

## Effect of mutation in *MC4R* gene on carcass quality in Pulawska pig included in conservation breeding programme

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In addition to highly productive breeds of pigs, Polish breeders keep local pigs subject to the conservation programme including Pulawska (P) pig. Analysis of records spanning 20 years showed that lean content of P carcass increased from 41.43% in 1983 to 45.68% in 2003, with a simultaneous decrease in fat content. Considering the relatively high rate of changes in these traits, it would be interesting to find out parameters that could serve as a criterion for evaluating the degree of heterozygosity in P pig. The aim of this study was, therefore, to determine the melanocortin receptor gene polymorphism in P pig and its effect on carcass quality. The study involved 66 P fatteners. After slaughter and 24-hour cooling at 4°C, linear measurements of carcasses were taken and dissection was made according to the Walstra and Merkus method. Genotype analysis showed the highest frequency of *MC4R*<sup>AG</sup> and the lowest of *MC4R*<sup>AA</sup> animals. Frequency of the allele *MC4R*<sup>A</sup> was only by 15.2 per cent units lower than that of the *MC4R*<sup>G</sup> allele.

The *MC4R*<sup>A</sup> allele showed a significant effect on increasing backfat thickness, especially over the loin and was significantly correlated with a greater fat amount of neck. Animals with the A allele at the *MC4R* locus were also characterized by a significantly lower amount of lean in this cut. The results obtained for frequency of different genotypes in P pig could serve as reference values for selection-induced changes, thus reflecting the level of genetic variation in the breed.

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In humans and animals, the melanocortin receptor gene (*MC4R*) is known as a factor maintaining body homeostasis by regulating the energy balance. In addition to other genes coding for hypothalamic hormones, *MC4R* is involved in appetite regulatory mechanisms, *i.e.* feelings of appetite and satiety [Barb *et al.* 2004]. Mutation in the melanocortin-4 receptor was shown to be a frequent, genetically determined cause of obesity in humans [Korner *et al.* 2003]. Widely studied in pigs has also been the effect of *MC4R* polymorphism on feed intake and carcass fatness traits. When studying the relationship between melanocortin receptor polymorphism and carcass fatness, feed intake and weight gain traits in pigs, Chen *et al.* [2004], Kim *et al.* [2006] and Meidtner *et al.* [2006] found that the *MC4R* gene can be used as a genetic marker for these traits in animal selection. Van den Maagdenberg *et al.* [2007] demonstrated that mutation in the melanocortin receptor gene affects the weight gain and carcass fatness in pigs, but not quality of their meat. In the population of Polish Large White and Polish Landrace pigs, Stachowiak *et al.* [2005] showed that *MCR4* gene polymorphism has no significant effect on feed intake or backfat thickness.

In addition to highly productive breeds, Polish pig breeders keep local breeds included in conservation programmes to which the Pulawska (P) pig, representing the meat-lard type belongs. Compared to highly productive breeds, P pigs are characterized by considerably thicker backfat and lower lean content of carcass. Analysis of the P pig records spanning 20 years showed that carcass lean content increased from 41.43% in 1983 to 45.68% in 2003, with a simultaneous fall of fat cover thickness [Borzuta *et al.* 2004]. Considering the relatively high rate of changes occurring in these traits, it would be desirable to find out parameters that could serve as a criterion for evaluating the degree of heterozygosity in this breed. The findings of Houston *et al.* [2004] suggest that the polymorphism of *MC4R* could serve this purpose. According to the authors cited, even short-term selection for such diverse traits as lean content and feed conversion makes the selected population diverse in terms of the frequency of this gene. In light of the reports cited above, it will be interesting to determine the melanocortin receptor gene polymorphism in Pulawska pigs and evaluate its effect on carcass quality.

### Materials and methods

Sixty-six Pulawska fatteners were investigated. Animals were slaughtered at an average body weight of 106 kg. On the day of slaughter, blood samples were collected to determine polymorphism of the melanocortin-4 receptor gene (*MC4R*). After slaughter and 24-hour cooling at 4°C, linear measurements of carcasses were taken and dissection was made according to the method used in meat plants [Walstra and Merkus 1996]. Right carcass-sides were subjected to linear measurements of backfat thickness at 5 points:

- over the shoulder at the point of maximum thickness;
- on the back over the joint between last thoracic and first lumbar vertebra;
- at three points over the edge of the cross-sectional area of the gluteus muscle (loin region):
  - rostral part (loin I),
  - middle part (loin II),
  - caudal part (loin III).

Carcasses were then divided into primary cuts (knuckle, leg, ham, loin, neck, shoulder, belly, loin end). These cuts were subjected to a detailed dissection into meat, fat, bone and skin tissue.

*MC4R* gene polymorphism was determined at the National Research Institute of Animal Production. DNA isolation from leukocytes was performed using the method of Kawasaki [1990] as modified by Coppieters *et al.* [1992]. Primers and probes for missense mutations in the *MC4R* gene were designed as described by Burgos [2005]. G/A 1426 *MC4R* was genotyped in a 7500 Real-Time PCR System (*Applied Biosystems*) with reference to Burgos [2005].

Statistical analysis was performed in order to determine the effect of individual *MC4R* genotypes on carcass fatness and muscling traits. Results obtained for gilts and castrated males were pooled, with sex as one of the factors in statistical model. Verifications were performed using two-way analysis of variance, taking into account covariance on right carcass-side weight as a concomitant variable. The General Linear Model (GLM) procedure of the SAS package was used.

## **Results and discussion**

Pulawska (P) pigs are now included in the conservation breeding programme, one aim of which is to maintain genetic diversity in the conserved population. This results in wide P pig inter-individual variation in carcass fatness and muscling confirmed by the present report in which the fatteners' carcasses showed a mean lean content of 50.5% (Tab. 1). Backfat was thickest over the shoulder and thinnest on loin II. The inter-individual differences in carcass muscling and fatness are evidenced by minimum and maximum values presented in Table 1 for individual linear measurements and weights of carcass cuts, as well as their coefficients of variation. Variation in carcass and carcass cuts fatness was higher ( $V=25-35\%$ ) for parameters of carcass and carcass cuts ( $V=15-22\%$ ), except for backfat thickness over the shoulder for which the coefficient of variation occurred relatively low (15.9%).

Surdacki *et al.* [1995] showed that Pulawska pigs had thickest backfat on loin III (41.7 mm) and over the shoulder (36.2 mm), and thinnest on loin II (28.4 mm). In turn, Eckert and Blicharski [2001] found that mean backfat thickness measured in Pulawska pigs at two carcass points with an Ultra-Fom 100 device averaged 19.2 mm (point 1) and 20.91 mm (point 2). Borzuta *et al.* [2004] when analysing changes in fatness that occurred over 20 years in Pulawska pig found that backfat thickness

**Table 1.** Means, standard deviations (SD) and coefficients of variation (V) for slaughter traits in a group of Pulawska pigs (n=66)

Item	Mean	SD	Min.	Max.	V (%)
Backfat thickness (mm)					
over shoulder	38.92	6.19	25.31	54.50	15.9
on back	24.99	6.10	8.00	41.41	24.41
on loin I	27.53	6.59	12.39	41.85	23.92
on loin II	21.04	6.68	5.55	36.07	31.74
on loin III	30.65	8.29	10.79	50.00	27.06
Knuckle lean (kg)	0.29	0.05	0.19	0.41	16.42
Knuckle fat (kg)	0.07	0.02	0.03	0.12	30.79
Leg lean (kg)	0.64	0.10	0.41	0.84	14.96
Leg fat (kg)	0.20	0.06	0.10	0.31	29.61
Ham lean (kg)	5.71	0.91	3.21	8.12	15.95
Ham fat (kg)	1.60	0.41	0.70	2.98	25.76
Loin lean (kg)	3.57	0.78	2.36	7.51	21.84
Loin fat (kg)	2.03	0.71	0.56	3.90	35.03
Neck lean (kg)	3.19	0.48	2.08	4.33	15.14
Neck fat (kg)	1.39	0.37	0.43	2.24	26.31
Shoulder lean (kg)	2.74	0.49	1.78	3.91	18.03
Shoulder fat (kg)	1.04	0.27	0.54	1.71	25.69
Belly lean (kg)	3.08	0.53	1.94	4.31	17.21
Belly fat (kg)	2.59	0.73	0.88	5.26	28.33
Loin end lean (kg)	0.70	0.13	0.44	0.99	19.02
Loin end fat (kg)	0.30	0.11	0.10	0.61	35.2
Carcass-side lean (kg)	20.59	3.12	12.82	26.59	15.18
Carcass-side lean (%)	50.53	7.67	40.09	60.77	15.17
Carcass-side fat (kg)	10.15	2.68	4.03	18.52	26.47
Carcass-side fat (%)	25.23	5.84	13.53	38.89	23.14

decreased, but was still highest over the shoulder (47.71 mm) and thinnest on loin II (31.18 mm). Our figures presented in Table 1 are in accordance with those results. The slightly lower fatness reached in this study is due to the fact that animals considered by the authors cited had slightly lower lean content of carcass compared to the pigs described in the present report. The lean content of carcass of those animals was 41.4-45.7% [Borzuta *et al.* 2004] and 47.8% [Eckert and Blicharski 2001] and was steadily rising, as confirmed by the results from Pig Testing Stations, according to which over the last 12 years the Pulawska carcass muscling increased from 51.7% in 1996 to 55.1% in 2008 [Różycki and Tyra 1997, 2009]. During the same period, fat cover expressed as mean backfat thickness from five measurements was observed to decrease from 2.93 cm to 1.96 cm. These data may provide good reason to fear that the Pulawska population is subjected to unintentional selection pressure that gives economic benefits to producers of slaughter material. This trend does not coincide with the objectives and directions of the genetic resources conservation programme that covers a large part of the Pulawska pig population. It should be emphasized that Pulawska pigs are predisposed to be included in the group of conservation breeds

specifically due to the level and unique distribution of fat (intramuscular fat) that determines good quality and taste of their meat as well as good reproductive and piglet rearing parameters.

Genotype and allele frequencies at the *MC4R* locus are presented in Table 2. Animals of the *MC4R*<sup>A/G</sup> genotype were the most, and those with the *MC4R*<sup>A/A</sup> genotype – the least frequent. The frequency of the *MC4R*<sup>A</sup> allele in this group was only by 15.2% (units) lower than that of the allele *MC4R*<sup>G</sup>. Kim *et al.* [2000] and Hernandez-Sanchez *et al.* [2003] demonstrated that in Landrace pigs, the *MC4R*<sup>G</sup> allele was much more frequent than the *MC4R*<sup>A</sup>. Meanwhile, in the Large White breed, the frequencies of both alleles (*MC4R*<sup>A</sup> and *MC4R*<sup>G</sup>) were similar [Kim *et al.* 2000, [Hernandez-Sanchez *et al.* 2003]. High frequency of the *MC4R*<sup>G</sup> allele in the Polish Landrace and of *MC4R*<sup>A</sup> in Polish Large White pigs was reported by Stachowiak *et al.* [2005] while Park *et al.* [2002] found that in Landrace × wild boar hybrids, the frequency of A allele was highly similar to that of allele G. Chinese Meishan pigs possess only one form of the allele – *MC4R*<sup>A</sup> [Chen *et al.* 2005]. Houston *et al.* [2004] demonstrated that selection pressure applied over several generations in two groups of animals chosen from a uniform population, directed to improve lean content and feed conversion, makes these groups diverse in terms of the frequency of *MC4R* alleles. The inter-breed differences cited above, result from the selection of the breeds. It can, therefore, be suggested that selection is the main factor of genetic fluctuations that often make a population homozygous, and this is associated with a decrease in the observed variation of productive traits. The rate of observed changes is directly proportional to their intensity, and in the case of selection based on genetic markers, it remains in direct proportion to the degree to which a gene product determines a given trait.

**Table 2.** Frequency of genotypes (%) and alleles (%) for melanocortin-4 receptor gene in a group of Pulawska pigs (n=66)

<i>MC4R</i> locus	Frequency of genotypes	
	<i>AA</i>	13.6
<i>AG</i>	57.6	
<i>GG</i>	28.8	
<i>MC4R</i> locus	Frequency of alleles	
	<i>A</i>	42.4
	<i>G</i>	57.6

In the present study, the effect was analysed of the *MC4R* genotype on Pulawska pig carcass quality traits. As suggested by many authors, *MC4R* genotype can be used as a genetic marker of feed intake and carcass fatness traits in pigs of different breeds [Chen *et al.* 2004, Kim *et al.* 2006, Meidtner *et al.* 2006]. The figures in Table 3 indicate that in Pulawska pigs, the A allele at the *MC4R* locus is responsible for increased backfat thickness, especially in hind parts of the carcass (loin I, II and III). Animals in whose genotype the A allele was found at the *MC4R* locus were characterized by thicker backfat measured at these points, compared to animals of the *MC4R*<sup>G/G</sup>

genotype. The observed differences were mostly significant or highly significant. The  $MC4R^A$  allele was also shown to have an effect on increased backfat thickness. Animals of the  $MC4R^{A/G}$  genotype had about 4 mm thicker backfat compared to those with the genotype  $MC4R^{G/G}$ , with a highly significant inter-genotype difference. No differences were observed between the analysed genotypes in backfat thickness over the shoulder.

Analysis was also made of the effect of the A allele at the  $MC4R$  locus on fatness and muscling of individual carcass cuts. Summarizing, fatteners with the  $MC4R^{A/A}$  genotype had a significantly higher fat amount of neck and belly compared to those with the  $MC4R^{G/G}$  genotype. Differences in the fatness of the other cuts were not significant, but a similar trend was observed for ham, loin and shoulder, *i.e.* animals with the  $MC4R^{A/A}$  genotype were characterized by a greater amount of fat in these cuts compared to pigs with the  $MC4R^{G/G}$  genotype. A similar trend was observed for carcass fatness.

A significant effect of the  $MC4R^A$  allele on increasing carcass fatness in Landrace, Large White, Large White  $\times$  Duroc [Kim *et al.* 2000, Hernandez-Sanchez *et al.* 2003], Landrace  $\times$  Lantang [Min Chen *et al.* 2004] and Hampshire  $\times$  Landrace pigs [Bruun *et al.* 2006] has been described. Melanocortin receptor polymorphism was also found to increase carcass fatness in Durocs [Kim *et al.* 2006] and in six lines of British Large White pigs [Houston *et al.* 2004]. A study by Van den Maagdenberg *et al.* [2007] with Large White  $\times$  Pietrain and Landrace  $\times$  Large White crossbreds confirmed that the mutation in the melanocortin receptor has a significant effect on increasing carcass fatness.

Analysis of the effect of the A allele at the  $MC4R$  on the muscling of different carcass cuts showed that the allele reduces the amount of meat in the neck and knuckle (Tab. 3). The differences between the mean values of traits in animals with the  $MC4R^{A/A}$  and  $MC4R^{G/G}$  genotypes were significant. Similar though non-significant relationships were observed for ham, loin, shoulder and belly, and for the whole carcass-side. Moreover, fatteners with the  $MC4R^{A/A}$  genotype were characterized by lower content of carcass lean compared to those with the genotype  $MC4R^{G/G}$ . A similar trend was also found for primary cuts as ham, loin, neck and shoulder. These results are in accordance with the findings of Van den Maagdenberg *et al.* [2007] and Kim *et al.* [2006], who showed a 2% increase in carcass lean content in  $MC4R^{G/G}$  compared to  $MC4R^{A/A}$  pigs.

It is concluded that in Pulawska pigs, the  $MC4R^A$  allele has a significant effect on increasing backfat thickness, especially in the loin area. The allele in question is significantly correlated with a greater amount of fat in the neck. Fatteners with the A allele at the  $MC4R$  locus also show significantly less meat in this cut.

The results obtained for the frequency of different genotypes could serve as an indicator of the genetic variation of Pulawska pigs, which should be maintained at a constant level that guarantees the *status quo* of the breed.

**Table 3.** Least squares means (LSM) and standard errors (SE) for individual genotypes of the MC4R gene for slaughter traits in a group of Pulawska pigs (n=66)

Item	MC4R					
	AA (n=9)		AG (n=38)		GG (n=19)	
	LSM	SE	LSM	SE	LSM	SE
Backfat thickness (mm)						
over shoulder	37.91	1.69	39.80	1.07	36.88	1.37
on back	23.91	1.41	26.30 <sup>A</sup>	1.07	22.41 <sup>A</sup>	1.27
on loin I	29.45 <sup>a</sup>	1.76	28.08 <sup>b</sup>	1.01	24.55 <sup>ab</sup>	1.63
on loin II	21.90	1.79	21.70 <sup>a</sup>	1.05	17.88 <sup>a</sup>	1.57
on loin III	33.26 <sup>A</sup>	3.05	31.57 <sup>B</sup>	1.27	26.43 <sup>AB</sup>	1.71
Knuckle lean (kg)	0.28 <sup>a</sup>	0.01	0.29 <sup>b</sup>	0.01	0.31 <sup>ab</sup>	0.01
Knuckle fat (kg)	0.08	0.01	0.07	0.01	0.07	0.01
Leg lean (kg)	0.66	0.03	0.64	0.02	0.66	0.02
Leg fat (kg)	0.19	0.02	0.20	0.01	0.18	0.01
Ham lean (kg)	5.67	0.25	5.73	0.16	5.89	0.17
Ham fat (kg)	1.70	0.17	1.62	0.06	1.46	0.10
Loin lean (kg)	3.39	0.16	3.64	0.14	3.70	0.16
Loin fat (kg)	2.06	0.24	2.11 <sup>a</sup>	0.12	1.79 <sup>a</sup>	0.14
Neck lean (kg)	3.12 <sup>b</sup>	0.15	3.16 <sup>A</sup>	0.07	3.40 <sup>bA</sup>	0.12
Neck fat (kg)	1.49 <sup>a</sup>	0.11	1.42 <sup>b</sup>	0.06	1.24 <sup>ab</sup>	0.08
Shoulder lean (kg)	2.64	0.14	2.72	0.08	2.90	0.12
Shoulder fat (kg)	1.08	0.10	1.03	0.04	0.97	0.06
Belly lean (kg)	3.04	0.19	3.09	0.09	3.18	0.12
Belly fat (kg)	2.67	0.32	2.66 <sup>a</sup>	0.11	2.31 <sup>a</sup>	0.15
Loin end lean (kg)	0.72	0.05	0.70	0.02	0.72	0.03
Loin end fat (kg)	0.33	0.03	0.30	0.02	0.26	0.02
Carcass-side lean (kg)	20.19	0.92	20.64	0.51	21.44	0.72
Carcass-side lean (%)	50.47	1.74	51.08	0.96	50.54	2.64
Carcass-side fat (kg)	10.47	1.03	10.30	0.42	9.30	0.61
Carcass-side fat (%)	25.81	2.03	25.50	0.93	23.15	1.36

<sup>aA</sup>Means within rows bearing the same superscripts are significantly different at: small letters – P≤0.05, capitals – P≤0.01.

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## Wpływ mutacji w *locus* MC4R na jakość tuszy świń rasy puławskiej utrzymywanych w hodowli zachowawczej

### Streszczenie

W krajowej hodowli poza rasami wysoko produkcyjnymi utrzymywane są lokalne rasy objęte programem hodowli zachowawczej. Jedną z nich jest rasa puławska. Na przestrzeni 20 lat zaszły w produktywności tej rasy znaczne zmiany w postaci wzrostu mięsności tusz z 41,43% (1983) do 45,68% (2003) przy jednoczesnym zmniejszeniu otłuszczenia. Mając na uwadze stosunkowo duże tempo zmian tych cech należałoby znaleźć kryterium bądź kryteria oceny poziomu heterozygotyczności rasy puławskiej. W tym świetle Autorzy zmierzali do określenia polimorfizmu genu receptora melanokortyny świń rasy puławskiej oraz jego wpływu na jakość tuszy. Materiał stanowiły tusze 66 tuczników omawianej rasy. Po uboju i 24 godzinnym schłodzeniu w 4°C dokonano liniowych pomiarów oraz przeprowadzono ich dysekcję zgodnie z metodą Walstry i Merkusa. Największą frekwencję odnotowano dla genotypu MC4R<sup>A</sup>/<sup>G</sup>, a najmniejszą dla genotypu MC4R<sup>A/A</sup>. Częstość występowania allelu MC4R<sup>A</sup> w badanej grupie świń była jedynie o 15,2% mniejsza niż allelu MC4R<sup>G</sup>.

Stwierdzono istotny wpływ allelu MC4R<sup>A</sup> na wzrost grubości słoniny grzbietowej, zwłaszcza w części krzyżowej. Wykazano także istotną zależność między występowaniem allelu MC4R<sup>A</sup> a większą ilością tłuszczu w karkówce. Nadto osobniki mające w swym genotypie allel A w *locus* MC4R cechowały się istotnie mniejszą ilością mięsa w tym wyrębie. Uzyskane wyniki dotyczące frekwencji poszczególnych genotypów w przypadku rasy puławskiej mogą być wartościami referencyjnymi zmian spowodowanych selekcją, a więc stanowić wskaźnik poziomu genetycznej różnorodności tej rasy.

