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ASSOCIATION OF GHRELIN GENE POLYMORPHISMS WITH SLAUGHTER TRAITS IN PIG*

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Abstract

It has been hypothesized that mutations in the ghrelin gene in pigs may play a similar role as in humans and may be associated with obesity. The aim of study was to analyse the polymorphisms of ghrelin gene and to evaluate its effect on pigs' carcass traits. The effect of *c.-93A>G, 4428T>C* and *g.4486C>T* polymorphisms at the ghrelin gene on slaughter performance was analysed in 346 gilts represented by three breeds (Polish Landrace, Duroc, Pietrain). Animals were fattened from 30 to 100 (±2.5) kg body weight. After slaughter, the carcasses were chilled for 24 hours (4ºC), weighed and the right half-carcasses were dissected and evaluated. A number of data were obtained including: meat weight in primary cuts, weight of ham, backfat thickness and carcass yield. From breeding and production point of view, the favourable results were obtained for pigs with the *GG* genotype at the *c.-93A>G* locus, characterized by better carcass results than those with the *AA* genotype, e.g. higher ham weight and lower average backfat thickness. In pigs with the *TT* genotype at the *g.4428T>C* locus, we found lower mean backfat thickness than in pigs with the *CC* genotype.

Key words: pigs, carcass quality, polymorphism, ghrelin, production traits

Food intake and energy homeostasis are under the control of several factors, including gastrointestinal hormones. They play a crucial role in the regulation of appetite. Acting as neurotransmitters, they converge the information about the body's energy status within the central nervous system. The main brain structure responsible for integration of neural and humoral signals that modulate food intake is the hypothalamus (Dong et al., 2023). Many studies have led to the recognition of the role of hypothalamic neuropeptides, adipose tissue products and digestive tract hormones as transducers of information about the energy balance delivered to the body during the last meal. These signals arise from the gastrointestinal tract (mainly from the abdomen and intestines) and are known as "satiety signals". The one exception is ghrelin (GHRL), which stimulates appetite. A breakthrough in the identification of these mechanisms was the discovery of leptin (LEP) and its role as a missing link between the peripheral and central nervous system. This paved the way for the melanocortin pathway (appetite regulation pathway), which consists of the satiety signalling pathway (regulated mainly by LEP, LEPR and MC4R) and the appetite signalling pathway (regulated among others by GHRL) (Męczekalski et al., 2008). Ghrelin is a 28-amino acid peptide, identified as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R), and it was isolated from the stomach of rats, humans and pigs (Kojima et al., 1999; Sun et al., 2019). Ghrelin conducts its biological activity through interaction with its specific cell surface receptor (GHS-R) that belongs to G protein-coupled receptors, and for which two subtypes have been described: GHS-R type 1a (functional) and GHS-R type 1b (non-functional) (van der Lely et al., 2004). Expression of GHS-R1a has been identified in a number of organs indicating a broad spectrum of biological activity of this peptide in the body (Zigman et al., 2006; Gnanapavan et al., 2002; Seim et al., 2017).

The *GHRL* gene was mapped on porcine SSC13 and was located close to QTL related to last rib backfat measurement (Kim et al., 2004). There was a significant homology between porcine SSC13 and human SSC3 on

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which the human *GHRL* gene is located (Van Poucke et al., 2001). In human, plasma ghrelin levels are inversely correlated with positive energy balance, body mass index (BMI), fat mass, adipocyte size, leptin level (Tschöp et al., 2001; Imaizumi et al., 2018). In obese subjects plasma ghrelin concentration is lower than in controls (Cummings et al., 2001). In human, mutations in ghrelin gene have been linked to obesity, suggesting their role in the aetiology of obesity. Several polymorphisms in the *GHRL* gene were reported in obese humans: Arg51Gln, Leu72Met and Arg604Gln (Ukkola et al., 2001; Imaizumi et al., 2018; Llamas-Covarrubias et al., 2017). The Leu72Met polymorphism was significantly associated with BMI and fat mass (Ukkola et al., 2002; Imaizumi et al., 2018).

Some polymorphisms within the porcine *GHRL* gene are related to body weight, daily gain (Dong et al., 2023) and carcass traits, such as backfat thickness and ham muscle weights (Kim et al., 2004; Wojtysiak and Kaczor, 2011; Dong et al., 2023), carcass weight, loin, and abdominal fat as well as to loin eye area (Wojtysiak and Kaczor, 2011). Based on biological function as well as localization on the chromosome SSC13, the *GHRL* gene can be considered a candidate gene for the pig fat deposition. These studies approached the topic in a fragmentary way. Therefore, our research included three mutations within the regulatory regions and the coding sequence of the porcine GHRL gene, and analysis of the association of selected SNPs with the characteristics of the carcass.

Material and methods

This study did not require Ethics Committee approval. The research was carried out on 346 gilts. They were purebred animals: Landrace – 188, Pietrain – 84, Duroc – 74. Pigs were maintained in the two Pig Test Stations of the National Research Institute of Animal Production, kept in the years 2011–2012 in the same housing and feeding system. Animals were fattened from 30 to 100 (±2.5) kg body weight. Blood samples were collected from each animal during the slaughter.

After slaughter, the carcasses were chilled for 24 hours (4ºC), weighed and the right half-carcasses were dissected and evaluated according to SKURTCh (Pig Performance Testing Station) procedures (Różycki and Tyra, 2010). The following carcass composition traits were evaluated: lean meat content (meatiness), carcass length, carcass yield, loin eye area, height and width of loin eye, tenderloin weight, loin weight, the weight of loin without backfat and skin, ham weight, the weight of ham without backfat and skin, and meat weight in primary cuts. Backfat thickness was measured at five locations: over the shoulder – at the thickest point, on the back above the joint between the last thoracic and first lumbar vertebrae and at three points above the loin: at the beginning (lower back I), at the centre (lower back II) and at the end of the gluteus medius muscle (lower back III). Those measurements were used to calculate the mean value of backfat thickness. Additionally between the last thoracic vertebra and the first lumbar vertebra backfat thickness was measured above the height measurement (C_1 point) and over the side edge of *longissimus dorsi* muscle (K point).

The total DNA from whole blood was isolated using Wizard DNA Purification Kit (Promega, Madison, USA). For all pigs, three polymorphisms within the *GHRL* gene were detected using the PCR-RFLP method. Analysed polymorphisms were identified using endonucleases: MwoI for *c.-93A>G*, localized in 5'gene flanking region; BslI for *g.4428T>C* and HgaI for *g.4486C>T*, both detected in 3'UTR region according to Ropka-Molik et al. (2011).

Statistical analyses

Statistical analyses were performed using the GLM (General Linear Model) procedure of the SAS (SAS Institute, Cary, NC, v.8.02.2001). The LSM (Least Squares Means) method was used to determine the statistical significance between genotype groups with Tukey-Kramer method for pairwise comparison. The differences in phenotype in particular genotype groups were presented as LSM ± SE. The linear model was:

$$Y_{ijkl} = \mu + s_i + b_j + g_k + (bg)_{jk} + \beta_{SW} + e_{ijkl}$$

where Y_{ijkl} is the observation, μ is the overall mean, s_i is the fixed effect of the experimental station, b_j is the fixed effect of breed, g_k is the fixed effect of different c.-93A>G, 4428T>C and g.4486C>T genotypes, $(bg)_{jk}$ is the interaction between genotype and breed (when significant differences occurred), β_{SW} is linear effect of slaughter weight as covariate, and e_{ijkl} is the random error.

The sire or sow effect was not included in this model because the animals were not related (we analysed offspring from 158 boars and 346 sows, so for one boar, 2.19 gilts were used in this study).

Additive and dominance effects were estimated for a total of 346 pigs of all breeds. Additive and dominance effects were calculated according to REG_A procedure (SAS v. 8.02), where the additive effect was denoted as −1 and 1 for genotypes *AA* (*CC*) and *GG* (*TT*), respectively, and the dominance effects are represented as −1 for heterozygotes *AG* (*CT*) and 1 for both homozygotes.

The Hardy–Weinberg equilibrium for each genotype and breed was estimated using Court Lab – HW calculator (https://www.scribd.com/doc/246388807/Court-Lab-HW-Calculator).

Results

In this study, the analysis revealed three different genotypes *AA, AG, GG* at locus *c.-93A>G* (69%, 27.2%, 3.8% respectively) and *CC, CT, TT* at locus *g.4428T>C* (49.4%, 39.3%, 11.3%) and *g.4486C>T* (12.7%, 38%, 50.3%) (Table 1). The frequency of the A allele for the

c.-93A>G mutation was over 80%, while in g.4428T>C the C allele was dominant (almost 70%). The T allele was found with a similar frequency in locus 4486C>T. A deviation from the Hardy-Weinberg equilibrium was found only in locus g.4486C>T. The analysis shows that the Duroc breed is responsible for this disproportion. This breed was characterized by an exceptionally even distribution of genotypes at locus g.4428T>C. In addition, no significant differences in the frequency of alleles and individual genotypes between the studied breeds were observed.

The breed has a significant effect (P<0.05) on all analysed slaughter traits (Table 2), but only for loin weight (with and without backfat and skin), ham (with and without backfat and skin) and average backfat thickness significance level reached P<0.01. The *GHRL* polymorphisms *g.4428T>C* and *c.-93A>G* were found (P<0.05) to be related with carcass yield, ham weight (with and without backfat and skin), average backfat thickness, and weight of main cuts. Moreover, in the *c.-93A>G* SNP, the significant effect (P<0.01) on tenderloin weight was observed, while *g.4428T>C* locus showed a significant effect (P<0.01) on loin weight without backfat and skin. Only one association was found between *g.4486C>T* polymorphism and tenderloin weight (P<0.05).

Table 1. The frequencies of genotypes and alleles according to three GRHL polymorphisms

SNP/breeds	Alleles (%)		Genotype observed			Genotype expected			Significance level
c.-93A>G	*A*	*G*	*AA*	*AG*	*GG*	*AA*	*AG*	*GG*	
ALL	82.6	17.4	221	87	12	218.7	91.7	9.6	NS
Landrace	86.1	13.9	127	44	2	128.4	41.3	3.3	NS
Duroc	90.3	9.7	60	10	2	58.7	12.6	0.7	NS
Pietrain	67.3	32.7	34	33	8	34	33	8	NS
g.4428T>C	*C*	T	CC	CT	TT	CC	CT	TT	
ALL	69.0	31.0	171	136	39	165.1	147.8	33.1	NS
Landrace	68.2	31.8	80	91	13	85.6	79.8	18.6	NS
Duroc	50.7	49.3	23	29	22	19.0	37.0	18.0	NS
Pietrain	86.4	13.6	68	16	4	65.6	20.8	1.60	NS
g.4486C>T	*C*	T	CC	CT	TT	CC	CT	TT	
ALL	31.1	68.9	39	114	155	29.9	132.2	145.9	*
Landrace	38.9	61.1	27	69	62	23.9	75.2	58.9	NS
Duroc	20.8	79.2	7	16	49	3.1	23.8	45.1	*
Pietrain	25.0	75.0	5	29	44	4.9	29.2	43.9	NS

NS – not significant; *P<0.05 – not consistent with HWE.

Table 2. Means ± standard deviation (SD) for carcass traits (n=346)

Traits	X	±SD	Significant level			
			Breed	*c.-93 A>C*	*g.4428 T>C*	*g.4486 C>T*
Carcass yield (%)	77.00	2.14	*	*	*	
Tenderloin weight (kg)	0.38	0.06	*	**		*
Loin weight (kg)	7.64	0.64	**			
Weight of loin without backfat and skin (kg)	6.19	0.63	**		**	
Ham weight (kg)	10.62	0.66	**	*	*	
Weight of ham without backfat and skin (kg)	9.16	0.80	**	*	*	
Carcass length (cm)	79.80	3.27	*			
Average backfat thickness (cm)	1.44	0.35	**	*	*	
Backfat thickness at point C_1 (cm)	0.91	0.30	*			
Backfat thickness at point K_1 (cm)	1.18	0.37	*	*		
Loin eye width (cm)	10.39	1.02	*			
Loin eye height (cm)	6.90	0.64	*		*	
Loin eye area (cm^2)	54.40	8.19	*			
Meatiness (%)	60.90	4.02	*	*		
Meat weight in primary cuts (kg)	23.96	2.02	*	*	*	

*P<0.05; **P≤0.01.

Table 3. Additive and dominance effect (±SE) obtained for the three *GRHL* polymorphisms for indicators of carcass yield and primary cuts content of carcasses

Traits	*c.-93A>G*		*g.4428T>C*		*g.4486C>T*	
	additive	dominance	additive	dominance	additive	dominance
Carcass yield (%)	1.16±0.31**	0.44±0.19*	–0.17±0.18	0.28±0.13*	0.10±0.19	–0.21±0.14
Tenderloin weight (kg)	0.04±0.01**	0.02±0.01**	–0.02±0.01**	–0.01±0.01	–0.01±0.01	–0.01±0.01
Loin weight (kg)	0.02±0.09	0.02±0.06	–0.09±0.06	0.03±0.04	0.01±0.05	0.01±0.04
Weight of loin without backfat and skin (kg)	0.21±0.09*	0.02±0.06	–0.14±0.06*	0.08±0.04*	0.01±0.06	–0.02±0.04
Ham weight (kg)	0.43±0.09**	0.08±0.06	–0.12±0.06*	0.14±0.04**	0.08±0.06	–0.01±0.04
Weight of ham without backfat and skin (kg)	0.55±0.11**	0.08±0.07	–0.18±0.07*	0.16±0.05**	0.13±0.07	–0.02±0.05
Carcass length (cm)	–1.02±0.48*	–0.24±0.30	0.60±0.29*	–0.20±0.20	–0.55±0.28	0.03±0.21
Average backfat thickness (cm)	–0.15±0.05**	–0.01±0.03	0.08±0.03*	0.02±0.02	–0.02±0.03	0.02±0.02
At point C_1	–0.09±0.04*	0.02±0.03	0.05±0.03	–0.01±0.02	–0.01±0.02	–0.02±0.02
At point K_1	–0.18±0.05**	–0.01±0.03	0.08±0.03*	–0.02±0.02	–0.04±0.03	0.01±0.03
Loin eye width (cm)	0.42±0.15**	0.05±0.09	–0.21±0.09*	–0.02±0.06	0.14±0.09	–0.11±0.07
Loin eye height (cm)	0.12±0.09	–0.04±0.06	–0.17±0.05**	0.07±0.04	0.08±0.06	–0.07±0.04
Loin eye area (cm^2)	2.79±1.18*	–0.08±0.73	–2.25±0.71**	0.81±0.49	0.83±0.73	–0.50±0.53
Meatiness (%)	2.33±0.57**	0.01±0.35	–1.35±0.34**	0.34±0.24	0.66±0.37	–0.18±0.27
Meat weight in primary cuts (kg)	1.32±0.28**	0.19±0.18	–0.53±0.17**	0.35±0.12**	0.29±0.17	–0.10±0.13

*, **means within rows bearing different superscripts differ significantly: *P<0.05; **P≤0.01.

Table 4. Differences in slaughtering traits between pigs with different genotypes at the locus *c.-93A>G* (LSM ± SE)

Traits	*c.-93A>G*		
	AA	*AG*	*GG*
Carcass yield (%)	77.10±0.14 a	77.00±0.19 a	78.30±0.74 b
Tenderloin weight (kg)	0.38±0.00 ABa	0.37±0.06 Ba	0.42±0.02 Ab
Loin weight (kg)	7.64±0.05	7.61±0.06	7.66±0.17
Weight of loin without backfat and skin (kg)	6.22±0.04	6.28±0.05	6.33±0.11
Ham weight (kg)	10.73±0.04 b	10.82±0.07 a	10.96±0.21 a
Weight of ham without backfat and skin (kg)	9.28±0.05 a	9.41±0.08 b	9.57±0.23 b
Carcass length (cm)	78.90±0.21	79.10±0.38	79.70±0.96
Average backfat thickness (cm)	1.46±0.02 a	1.40±0.03 ab	1.34±0.11 b
Backfat thickness at point C_1 (cm)	0.92±0.02	0.84±0.02	0.86±0.07
Backfat thickness at point K_1 (cm)	1.16±0.02 a	1.06±0.04 b	1.05±0.08 ab
Loin eye width (cm)	10.42±0.07	10.43±0.08	10.52±0.31
Loin eye height (cm)	6.96±0.04	7.02±0.06	6.79±0.16
Loin eye area (cm^2)	55.20±0.53	55.60±0.82	54.70±2.10
Meatiness (%)	61.30±0.26 Aa	62.20±0.37 Bb	61.90±1.47 Ab
Meat weight in primary cuts (kg)	24.20±0.13 a	24.50±0.18 a	24.80±0.58 b

The means in rows marked with the different letters differ significantly; capital letters denote the significance of difference at P≤0.01, whereas small letters denote the significance of difference at P<0.05.

Significant results of the additive positive effect on slaughter traits of pigs (Table 3) were obtained at the locus *c.-93A>G* for carcass yield estimated to be P<0.01, tenderloin weight (P<0.01), the weight of loin without backfat and skin (P<0.05), ham weight (P<0.01), loin eye width (P<0.01), loin eye area (P<0.05) and meatiness and meat weight in primary cuts (P<0.01). Moreover, additive negative effects were found in carcass length (P<0.05), average backfat thickness (P<0.01), backfat thickness at points C_1 (P<0.05) and K_1 (P<0.01). Dominance effect was smaller than additive effect, and significant results were found only in carcass yield (P<0.05) and tenderloin weight (P<0.01). The additive negative allelic effect was prevalent in slaughter traits at the locus *g.4428T>C*. The significant results (P<0.01) were found in tenderloin weight, the weight of loin without backfat and skin, ham weight, ham weight without bones and skin, loin eye width, loin height, loin eye area, meatiness and meat weight in primary cuts. Obtained results allowed us to detect the dominance positive effect on carcass yield (P<0.05), loin weight with-

out bones and skin (P<0.05), ham weight (P<0.01), ham weight without bones and skin (P<0.01) and weight of primary cuts (P<0.01). The ghrelin polymorphism at the *g.4486C>T* position showed no significant additive or dominance effect on any of the analysed slaughter traits.

Table 4 shows significant differences between genotypes in the *c.-93A>G* locus for some of the analysed slaughter traits. The *GG* genotype individuals had a higher weight of ham, weight of ham without lard and skin and the lower backfat thickness than pigs with the *AA* genotype (P<0.05). Additionally, animals with the *GG* genotype had a higher slaughter efficiency (P<0.05), tenderloin weight (P<0.05 and P<0.01, respectively) and meat weight of the primary cuts (P<0.05) compared to *AA* and *AG* pigs. In our study, the least numerous group of individuals with the *TT* genotype at the *g.4428T>C* locus (Table 5) showed higher carcass yield (P<0.05) and ham weight (P<0.05) compared to pigs with *TC* genotype The *CC* pigs obtained the greatest loin weight without bones and skin (P<0.01), ham weight without skin and bones (P<0.05), loin eye height (P<0.05) and primary meat cuts (P<0.05) compared to heterozygote pigs. Compared to the other groups *TC* and *TT*, the mean backfat thickness reached the highest value in homozygotes *CC* (P<0.05).

Analysis of porcine ghrelin polymorphism at the locus *g.4486C>T* (Table 6) showed a significant difference (P<0.05) in tenderloin weight between *CT* heterozygotes and *TT* pigs. Other analysed traits showed no differences between analysed genotypes.

Table 5. Differences in slaughtering traits between pigs with different genotypes at the locus *g.4428T>C* (LSM ± SE)

Traits	*g.4428T>C*		
	CC	*TC*	*TT*
Carcass yield (%)	77.12±0.15 ab	76.90±0.18 a	77.67±0.34 b
Tenderloin weight (kg)	0.37±0.00	0.38±0.01	0.38±0.01
Loin weight (kg)	7.72±0.05	7.56±0.05	7.52±0.11
Weight of loin without backfat and skin (kg)	6.31±0.04 A	6.11±0.06 B	6.20±0.10 AB
Ham weight (kg)	10.78±0.05 ab	10.65±0.05 a	10.85±0.07 b
Weight of ham without backfat and skin (kg)	9.36±0.06 a	9.19±0.07 b	9.41±0.08 ab
Carcass length (cm)	79.05±0.26	78.93±0.24	79.54±0.46
Average backfat thickness (cm)	1.46±0.03 a	1.43±0.02 b	1.43±0.05 b
Backfat thickness at point C_1 (cm)	0.90±0.03	0.92±0.03	0.90±0.05
Backfat thickness at point K_1 (cm)	1.12±0.03	1.15±0.03	1.17±0.04
Loin eye width (cm)	10.33±0.09	10.48±0.07	10.56±0.10
Loin eye height (cm)	7.03±0.05 a	6.85±0.05 b	6.88±0.09 ab
Loin eye area (cm^2)	55.40±0.65	54.20±0.63	55.40±0.94
Meatiness (%)	61.80±0.81	61.40±0.33	61.20±0.32
Meat weight in primary cuts (kg)	24.40±0.15 a	23.90±0.17 b	24.58±0.23 ab

The means in rows marked with the different letters differ significantly; capital letters denote the significance of difference at P≤0.01, whereas small letters denote the significance of difference at P<0.05.

Table 6. Differences in slaughtering traits between pigs with different genotypes at the locus *g.4486C>T* (LSM ± SE)

Traits	*g.4486C>T*		
	CC	*CT*	*TT*
Carcass yield (%)	76.93±0.28	77.30±0.21	76.40±0.18
Tenderloin weight (kg)	0.38±0.01 ab	0.39±0.01 a	0.37±0.00 b
Loin weight (kg)	7.60±0.10	7.59±0.06	7.64±0.05
Weight of loin without backfat and skin (kg)	6.23±0.07	6.22±0.04	6.17±0.06
Ham weight (kg)	10.78±0.08	10.74±0.06	10.73±0.05
Weight of ham without backfat and skin (kg)	9.28±0.10	9.29±0.08	9.24±0.07
Carcass length (cm)	79.02±0.50	78.8±0.29	78.0±0.25
Average backfat thickness (cm)	1.46±0.07	1.45±0.03	1.44±0.03
Backfat thickness at point C_1 (cm)	0.90±0.04	0.95±0.03	0.91±0.02
Backfat thickness at point K_1 (cm)	1.15±0.06	1.14±0.04	1.15±0.03
Loin eye width (cm)	10.21±0.28	10.43±0.08	10.42±0.07
Loin eye height (cm)	6.85±0.09	6.99±0.05	6.91±0.05
Loin eye area (cm^2)	54.4±1.11	54.9±0.75	54.7±0.68
Meatiness (%)	61.30±0.62	61.50±0.41	61.40±0.31
Meat weight in primary cuts (kg)	24.14±0.26	24.24±0.19	24.15±0.17

The means in rows marked with the different letters differ significantly; capital letters denote the significance of difference at P≤0.01, whereas small letters denote the significance of difference at P<0.05.

Discussion

Recent study results suggested that SNPs of the ghrelin gene can be used in the breeding programme as selection markers for economically important traits (e.g. meat quality) in livestock animals such as poultry (Kaczor et al., 2017), cattle (Braz et al., 2015; Zhang et al., 2009) and pigs (Wojtysiak and Kaczor, 2011; Dong et al., 2023). Additionally, porcine polymorphisms (*g.253G>A*, *c.-93A>G*, *g.4486C>T*, *g.4428T>C*, *g.31C>T*) of the ghrelin gene were previously found in Polish pig breeds and described by Ropka-Molik et al. (2011) but until now no research has been done on their relationship to slaughter traits.

The genetic disequilibrium found in the study in the locus *g.4486C>T*, according to the Hardy–Weinberg equilibrium, may indicate potential genotyping errors, but also a real relationship with the analysed trait (Turner et al., 2011). The test protocol should eliminate genotyping error. In addition, it has been shown that allele-based genotyping errors do not upset the Hardy–Weinberg equilibrium in genotype distribution (Moskvina and Schmidt, 2006). However, the lack of accordance with the Hardy–Weinberg law may be caused by long-term selection aimed at improving fattening and slaughter characteristics. The animals included in the study came from nucleus herds (active population), in which intensive selection is carried out on the basis of the assessment of the breeding value of pigs using the BLUP method (for the percentage of meat in the carcass and daily gains), which seems to be the cause of the described imbalance.

The conducted studies showed a positive additive effect within the c.-93A>G locus on parameters related to the performance of the carcass and its cuts (except for loin weight and loin eye height) and a negative effect on the features characterizing the fatness of the carcass. Studies on the human population have also shown that *GHRL* polymorphism is associated with obesity (Ukkola et al., 2001; Gueorguiev et al., 2009; Li et al., 2014). In addition, a negative correlation was found between plasma ghrelin levels and percent body fat (Tschöp et al., 2001). In the case of the dominance effect, significant results were obtained in carcass yield and tenderloin weight. The additive negative allelic effect was prevalent in slaughter traits at the locus *g.4428T>C*. It is possible that this association also explains the higher overall liking scores for animals with *c.-93A>G* mutation.

From breeding and production point of view, favourable results were obtained for pigs with the *GG* genotype at the *c.-93A>G* locus, which were characterized by better carcass results than individuals with the *AA* genotype of this gene, e.g. higher ham weight by 0.23 kg and lower average backfat thickness by 0.12 cm. A similar result was obtained by Kim et al. (2004) where *GHRL* polymorphism (BsrI) was associated with backfat, bone-in loin and bone-in ham muscle weight (not with boneless muscle weight) and marbling. Nowadays, these traits are desired by consumer requirements and trends. In general, pig selection programmes are designed to improve meatiness and increase the meat weight of primary cuts with simultaneous reduction of carcass fat content (Borzuta and Lisiak, 2016; Szulc et al., 2012; Blanchard et al., 2000). Wojtysiak and Kaczor (2011) showed significant differences between analysed genotypes (*GHRL* /BsrI) and carcass weight, backfat thickness and both loin weight and loin eye area.

Our results showed significant or highly significant differences between *CC* or *TT* homozygotes and heterozygotes at the *g.4428T>C* locus for some slaughter traits. However, the average backfat thickness in pigs with the *CC* genotype was only 0.03 cm higher compared to other genotypes, which gives no hope for using such a marker in breeding practice. In previous studies, the association between *GHRL* polymorphism and backfat thickness was not clear. Fontanesi et al. (2012) showed an association with the porcine *GHRL* polymorphism at 287 position (*A>C*) and backfat thickness, while Tanaka et al. (2015) found that the *c.335A>C* polymorphism in porcine ghrelin was not related to backfat thickness. Recent findings suggest that the polymorphism *g.3734 G>T* and *g.7691 A>C* in the porcine *GHRL* gene may be one of the useful genetic markers in pig breeding due to its association with backfat thickness (Dong et al., 2023).

In poultry, the polymorphism within the ghrelin receptor *GHSR* gene had a significant association with carcass traits such as: carcasses weight, breast weight, breast muscle weight, wings weight and drumstick muscle weight (Khaerunnisa et al., 2017). Moreover, the growth hormone secretagogue receptor gene was significantly associated with the chicken fat traits (Lei et al., 2007). This is also confirmed by studies on the expression patterns of ghrelin and ghrelin receptor genes and their impact on the fat deposition effect in chickens and ducks (Nie et al., 2009). Some SNPs were found to be associated with fat traits such as abdominal fat mass and subcutaneous fat thickness in chickens and ducks.

Conclusions

In conclusion, our results indicate that porcine *GHRL* polymorphism allows its use in breeding programmes to improve selected carcasses characteristics. It was demonstrated that carcasses composition traits were affected by *c.-93A>G* and *g.4428T>C* polymorphisms. At the same time, the frequency of advantageous genotypes of these mutations is at a level guaranteeing the possibility of long-term use in breeding practice. In the case of the c.-93A>G polymorphisms, we observe a linear effect on the analysed features (and at the same time favourable in the analysis of the feature group), which gives more hope for the possibility of using it in selection. These results emphasize the importance of further research on a larger population, with different sexes and breeds.

Conflicts of Interest statement

None of the authors have a financial or other relationship with other people or organizations that may inappropriately influence this work.

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