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Effect of polymorphisms at the ghrelin gene locus on carcass, microstructure and physicochemical properties of *longissimus lumborum* muscle of Polish Landrace pigs

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ABSTRACT

The influence of RFLP–*Bsr*I polymorphisms at the ghrelin gene locus on carcass, meat quality parameters and muscle fiber characteristics of *longissimus lumborum* was studied in 168 barrows of the Polish Landrace breed. Analysis revealed a high frequency of the 1 allele (0.60) with the frequencies of the 11, 12 and 22 genotypes being 0.45, 0.30 and 0.25, respectively. The most favorable parameters of meat traits were characteristic of pigs with the 22 genotype, together with a higher carcass and loin weight and lower thermal loss compared to 12 heterozygotes. The highest fat content was found in pigs with the 11 genotype, which had the highest abdominal fat weight and mean backfat thickness. Meanwhile, the 12 heterozygotes were characterized by the largest loin eye areas, highest lightness (L*) and yellowness (b*) values, and lowest redness (a*) values, as well as the greatest hardness and chewiness and largest diameter of type IIB muscle fibers compared to the other genotypes.

1. Introduction

The aim of today's pork producers is high lean content paralleled by decreased fat content. Genes of the somatotropic axis are some of the many genes considered to improve this trait. They play a significant role in regulating metabolism, and some SNPs identified at the loci of these genes may be associated with the growth of animals (Amillis et al., 2003; Qiu et al., 2006), thus determining meat quality traits, deposition of fat, or muscle fiber number and size (Scheuermann, Bilgili, Tuzun, & Mulvaney, 2004). For this reason, they are eligible to be called markers for meat quality traits, understood as a complex of structural and functional processes dependent on the species, breed, genetic factors, antemortem metabolic status or environmental conditions (Lei et al., 2007). One of the somatotropic axis genes is ghrelin, which, in addition to obestatin, is a product of preproghrelin processing. Ghrelin belongs to a group of conservative factors that are characterized by strong interspecies homology of amino acid combination. In mammals, ghrelin is a 27 or 28-amino-acid octanoylated peptide hormone extracted from rat stomach (Kojima et al., 1999) as an endogenous ligand of the growth hormone secretagogue receptor (GHSR). Ghrelin is an orexigenic peptide that antagonizes leptin action through the activation of the hypothalamic neuropeptide Y/Y1 receptor pathway and plays an important role in the regulation of energy homeostasis (Shintani et al., 2001). This gastric hormone plays an important role in regulating lipid deposition in white adipose tissue, although ghrelin's effect on adipocytes is controversial because it can increase or inhibit adipogenesis in rats (Choi et al., 2003), increase fat deposition and triglyceride content and reduce lipolysis (Tsubone et al., 2005). Ghrelin and leptin have been shown to act antagonistically in regulating body homeostasis, and leptin blocks ghrelin's ability to increase appetite and vice versa (Barazzoni et al., 2003). Ghrelin blocks glucose absorption in muscles and adipose tissue and stimulates glucose production by the liver. In humans, mutations in the preproghrelin (Leu72Met) or ghrelin gene (Arg51Gln) are associated with obesity (Ukkola et al., 2001) and the incidence of metabolic syndromes (Tang et al., 2008). The ghrelin variant g.A265T showed an association with obesity, and the variant g. A604G showed an association with insulin levels in post-oral glucose tolerance tests in obese families (Gueorguiev et al., 2009).

Most studies on the relationship between *GHRS* and *GHRL* loci polymorphism and meat traits have been conducted in poultry (Fang, Nie, Luo, Hang, & Hang, 2007; Lei et al., 2007; Qiu et al., 2006) and cattle (Sherman et al., 2008; Zhang et al., 2009). However, it should be noted that unlike in mammals, ghrelin blocks feed consumption in poultry (Fang et al., 2007). In Berkshire and Yorkshire pigs, only one RFLP–*Bsr*I polymorphism was identified in the preproghrelin gene (*GHRL*), which showed an association with bone-in loin, backfat thickness and marbling of meat (Kim et al., 2004). The correlation between the growth of farm animals and variation at the locus of genes coding for proteins of the neuroendocrine axis plays a key role in identifying these processes and in understanding factors affecting meat quality.

Therefore, the purpose of the present study was to determine the effect of the RFLP–*Bsr*I polymorphism at the ghrelin gene locus on selected parameters of meat production and *m. longissimus lumborum* quality and microstructure in Polish Landrace pigs.

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2. Materials and methods

2.1. Animals

168 Polish Landrace fattening pigs (barrows) were kept in individual pens equipped with automatic drinkers and fed ad libitum. Animals were fattened from 50 to 105 kg body weight using a complete diet containing 13.3 MJ metabolizable energy and 161 g crude protein. This level was calculated based on the diet composition using tabular data for individual components (Polish Feeding Standards, 1993). All animals used in this study did not carry the g.1843C>T mutation and had the CC genotype in the ryanodine receptor 1 gene. The animals were acquired from the Agricultural Production Cooperative in Kędzierzyn-Koźle, Poland. When the pigs had attained the appropriate slaughter weight (105 kg), they were electrically stunned and exsanguinated. Rather than being transported, pigs were slaughtered at a commercial slaughterhouse belonging to the Agricultural Production Cooperative in Kędzierzyn-Koźle. Feed was withdrawn 12 h before slaughter but water was freely available in lairage. Moreover, daily gains and feed conversions in the test were determined from the 50–105 kg body weight range.

2.2. Genotyping

DNA was isolated from whole blood drawn from the animals' jugular vein by an authorized veterinarian into sterile test tubes containing K_3EDTA and stored at −25 °C until required. Isolation was carried out using a MasterPure™ Genomic DNA Purification Kit (Epicentre Technologies, USA) according to manufacturer's instructions. Polymorphisms at exon III of the ghrelin (*GHRL)* were identified using the PCR–RLFP method described by Kim et al. (2004). The volume of the reaction mixture was 25 μl and contained 1× PCR reaction buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 15 pmol each primer, 0.6 U Taq polymerase (MBI Fermentas, Lithuania) and approximately 200 ng DNA. A PCR thermal profile consisted of pre-denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 1 min, and elongation at 72 °C for 1 min and a final extension at 72 °C for 5 min using a Mastercycler Ep Grad S (Eppendorf, Germany). Amplified fragments were digested with the restriction enzyme *Bsr*I (4U, MBI Fermentas, Lithuania) at 65 °C, overnight. The digestion products were separated on 3% agarose (Agarose Type I-A, Low EEO, Sigma) gels in TBE buffer stained with ethidium bromide, visualized and scanned on a UVI-KS 400i/Image PC (Syngen Biotech, Poland).

2.3. Carcass quality measurements

The carcasses were chilled for 24 h at 5 °C and weighed. After that, the right half of each carcass was divided into pieces following the procedure described by Różycki (1996), and the weight of each piece was determined.

The weights of ham, loin, belly and abdominal fat, as well as of mean backfat thickness (with calipers) were determined from five measurements, that is, over the shoulder at the point of maximum thickness, on the back over the join between last thoracic and first lumbar vertebra and at three points over the edge of the cross-sectional area of the gluteus muscle (loin region): the rostral part (loin I), middle part (loin II) and caudal part (loin III). In addition, the contour of the cross-sectional area of the *longissimus dorsi* muscle was traced behind the last thoracic vertebra and on the rostral surface, and then the cross-sectional area (loin eye area) was determined using Multi Scan Base v.8.08 image analysis software.

2.4. Meat quality measurements

The samples for meat quality assessment were taken from the right carcass side from the *longissimus lumborum* muscle at the level of the 2nd–4th lumbar vertebrae. Muscle pH was measured using a Matthäus (Germany) pH meter with a glass electrode standardized for pH 4.0 and 7.0 according to Polish Standard PN-77/a-82058 with automatic correction for muscle temperature at 45 min (pH_{45}) and 24 h (pH_{24}) *postmortem*. The meat color was assessed 24 h *postmortem* by the L* (lightness), a* (redness) and b* (yellowness) system (CIE 1976) using a Minolta colorimeter (Chroma Meter CR-310, Minolta Camera C, Osaka, Japan). Water holding capacity (WHC) was determined as the amount of free water according to the filter paper press method of Grau and Hamm (1952) 24 h *postmortem*. The sample values were means from three measurements. The intramuscular fat content (IMF) of the *longissimus lumborum* muscle was determined in duplicate according to the procedure recommended by AOAC No. 991.36 (AOAC, 2000).

Meat samples for Warner–Bratzler shear force (WB) and texture profile analysis (TPA) were taken after 24 h of cooling at 4 °C. Next, they were roasted at 180 °C to reach an internal temperature of 78 °C and then cooled to room temperature and weighed for thermal loss determination. Next, ten 14 mm in diameter cores were taken from each chop parallel to the muscle fiber orientation. Shear force was measured using a Texture Analyser TA-XT2 (Stable-Micro Systems, UK) with a Warner–Bratzler unit and a triangular blade. The cores for texture profile (TPA) determination were doubly compressed by a cylinder (SMS P/25, base diameter 50 mm) to 70% of their height at a rate of 2 mm/s with a 3-second break between the storage of compression. In all cases, measurement was carried out at 6 ± 1 °C. The following parameters (TPA) were defined: hardness, springiness, cohesiveness, chewiness and resilience. The TPA parameters were determined using a Texture Analyser TA-XT2 (Stable-Micro Systems, UK).

2.5. Histochemical and immunohistochemical analysis

Within 45 min *postmortem,* muscle samples for histochemical and immunohistochemical analysis were taken from the right side of the carcass from the *longissimus lumborum* muscle at the level of the 5th lumbar vertebra and deep within the muscle. Muscle samples were cut into 1 cm^3 pieces (parallel to the muscle fibers) and frozen in isopentane that was cooled using liquid nitrogen and stored at −80 °C until subsequent analyses. Samples were mounted on a cryostat chuck with a few drops of tissue-freezing medium (Tissue-Tek; Sakura Finetek Europe, Zoeterwoude, The Netherlands). Transverse sections (10-μm thick) were cut at −20 °C in a cryostat (Slee MEV, Germany). To determine the muscle histochemical composition, we used a modified combined method of NADH-tetrazolium reductase activity and immunohistochemical determination of the slow myosin heavy chain on the same section (Wojtysiak & Kaczor, 2011). First, sections were air-dried for 1 h and incubated for 1 h at 37 °C with medium for the determination of NADH-tetrazolium reductase (Dubovitz & Brooke, 1973). Next, on the same section, the immunohistochemical staining with monoclonal antibodies against the skeletal slow myosin heavy chain was performed for 1 h at RT (clone WB-MHCs Leica, Germany, dilution 1:80). The reaction was visualized by NovoLink™ Polymer Detection System (Leica, Germany) according to the manufacturer's instruction. Finally, all sections were dehydrated in a graded series of ethyl alcohol, cleared in xylene and mounted in DPX mounting medium (Fluka, Buchs, Switzerland). A minimum of 300 fibers were counted in each section using a NIKON E600 light microscope. The percentage and diameter of muscle fiber types were quantified with an image analysis system using Multi Scan v. 14.02 computer program.

2.6. Statistical analysis

Associations of the pig genotype at the *GHRL*/*Bsr*I locus with parameters of growth, carcass, physicochemical and muscle fiber traits were determined using the General Linear Model (GLM) procedure of the Statistical Analysis System program (SAS v. 8.02, 2001). The data were presented as LSM ± SE.