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Influence of chosen pre- and post-slaughter factors on heme iron content in selected beef muscles of crossbred bulls and steers

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ABSTRACT

The experiment aimed to assess the effect of selected factors on the content of heme iron (Fe$^{3+}$) in beef muscle. The research material included selected beef muscles acquired from steers and bulls obtained by crossing dairy cows with meat-bred bulls (Limousin, Charolais, Hereford). Analysis of the preslaughter factors showed a significant effect of paternal breed, slaughter age, diet, and hormonal status of animals on the content of Fe$^{3+}$ ($\alpha = 0.05$). Significantly more Fe$^{3+}$ was observed in longissimus lumborum derived from older animals. Regarding paternal breed, higher Fe$^{3+}$ content was observed in the glutes medius muscle obtained from Herefords, compared with Charolais and Limousin. Furthermore, meat obtained from bulls and semi-intensively fed animals contained more Fe$^{3+}$ compared with meat obtained from steers and intensively fed animals. In the preparation process, Fe$^{3+}$ content significantly decreased during meat aging, meat grilling also caused a loss of Fe$^{3+}$ by approximately 19% on average.

1. Introduction

The consumption of meat products is a good opportunity to increase the content of heme iron in the diet (Purchas, Rutherfurd, Pearce, Vather, & Wilkinson, 2004). Of the most commonly consumed meat types, beef contains the highest amount of heme iron in the total Fe pool. A portion of beef (150 g), recognized as skeletal beef muscles, covers 21% of the heme Recommended Daily Allowance, on average (Regulation of Ministry of Health, 2010). Iron is a necessary ingredient for the proper functioning of all living organisms, as it participates in many metabolic processes such as oxygen transport, DNA synthesis, and electron transport (Lieu, Heitskala, Peterson, & Yang, 2001). The most characteristic symptom of Fe deficiency is the occurrence of anemia. Iron deficiency occurs in approximately 30% of humans (Geissler & Singh, 2011; Lynch, 2011; Umbreit, 2005). Iron deficiency can affect many components of the human physiology and function. Iron is involved in the synthesis of neurotransmitters like serotonin, dopamine, and noradrenaline. Accordingly, insufficient levels of iron in the body lead to lower synthesis of these compounds. Studies conducted on rats suggest that iron deficiency in early life can affect central nervous system development (metabolism and neurotransmission in the basal ganglion and hippocampus), have a negative impact on the process of myelination of white matter, and additional consequences can be reflected in central nervous system functioning in adults (Beard, 2003, 2008; Lozoff & Georgieff, 2006).
Diversity of meat quality is determined by the animal’s breed, environmental conditions, method of feeding, physical activity, age at slaughter, sex, physiological state, castrating, and many other unidentified factors (Lawrie, 2006; Nowak, Palka, & Troy, 2005; Pisula & Pospišil, 2011). The influence of factors other than the diet of the animal (such as genetics and breeding) on the content of iron in meat is also unexplained. In contemporary literature there is no information about the losses of the most valuable nutrients in beef as a result of culinary processes (Driskell et al., 2011; Florek, Litwińczuk, Kędzierska-Matsysek, Grodzicki, & Śkałecki, 2007; Gerber, Scheeder, & Wenk, 2009; Leheska et al., 2008; Leonhardt & Wenk, 1997; Lombardi-Boccia, Lanzi, & Aguzzo, 2005; Reykdal, Rabieh, Steingrimsdottir, & Gunnlaugsdottir, 2011). However, there is a very high diversity of published data related to the nutritional value of beef due to differences in the meat researched, the genotype of animals, the feeding method, the content of various nutrients in the environment, various techniques of slaughtering, and post-slaughter processing, sampling, and from the selection of analytical techniques (Kerry & Ledward, 2009; Lawrie, 2006; Pisula & Pospišil, 2011).

Among the various breeds of cattle directed to artificial fattening, only meat cattle and its hybrids obtain satisfactory production results and culinary grade beef, which meet the consumers’ requirements. Increasing the quantity of beef produced and improving the quality can be achieved by crossing commodity dairy cows with meat-bred bulls. The majority of available literature data are related to meat from meat-bred animals, while little data are related to meat obtained from animals derived by crossing commodity dairy cows with meat-bred bulls. This is surprising as the meat obtained from these animals is a substantial part of the consumed meat in many countries.

The aim of this study was to evaluate the effect of selected factors, such as muscle anatomy, paternal breed, diet, slaughter age, castrating, and the processes of aging and grilling of meat on the heme iron content in beef.

2. Materials and methods

2.1. Animals, samples, and experimental designs

The research material included selected beef muscles acquired from steers and bulls obtained by crossing Polish Holstein-Friesian cows with meat-bred bulls (Limousin, Charolais, Hereford). A detailed study arrangement is shown in Figure 1. The animals were raised on the same farm and were fed according to the guidelines for intensive and semi-intensive fattening under strictly controlled conditions. In the intensive and semi-intensive fattening regimen, the animals were fed ad libitum with grass silage and the addition of a mixture of concentrate (post-extraction rapeseed meal, middlings triticale, and mineral supplement). The amount of concentrate in each dose was calculated based on the energy density of the dose recommended in the system of evaluation and nutrition INRA (Institut national de la recherche agronomique [French National Institute for Agricultural Research]) (Dobrowolska, 1993), according to the models provided for animals of beef breeds or animals obtained by crossing. The intake of concentrate depended on the level of fattening desired – animals fed intensively received a greater quantity of the concentrate compared with animals fed semi-intensively. In the semi-intensive fattening system animals were slaughtered at the age of

Figure 1. Schematic diagram of the research.

Figura 1. Diagrama esquemático de la investigación.
18 months, when their mass reached 550 kg (average daily gain (ADG) of animals: 1000 g/day). In the intensive fattening system animals were slaughtered at the age of 18 months, when their mass reached 600 kg (ADG of animals: 1300 g/day). The composition of the initial mix intended for intensively fed bulls weighing from 250 to 450 kg was as follows: 585 g/kg triticate, 390 g/kg rapeseed extraction meal, and 25 g/kg mineral-vitamin premix. In the case of semi-intensively fed animals in the aforementioned weight range, the mixture consisted of 315 g/kg triticate, 660 g/kg rapeseed extraction meal, and 25 g/kg mineral–vitamin premix. The mixture intended for intensively fed bulls weighing more than 450 kg consisted of 749 g/kg triticate, 226 g/kg rapeseed extraction meal, and 25 g/kg mineral–vitamin premix. Lastly, for semi-intensively fed animals weighing more than 450 kg this mixture consisted of 645 g/kg triticate, 330 g/kg rapeseed extraction meal, and 25 g/kg mineral–vitamin premix. The mineral–vitamin premix contained in 1 kg: Fe – 500 g, Ca – 235 g, Na – 79 g, Mg – 28 g, P – 48 g, Mn – 2000 mg, Zn – 3750 mg, Cu – 375 mg, Co – 12.5 mg, I – 50 mg, Se – 12.50 mg, vitamins: A – 250,050 IU, D3 – 50,000 IU, DL-alpha tocopherol – 909 mg, E – 1000 mg. ADG and daily dry matter intake (DMI) were calculated for each animal. The feed conversion ratio was calculated as daily DMI divided by ADG (Nogalski et al., 2013).

The animals were transported to a local slaughterhouse. After rigor mortis (48 h, 2 ± 1°C), the muscles were removed from each carcass. The muscles were then subjected to a ‘wet’ aging process, through vacuum packaging into barrier polyethylene bags and refrigerated storage (2 ± 1°C) for 7, 14, and 21 days. After each aging period, samples were frozen (−22 ± 1°C) using a Küppersbusch ‘blast-freezer’ and stored at −18°C until analysis. The thawing process was conducted at 2 ± 1°C for 24 h. After the defrosting process, the muscles were removed from their packages and 2.54-cm thick steaks were sliced. Anatomical muscles, which were wholly ground in order to standardize the research sample, were prepared from the individual carcasses.

The aging process was performed (after vacuum packing the beef muscles) at 2 ± 1°C at three time points after slaughter: 7, 14, and 21 days. In order to carry out a controlled grilling process, the beef muscles were cut into steaks with a thickness of 2.5 cm after 14 days of aging. The steaks were then heat-treated using an electric grill with a grooved top cover (S-165 K ELEKTROGERÄTE GmbH 59757 Arnsberg, Germany) having a temperature of 190°C at the top and 210°C at the bottom. The process was conducted until the temperature at the geometric center of the product reached 70°C (thermocouple thermometers: NiCr–NiAl, type TP-151 with EMT-50 K CZAKI, THERMO PRODUCT, Poland). Then, the steaks were subjected to a 6-min relaxation process at 60°C.

### 2.2. Determination of heme iron content

The content of heme iron (Fe\(^{III}\)) in beef was determined using the colorimetric method as described by Hornsey (1956), with some modifications proposed by Lombardi-Boccia, Martinez-Dominguez, Aguzzi, and Rincón-león (2002). The frozen sample of beef was sliced into cubes (side length: 2–3 mm) and a weight of 3 ± 0.1 g was added into a 50-ml PP test tube. A volume of 15 ml of an extraction mixture consisting of 80% ACN (acetonitrile) (p.a.): 3% HCl (36%, p.a.): 17% H\(_2\)O (volume ratio), was added to the test tube. The meat was homogenized (duration: 2 min; mandrel diameter: 1.5 cm, speed: 8000 rpm), the mixture stem rinsed (2 × 1.5 ml), and then the sample was left for 2 h in a dark place (ambient temperature approx. 22°C). The mixture was then centrifuged (8944 × g for 15 min, temp. 4°C; centrifuge MPW-380R, Med. Instruments, Warsaw, Poland), and the supernatant was subsequently transferred into a 25 ml volumetric flask. The sample was mixed again and the solution was filtered using a filter paper. The absorbance was determined using a spectrophotometer (Helios Gamma, Thermo Scientific, Anch.comp., Warsaw, Poland) at λ = 640 nm; prior to the measurement, the spectrophotometer was zero-adjusted on the extraction mixture. Quantification of the absorbance was performed using a six-point standard curve, and the standard was hemin (H9039 SIGMA) solution in the extraction mixture. The content of Fe\(^{III}\) was calculated based on the chemical composition. The results are reported as milligrams (mg) of Fe\(^{III}\) per kilograms (kg) of raw muscle/grilled muscle/dry matter. The measurement of the Fe\(^{III}\) content for a sample of beef was calculated as arithmetic average of six independent measurements. The procedure to determine the ferrous iron content in beef included parameters such as range of linearity and repeatability. As a standard, the linear relation for hemin, based on the Fe\(^{III}\) determined in the concentration range of 0.52–10.48 μg/ml (r\(^2\) > 0.999) was 0.73–2.87 μg/ml. The experimental repeatability, expressed by the relative standard deviation did not exceed 5.5%.

### 2.3. Statistical analysis of the results

Statistical analysis of the results was performed using either STATISTICA 10 software (Experiment 2) or STATGRAPHISC Plus 5.1 software (Experiments 1 and 3). To assess the effect of factors such as paternal breed, diet, slaughter age, and castrating on the content of Fe\(^{III}\) in beef, a Student’s t-test and factorial analysis of variance was employed in a completely random design. To assess the effect of factors such as muscle anatomy and the processes of meat aging and grilling on the Fe\(^{IV}\) content, a factorial analysis of variance was employed in a completely randomized block design to eliminate the impact of the uncontrolled factor of the individual variability of the animals. To study the differences between groups, a Duncan test (α = 0.05) was used.

Lastly, to determine what factors which are most significantly differentiating the content of Fe\(^{III}\) in bovine meat, classification and regression tree models were used.

### 3. Results

#### 3.1. Experiment 1 – influence of muscle type

Heme iron content varied in the selected anatomic muscles (Table 1), with the lowest content of heme iron found in the semitendinosus and the highest in the longissimus lumborum. There were six homogeneous groups, where statistically significant differences in the iron content were found (p < 0.05).

Table 1 shows also the Fe\(^{III}\) content calculated on the dry matter of muscles. A similar differentiation was measured as in the case of fresh muscle content.

#### 3.2. Experiment 2 – influence of paternal breed, castration, fattening method, and slaughter age

Table 2 shows the content of heme iron (in raw muscle, as well as calculated on dry matter) in two selected muscles
Table 1. The content of Fe\textsuperscript{II} in various raw beef muscles.

| Muscles                      | Fe\textsuperscript{II} content X\textsubscript{av.} (SEM) |
|------------------------------|---------------------------------------------------------|
|                             | mg/kg of raw muscle | mg/100 g of dry matter |
| Longissimus thoracis         | 25.6\textsuperscript{ab} (1.2) | 99.4\textsuperscript{ab} (4.0) |
| Biceps femoris               | 23.7\textsuperscript{abc} (0.3) | 99.5\textsuperscript{ab} (4.3) |
| Tensor fasciae latae         | 25.2\textsuperscript{ab} (0.8) | 105.5\textsuperscript{a} (5.5) |
| Gluteus medius               | 25.7\textsuperscript{ab} (1.3) | 101.7\textsuperscript{ab} (6.5) |
| Semimembranosus              | 15.7\textsuperscript{b} (2.6) | 66.0\textsuperscript{b} (11.2) |
| Psoas major                  | 22.6\textsuperscript{ab} (0.1) | 95.7\textsuperscript{ab} (2.1) |
| Longissimus lumborum         | 27.9\textsuperscript{ab} (0.4) | 106.6\textsuperscript{ab} (2.0) |
| Pectineus                    | 17.6\textsuperscript{b} (1.7) | 75.5\textsuperscript{b} (7.7) |
| Sartorius                    | 20.6\textsuperscript{ab} (1.4) | 91.2\textsuperscript{ab} (7.1) |
| Semimembranosus              | 26.1\textsuperscript{ab} (1.1) | 100.2\textsuperscript{ab} (4.0) |
| Adductor femoris             | 25.0\textsuperscript{ab} (1.9) | 102.0\textsuperscript{ab} (7.4) |
| Gracilis                     | 19.2\textsuperscript{ab} (1.4) | 83.8\textsuperscript{ab} (6.0) |
| **Average content in muscles** | **22.9 (0.5)** | **93.9 (2.0)** |

X\textsubscript{av} – average content; SEM – standard error; a–f – mean values in columns indicated with different letters differed significantly (a = 0.05).

The content of heme iron present in the tested muscles after aging and the grilling processes is shown in Table 3.

3.3. Experiment 3 – influence of aging and grilling processes

The content of heme iron present in the tested muscles after aging and the grilling processes is shown in Table 3.

With the longer aging period, the content of Fe\textsuperscript{II} in muscles decreased significantly on average. However, when taking into account the average dry matter within muscles, this difference is not significant, which can be explained by the presence of natural leakage averaging 4%. As expected, the content of Fe\textsuperscript{II} in muscles also changed after the grilling process. Meat following culinary preparation was characterized by a higher iron content compared with its content in raw muscle (Table 3). This is associated with significant thermal leakage (30% on average), and consequently with the efficacy of the grilling process, which was observed to range from about 64% to 81% depending on the anatomical type of muscle. Taking into account dry matter we can conclude that the grilling process causes a significant loss of Fe\textsuperscript{II} in meat, averaging a loss of 19%.

In this study, we performed a statistical analysis involving the construction of classification and regression trees, including ranking the factors affecting the content of heme iron in order of importance. The analysis created on the basis of the content of heme iron, depending on pre- and post-slaughter factors is presented in Figure 2. A key factor differentiating the content of Fe\textsuperscript{II} in the tested muscles was type of muscle, heat treatment (grilling), and the aging process of the meat. Based on the classification tree analysis, the highest content of Fe\textsuperscript{II} was observed in the longissimus lumborum/thoracis and semimembranosus muscle. In addition, a significantly high content of Fe\textsuperscript{II} was observed in the muscles (gluteus medius, longissimus lumborum) obtained from animals of paternal Hereford breed.

4. Discussion

Red meat is a good source of iron in the diet, although there are other products that can provide a significant amount of this ingredient, e.g.: fish, grains, beans, nuts, eggs, dark green vegetables, and fortified foods (European Food Safety Authority [EFSA], 2015). From the point of view of nutrition it should be remembered however, that a high intake of red meat and products of its processing (the...
main source of heme) is directly associated with excessive iron levels in the body that contribute to the development of diseases such as cancer, diabetes, cardiovascular disease (Bao, Rong, Rong, & Liu, 2012; Czerwonka & Tokarz, 2017). It is therefore important to know the conditions of the content of this ingredient in the meat, which was the aim of this work.

The research indicates that the content of heme iron in bovine meat may also be conditioned by other pre- and post-slaughter factors such as slaughter age, paternal breed of animals, and the degree of meat aging (Patten et al., 2008; Purchas et al., 2004; Ramos, Cabrera, & Saadoun, 2012). Lawrie (2006) reports that the heme iron content of the meat is affected primarily by factors, which occur mainly at the stage of breeding.

Other studies also show that diverse concentrations and chemical form of the main heme pigment (myoglobin) influence the differences in the amount of heme iron in muscles (Gurzau, Neagu, & Gurzau, 2003; Kolczak, 2008; Kukowski, Maddock, & Wulf, 2004; Lawrie, 2006).

Table 3. The content of Fe\(^{H}\) in the tested muscles after being subjected to aging and grilling process.

| Aging period (days); grilling process/muscles | Gluteus medius | Semitendinosus | Longissimus thoracis | Psoas major | Semimembranosus | Longissimus lumbrorum | Average within muscles |
|---------------------------------------------|----------------|---------------|----------------------|-------------|-----------------|-----------------------|------------------------|
| mg Fe\(^{H}\)/kg raw                        |                |               |                      |             |                 |                       |                        |
| 7                                           | 21.6\(^{a}\)   | 22.1\(^{a}\)  | 25.9\(^{a}\)         | 25.3\(^{a}\) | 26.0\(^{a}\)    | 28.2\(^{a}\)           | 27.2\(^{a}\)           |
| 14                                          | 25.7\(^{a}\)   | 15.7\(^{b}\)  | 26.6\(^{b}\)         | 26.1\(^{a}\) | 27.9\(^{a}\)    | 29.8\(^{b}\)           | 23.9\(^{a}\)           |
| 21                                          | 25.5\(^{a}\)   | 15.0\(^{b}\)  | 18.6\(^{b}\)         | 22.9\(^{b}\) | 21.3\(^{b}\)    | 27.0\(^{a}\)           | 21.7\(^{b}\)           |
| mg Fe\(^{H}\)/kg dry                        |                |               |                      |             |                 |                       |                        |
| 7                                           | 84.4\(^{a}\)   | 92.7\(^{a}\)  | 93.4\(^{a}\)         | 95.7\(^{a}\) | 95.7\(^{a}\)    | 112.0\(^{a}\)          | 99.0\(^{a}\)           |
| 14                                          | 101.7\(^{a}\)  | 66.0\(^{b}\)  | 99.4\(^{b}\)         | 95.7\(^{b}\) | 100.2\(^{b}\)   | 106.6\(^{b}\)          | 94.9\(^{b}\)           |
| 21                                          | 104.1\(^{a}\)  | 61.6\(^{b}\)  | 93.1\(^{b}\)         | 102.5\(^{b}\) | 86.5\(^{b}\)    | 108.2\(^{a}\)          | 88.3\(^{b}\)           |
| mg Fe\(^{H}\)/kg raw                        |                |               |                      |             |                 |                       |                        |
| raw                                         | 2.57\(^{a}\)   | 1.57\(^{b}\)  | 2.56\(^{b}\)         | 2.26\(^{b}\) | 3.00\(^{b}\)    | 2.79\(^{a}\)           | 2.79\(^{b}\)           |
| Grilled                                     | 2.50\(^{a}\)   | 2.04\(^{b}\)  | 2.91\(^{a}\)         | 2.79\(^{b}\) | 3.00\(^{b}\)    | 3.21\(^{a}\)           | 2.74\(^{b}\)           |
| mg Fe\(^{H}\)/kg dry                        |                |               |                      |             |                 |                       |                        |
| Raw                                         | 101.7\(^{a}\)  | 66.0\(^{b}\)  | 99.4\(^{b}\)         | 95.7\(^{b}\) | 100.2\(^{b}\)   | 106.6\(^{b}\)          | 94.9\(^{b}\)           |
| Grilled                                     | 74.6\(^{a}\)   | 65.0\(^{b}\)  | 84.1\(^{b}\)         | 95.8\(^{b}\) | 98.5\(^{b}\)    | 85.5\(^{b}\)           | 85.5\(^{b}\)           |

X\(^{av}\) – average content; SEM – standard error; *p < 0.05; **p < 0.01; a–b – mean values in columns within each muscle indicated with different letters differed significantly (α = 0.05).

Figura 2. Árbol de clasificación y regresión, creado con base en el contenido de hierro hem, dependiente de factores seleccionados antes y después del sacrificio; Av. = Promedio [mg Fe\(^{H}\)/kg carne cruda].
A high iron level is related to the higher content of myoglobin in this type of muscle and the need to secure the aerobic metabolism. Furthermore, different meat cuts have different contents of connective tissue and fat, which also largely determines the content of iron (Chikuni et al., 2010; Czerwonka & Tokarz, 2017; Ramos et al., 2012).

Heme iron represents a significant share of the total iron in red meat. The ratio of heme form to total content of the element ranges between 40% and 90%, but the most common value is about 70%, higher for beef than pork (Lombardi-Boccia, Martinez-Dominguez, & Aguzzi, 2002; López-Alonso et al., 2004; Ramos et al., 2012; U.S. Department of Agriculture [USDA], 2015).

Heme iron content within the limits 16–28 mg/kg of raw muscle coincide with the content of the component described in literature (Cabrera, Ramos, Saadoun, & Brito, 2010; Driskell et al., 2011; Gerber et al., 2009; Ramos et al., 2012).

In this study, the semitendinosus muscle, which is characterized by high intravital activity (intense working), contained low amounts of heme iron as compared with other muscles. Other studies also show that diverse concentrations and the chemical form of myoglobin, the main heme pigment, influence differences in the amount of heme iron in muscles. In addition, myoglobin levels are affected by the animal’s physical activity (Gurzau et al., 2003; Kolczak, 2008; Kukowski et al., 2004; Lawrie, 2006).

Patten et al. (2008) studied the amount of heme iron in different anatomical parts of beef carcass (cows and young cattle). The average amount of heme iron present was relatively high, especially in the muscles of cows (30 mg/kg of raw muscle), and the differences between elements were more noticeable than those obtained in the present work.

The highest content of FeIV was observed in the longissimus lumborum muscle and it strongly varied from the semitendinosus muscle and selected topside muscles.

In other previous experiments, Ramos et al. (2012) estimated the Fe content in three muscles (psoas major, longissimus lumborum, glutaeus medius), which were obtained from Hereford and Braford animals. The content of FeIV measured in meat was higher than the one observed in the current work (an average of 29 mg FeIV/kg of raw muscle). Favorably, Lombardi-Boccia, Martinez-Dominguez, and Aguzzi (2002) observed a similar FeIV amount (average 21.4 mg/kg of raw muscle) to that measured in the current study (an average of 22.9 mg/kg of raw muscle).

Anatomical differentiation between beef muscles is demonstrated in a series of publications (Daley, Abbott, Doyle, Nader, & Larson, 2010; Driskell et al., 2011; Dugan, Aldai, Aalhus, Rolland, & Kramer, 2011; Florek et al., 2007; Gerber et al., 2009; Leheska et al., 2008; Lombardi-Boccia et al., 2005; Raes et al., 2004; Reykdal et al., 2011). Also, other researchers have shown that differences in the content of heme iron in individual muscles result from variations in concentration and chemical form of myoglobin, which is the main heme pigment. Its levels in skeletal muscles are affected by physical activity of animals during their lifetime (Kolczak, 2008; Lawrie, 2006).

Beef is the best source of heme iron among the most popular types of meat in Poland, and the content of FeIV is most differentiated by the type of tissue and muscle. Furthermore, the current research indicates that the content of heme iron in bovine meat may also be conditioned by other pre- and post-slaughter factors such as slaughter age, paternal breed of animals, and the degree of meat aging (Patten et al., 2008; Purchas et al., 2004; Ramos et al., 2012).

The quality of beef derived from animals of different breeds has been the subject of many previous studies by various authors. Racial differences affect metabolism, which thereby results in a differentiated muscle demand for specific nutrients and a different degree of their utilization (Lawrie, 2006). Even subtle differences in genotype can cause the raw material derived from two individuals to never have the same chemical composition (Kerry & Ledward, 2009).

After analyzing the impact of paternal breed on the content of heme iron in meat, in view of available literature, it was found that this problem has not been sufficiently or clearly explained. Often different data are quite controversial, and sometimes even contradictory. Two scientific experiments described in Ramos et al. (2012) and Cabrera et al. (2010) analyzed the contents of minerals (including iron) in anatomical parts of carcass beef derived from Hereford and Braford animals. The Ramos et al. (2012) study showed differences in iron content between the meat derived from animals of the two breeds. In contrast, Cabrera et al. (2010) did not observe any differences between the content of this compound in meat derived from both breeds.

In the current study, differences in the content of heme iron in meat obtained from animals of different paternal breeds were observed only in the case of the glutaeus medius muscle. This tendency did not occur for the longissimus lumborum muscle.

Another factor that may have an impact on the nutritional value of beef is gender, often cited as the hormonal status of the animal. Steer meat is regarded as more valuable in terms of palatability and sold at a higher price on specialty markets and in restaurants (Nogalski et al., 2013). On a national level, the castration of bulls is not a common practice among cattle producers. This is due to higher financial outlays that breeders would be obliged to incur in connection with breeding steers. However it is expected that potential consumers would pay a higher price for higher beef quality, as was concluded in parallel studies conducted as part of the ProOptiBeef Project, under which this work was also carried out.

Many previous publications have studied the effect of bull castration on the quality of meat. However, these studies mainly concerned animals of pure meat breeds and not those from crossbreeds. In this paper, we analyzed the meat obtained from bulls and steers born out of crossing female dairy cattle with meat Charolaise males that were bred intensively under strictly controlled conditions. The results indicate that meat obtained from bulls contained more FeIV than the meat obtained from steers.

The content of heme iron can also be determined by the gender of an animal, however these data are ambiguous. López-Alonso et al. (2004) observed a higher Fe content in heifer’s meat, compared with bull’s meat. On the other hand, Florek et al. (2007) showed an inverse relationship: the iron content was higher in muscles (longissimus thoracis) derived from bulls, compared with heifers.

Available data in the published literature regarding the possibilities of increasing the amount of iron in bovine meat by changing animal feeding are poor and quite divergent.
According to some authors animal diet has little impact on the content of iron in beef muscles. Leheska et al. (2008) studied the effect of feeding and intensive grazing (based on concentrates) on the amount of selected components (including iron) in bovine meat (minced meat and steak tenderloin), and Lozicki, Dymicka, Arkuszewska, and Pustkowiak (2012) published research on the nutritional value of the longissimus thoracis muscle derived from Herefords fattened on pastures or using corn silage. Both researchers found that the iron content was significantly higher in meat obtained from animals fed on pastures.

Currently, no data exists describing the impact of feeding cattle on the resulting heme iron content in bovine meat. In this study, we observed a significant effect of the employed feeding system on the resulting content of heme iron in beef, with higher values recorded in the case of meat from animals fed in a semi-intensive system.

Another factor influencing the nutrient content in meat is the age of the animal during the slaughter. The data found in available literature suggests that meat from older animals is characterized by a higher content of heme iron (Wadhwani, Comforth, Murdia, & Whittier, 2011). Despite mechanisms controlling the content of Fe in the body, it is noted that the content of this element actually increased throughout the lifetime of the animal (Chambaz, Scheeder, Kreuzer, & Dufey, 2003). In our experiments, this tendency was only observed in the case of longissimus lumborum.

Post-slaughter factors include different treatments of meat after slaughter. An important factor determining the quality of the meat is the aging process, in which a series of physicochemical changes occur. The meat after slaughter gradually achieves more favorable sensory, nutritional, and technology qualities. In addition, aging of meat gradually increases the pH value, which is caused by proteolytic degradation of muscle fiber and the decomposition of proteins present in the meat (Gašperlin, Žlender, & Abram, 2001; Lawrie, 2006). In addition, the aging process has a significant impact on the amount of free leakage and hence on losses of different bioactive compounds that are soluble in water (Niedźwiedź, Zmięciński, Ostoja, & Cierach, 2011).

Beef aging causes either a slight decrease or no change of the amount of heme iron (FeH) present depending on the type of muscle, as confirmed by Ramos et al. (2012). This may result from the fact that during the process of meat aging we can observe a reduction in the rate of accumulation of the main carrier of heme iron in meat, namely metmyoglobin. During aging, there are also changes in the degree of oxygen utilization by the aging muscles (Lindahl, 2011).

Raw meat is rarely consumed. In order to obtain unique flavor attributes, improved protein digestibility, and microbiological safety, the meat is subjected to a heat treatment process. However, cooking of meat can lead to significant modifications of iron level, which depend on the type of thermal process and its parameters. Iron in meat is mainly bounded to protein structures and, thus, cooking treatment such as roasting, frying, and grilling cause element condensation in the final product, as a result of thermal leakage. The iron content in 100 g of ready-to-eat product is 10–40% higher than in 100 g of raw material (Czerwonka & Szterk, 2015; Lombardi-Boccia et al., 2005; USDA, 2015). Simultaneously, the share of heme iron during thermal process is reduced by about 4–25% (Czerwonka & Szterk, 2015; Lombardi-Boccia, Martinez-Dominguez, Aguzzi, & Rinco n-León, 2002; Purchas & Busboom, 2005).

Lombardi-Boccia, Martinez-Dominguez, Aguzzi, and Rinco n-León (2002), Lombardi-Boccia, Martinez-Dominguez, and Aguzzi (2002) found that the technological process such as grilling causes significant losses in heme iron content in bovine meat (García-Vaquero, Miranda, Benedetto, Blancone, Penedo, & López-Alonso, 2011; Lombardi-Boccia, Martínez-Dominguez, & Aguzzi, 2002). This tendency was also demonstrated in this study. Losses of heme iron are mainly due to cooking loss occurred during grilling, which in this study was about 30%. Heme iron is soluble in water and therefore it is wasted with the cooking loss.

According to Schönfeldt and Hall (2011), the average bioavailability of heme iron in meat is 23%, and nonheme is 3%. Assuming an average content form of heme in the overall iron concentration is 70% (Czerwonka & Szterk, 2015; Lombardi-Boccia, Martinez-Dominguez, Aguzzi, & Rinco n-León, 2002), and total iron content of the ready-to-eat portions of red meat (120 g) is about 1.8 mg, the level of absorption of iron from red meat portions will be approximately 0.3 mg.

5. Conclusions

The distribution of heme iron in the beef muscle is inconsistent and ranges from 15.7 mg FeH/kg of raw muscle in Semitendinosus muscle to 27.9 mg FeH/kg of raw muscle in the Longissimus lumborum muscle. These differences can result from different functions, as well as from the intensity of activity of the tested muscles. Pre- and post-slaughter factors have a varied impact on the heme iron content in beef muscles. The analysis of preslaughter factors showed a significant effect of the paternal breed, slaughter age, diet, and hormonal status of animals on the content of FeH measured (α = 0.05). In terms of individual anatomical muscles, significantly more heme iron was observed in the longissimus lumborum derived from older animals (18 months). Significant differences in the content of heme iron in terms of paternal breed were observed in the case of the glutaeus medius muscle. Moreover, higher iron content in the muscles obtained from animals derived from the paternal Hereford breed was also observed compared with animals derived from the paternal breeds Charolais and Limousin. Meat obtained from bulls and semi-intensive feeding animals contained more FeH compared with meat obtained from steers and intensive feeding animals. In addition, FeH content decreased during the meat aging process, the drop varied dependently of the aging time. Lastly, meat grilling caused significant losses of approximately 19% in the content of FeH.

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