure for meat treatment should be regarded as beneficial. Zgur et al. (2003) showed that the thermal stability of collagen was primarily associated with the possibility of obtaining higher daily gains during fattening. The content of total collagen in the muscles of Limousin cattle (4.16 mg/g) indicated by Monson et al. (2004) appeared to be similar to that obtained in the presented study. However, these authors obtained a markedly higher value for soluble collagen (41.87% of the total collagen). This tendency is also confirmed by Stolowski et al. (2006). The authors of that study also proved that the muscle MLD, in relation to MST, was characterized by a smaller proportion of connective tissue and its lower temperature stability, and consequently, also a higher content of soluble collagen. The study of Archile-Contreras et al. (2010) concerning *m. longissimus dorsi*, showed the relationships between the intensity of growth and the proportion of total collagen (r= from 0.266 to –0.406), as well as between growth intensity and soluble collagen (r= 0.499 to –0.494). In the study conducted by Bowers et al. (2012), the authors observed a worsening of meat tenderness already in the range of temperatures from 71.7°C to 76.7°C. Powell et al. (2000) and Obuz et al. (2004), in turn, claimed that the use of dry-heat cooking results in a serious worsening of tenderness in muscles with a higher proportion of connective tissue, and thus collagen (*m. semitendinosus*) than in muscles containing less of this component (*m. longissimus lumborum*). Bowers et al. (2012) subjecting *m. longissimus lumborum* to roasting in a water vapor environment showed a smaller effect of the applied method on the tenderness of this muscle than in *m. biceps femo-*

ris and *m. pectoralis*. In this way, the authors confirmed that cooking has a significant effect on tenderness when muscles have much connective tissue. Kolle et al. (2004) indicated that *m. rectus femoris* and *m. semitendinosus* were characterized by a more favorable tenderness when cooked with moist heat than in dry heat. It is supposed that the use of increased pressure in the thermal treatment of meat may induce enzymatic mechanisms improving its tenderness (Sikes et al., 2010). The dissolution of collagen in water is possible at higher temperatures, however, such temperatures inactivate enzyme functions, and therefore looking for different methods for increasing collagen solubility during the preparation of meat for consumption is still a very topical field of study. The results of the studies by Ma and Ledward (2004) indicate that, in a dry heat environment, collagen has been denatured only at a pressure of 200 MPa and temperature about 60–70°C, which allowed for obtaining a more favorable tenderness of beef. The results of that study (Ma and Ledward, 2004) are consistent with those obtained earlier by Galazka et al. (2000) who showed that in the range of temperatures of about 60°C, only the application of pressure above 400 MPa resulted in the degradation of the structures of collagen fibers. The issues concerning the effect of treatment temperature on the performance of raw material subjected to cooking have been the object of scientific research for many years and a tendency to increase the weight loss of cooked meat with increasing time and temperature was observed (Bowers et al., 2012). This tendency was also proved in our study. The results of the presented experiment also show that a decrease in thermal drip is possible by reducing the shrinkage of collagen fibers during cooking, which was obtained by increasing the pressure of water vapor from 0.1 to 0.2 MPa.

Based on the results obtained in the presented experiment, it may be concluded that in respect of cooking parameters of beef, which are determined by, among other things, collagen quality, low pressure combined with high-temperature values during meat heat treatment enables the improvement of its tenderness and created more favorable values of water holding capacity. And because the tenderness and water holding capacity are some of the basic meat quality characteristics essential for both the consumer and the processing industry, to fully confirm a beneficial effect of the simultaneous action of pressure and heat on meat quality traits, it is necessary to conduct further research in this direction.

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