Animal Science Papers and Reports vol. 33 (2015) no. 3, 267-276
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Association of *MC4R* and *LEPR loci* with reproductive performance and milk composition of sows

Magdalena Szyndler-Nędza[1]*, Katarzyna Ropka -Molik[2]

[1] Department of Animal Genetics and Breeding,
National Research Institute of Animal Production,
Krakowska 1, 32-083 Balice, Poland

[2] Laboratory of Genomics, National Research Institute of Animal Production,
Krakowska 1, 32-083 Balice, Poland

(Accepted August 3, 2015)

Porcine *MC4R* and *LEPR* gene polymorphisms have been implicated in traits associated with feed intake and carcass fatness. These genes could also indirectly regulate reproductive processes. The objective of this study was to determine the *MC4R* and *LEPR* effects on basic components of colostrum and milk from sows on their reproductive performance. The experiment was performed on 230 sows of breeds used in the breeding program as a maternal line: Polish Large White (PLW) and Polish Landrace (PL). The animals were maintained under the same feeding and housing conditions, according to the test station procedure and they were adapted for use as lactating sows. Colostrum and milk of sows were collected at 1, 7, 14 and 21 days of lactation to assay solids, total protein, fat and lactose. Data on piglet rearing performance were collected at 1, 7 and 21 days of lactation. The present study showed that *MC4R* and *LEPR* genes affected carcass fat deposition in sows and influenced production of nutrient-rich milk. Sows of the $MC4R^{AA}$ genotype, as well as sows of the $LEPR^{BB}$ genotype, characterized by a greater backfat thickness, produced colostrum with a lower content of fat, protein and solids. In addition, the fact that the investigated polymorphisms have no effect on piglet rearing results suggests that these markers could be used in selecting pigs for decreased backfat thickness, without fear of reducing the quality of milk and thereby piglet rearing performance.

KEY WORDS: maternal breeds / *LEPR* / *MC4R* / milk composition / rearing of piglets

*Corresponding author. magdalena.szyndler@izoo.krakow.pl

The body weight and weight gains of piglets during the maternal nursing period are factors affecting the economic performance of production farms. During the first weeks of life, these parameters are influenced, among others, by the composition of colostrum and milk. Their abundance of nutrients and immunoglobulins enhances passive immunity in piglets. Many studies [e.g. Alston-Mills *et al*. 2000] identified factors affecting the quality of sow milk, the composition of which was found to be influenced by breed. This is related to differences in resistance to environmental conditions [Gourdine *et al.* 2006], which has a direct effect on the course of lactation. When ambient temperatures and humidity are high, not only the quantity, but also the quality of milk is affected [Babicz *et al*. 2012]. Many authors recognize diet as the most important determinant of milk quality [Beyer *et al*. 2007, Ariza-Nieto *et al.* 2011].

In research concerning expression of genes in different pig breeds, significant relationships between transcript abundance of *GHR, IGF1, IGF2, IGF1R* genes and carcass composition traits were observed [Pierzchała *et al*. 2012]. Furthermore, the expression levels of both *MC4R* and *LEPR* genes were associated with intramuscular fat content and fatty acid composition in the *longissimus dorsi* muscle [Wang *et al*. 2013]. Genetic merit of sows may also have some influence on the chemical composition of their milk, and thereby on rearing of piglets. Porcine *MC4R* gene polymorphisms have also been implicated in traits associated with feed intake, carcass fatness and meat quality traits. In contrast to allele *G* (*MC4R* p.298Asn), the *A* allele is associated with higher daily gains, higher feed intake, greater carcass fatness, better flavor and less shear force of meat [Meidtner *et al*. 2006, Piórkowska *et al.* 2010, Zhang *et al*. 2014]. Within the *LEPR locus*, several polymorphisms have been identified so far. Their association with porcine production traits was investigated and confirmed by many authors. Li *et al*. [2010] showed an effect of *LEPR Ava*II polymorphism e.g. on cholesterol and intramuscular fat content. Also Óvilo *et al*. [2002] and Tyra and Ropka-Molik [2011] demonstrated a significant effect of the *LEPR*/HpaII polymorphism on backfat thickness and intramuscular fat percentage (IMF). Through their role in the melanocortin pathway initiated by leptin [Hausman and Barb 2010], the *LEPR* and *MC4R* genes indirectly regulate reproductive processes [Barb *et al*. 2005]. Furthermore, Szyndler-Nędza *et al*. [2013b] showed a significant association between both genes and colostrum and milk compositions in pigs. The promising results indicated the need for further research in this area.

Thus, the objective of the present study was to determine the effects of *MC4R* and *LEPR* genes on body weight, as well as backfat thickness and reproductive performance of sows of maternal breeds. The association between both loci and basic components of colostrum and milk was also investigated.

Material and methods

The analysis was performed on the second and third litters of sows of maternal breeds: Polish Large White (PLW) and Polish Landrace (PL), (109 and 121 sows;

respectively). All sows were kept at the Experimental Station of the National Research Institute of Animal Production Ltd. in Żerniki Wielkie (Poland). In the experiment, only sows in a similar condition on the day of mating were used. Their condition was determined based on body weight and last rib (P2) backfat thickness, measured with an ultrasonic device (Piglog 105). For each sow, the standard weaning-to-rebreeding intervals were applied, which were on average 149.6 days (SD=16.10; V=9.29). Blood samples were collected from all sows to determine polymorphisms of the melanocortin 4 receptor (*MC4R*) and leptin receptor (*LEPR*) genes. The animals were maintained under the same feeding and housing conditions, according to the test station procedure and they were adapted for use as lactating sows. The food ration contained 12.94 MJ metabolizable energy, 15% crude protein and 6.2% crude fat. Colostrum was collected after parturition and expulsion of the placenta. Milk was collected at the 7, 14 and 21 days of lactation, two hours after the morning feeding. Milk samples (50 ml per sample) were taken from the first, third and sixth teats following administration of 2 ml oxitocin. Next, the samples were labelled and chilled to 4°C. Cooled samples of fresh milk were transported to the Milk Testing Laboratory of the University of Environmental and Life Sciences in Wrocław to determine the basic composition of milk: solids, total protein, fat, and lactose.

Reproductive performance of the experimental sows was determined based on 230 litters, which were analysed for the number and body weight of piglets at birth and at 7 and 21 days of age.

DNA was isolated from whole blood using the Wizard Genomic Purification Kit (Promega, Madison, WI, USA). Mutation in the *MC4R* gene was genotyped for each animal using the Allelic Discrimination method on a 7500 Real-Time PCR System (Applied Biosystems). Primers and probes for detecting the mutation in the *MC4R* gene (G1426A missense mutation) were designed as described by Burgos **et al.** [2006]. Primers to detect mutations in the *LEPR* gene using enzyme *Hpa*II (*Msp*I) (F GGAAGGCATTTGTTTCAGCAGTTA and R CAAGTCCTCTTTCATCCAGCACTG) were designed following the method described by Stratil *et al*. [1998]. After restriction digestion, the PCR products were separated on 2% agarose gel.

The data were statistically analysed using the procedures of Statistica version 10 (2011, StatSoft Inc.). The differences between groups were estimated by the ANOVA procedure according to the following statistical model:

$$y_{ijkl} = a_i + b_j + c_k + e_{ijkl}$$

where:

y_{ijkl} – trait observation of *ijkl-th* individual;

a_i – fixed effect of *i-th* breed (i = 1, 2);

b_j – fixed effect of *j-th* lactation (j=1, 2);

c_k – fixed effect of *k-th* genotype (k= 1, 2, 3).

Differences between the means of individual traits were tested using Duncan's multiple range test.

Results and discussion

The PLW and PL breeds raised in Poland differ in the frequency of the $MC4R^{A}$ and $MC4R^{G}$ alleles [Szyndler-Nędza *et al*. 2013b, Piórkowska *et al*. 2010, Stachowiak *et al*. 2006]. The $MC4R^{G}$ allele is more frequent in the PL breed, while the $MC4R^{A}$ allele is more common in the PLW breed. In turn, both alleles of the *LEPR* gene have an almost similar frequency in both breeds. Animals of the $LEPR^{AA}$ genotype are least frequent in the two breeds [Szyndler-Nędza *et al*. 2013b, Tyra and Ropka Molik 2011]. A previous study, investigating the effect of *MC4R* and *LEPR* polymorphisms on the composition of colostrum from PLW and PL sows [Szyndler-Nędza *et al*. 2013b], demonstrated that the analyzed polymorphisms have a similar effect in both breeds. The $MC4R^{A}$ and $LEPR^{B}$ alleles (in the PLW and PL breeds) reduce the content of fat, protein and solids in the colostrum of sows. Thus, in the present study molecular analyses were performed to determine the association of the *MC4R* and *LEPR* genes with milk composition in both maternal breeds (PLW and PL). Most of the animals had the $MC4R^{GG}$ genotype (34.96%), followed by $MC4R^{AA}$ (32.74%) and $MC4R^{AG}$ (32.30%). As regards the *LEPR* gene, most animals had the $LEPR^{AB}$ and $LEPR^{BB}$ genotypes (47.83%, 46.09%, respectively), with the $LEPR^{AA}$ genotype being the least frequent (6.09%) – most analyzed animals had the $LEPR^{G}$ allele (70%). The lack of the Hardy-Weinberg equilibrium (HWE) in the analyzed populations may be due to the effect of selection aimed at improving sows reproduction traits on genotype frequencies of both genes. Analysis of genotype frequency showed a difference in the analyzed populations between the observed and expected frequencies of *MC4R* and *LEPR* genotypes ($P \leq 0.01$) (HWE p-values 0.006 and 0.0011, for MC4R and LEPR, respectively).

The estimated effects of polymorphisms of the *MC4R* and *LEPR* genes are presented in Tables 1-2. Within the *MC4R* polymorphic groups (Tab. 1), sows of maternal breeds were characterized by similar body weight and backfat thickness (non-significant differences). Likewise, Galve *et al*. [2012] and Kim [2006] reported no significant effect of the *MC4R* polymorphism on backfat thickness. However, Kim *et al*. [2006] estimated the significant differences for backfat thickness in animals with various diplotypes of two genes: *HMGA1* and $MC4R^{AA}$. Opposite results were obtained by Van den Maagdenberg et al. [2007] and Piórkowska *et al*. [2010], who showed that animals of the $MC4R^{AA}$ genotype have a significantly thicker backfat compared to the $MC4R^{GG}$ animals.

Milk quality is one of the main factors which affects daily gain and weight of weaned piglets, so the objective of the present study was to examine the effect of the polymorphisms of the *LEPR* and *MC4R* genes on the chemical composition of colostrum and milk from sows of these maternal breeds. The results obtained in this study showed that animals with the $MC4R^{A}$ allele ($MC4R^{AA}$ and $MC4R^{AG}$) in their genotype had a significantly lower content of fat, protein and solids in colostrum compared to animals with the $MC4R^{GG}$ genotype. Differences in the contents of these components between the above mentioned polymorphic groups ($AA<AG$, $AA<GG$) were 0.21% and 1.4% ($P \leq 0.01$) for fat, 1.24% ($P \leq 0.05$) and 2.3% ($P \leq 0.01$) for

Table 1. Means and standard deviations for sows' condition on the day of mating, chemical composition of colostrum and milk from 21-day lactation, and rearing performance of piglets from sows of maternal breeds with different *MC4R* genotypes

Item	PLW and PL		
	MC4R		
	AA (n=74)	*AG* (n=73)	*GG* (n=79)
Condition			
body weight at mating (kg)	193.2±26.3	189.0±27.7	187.2±23.9
backfat thickness (P2) (mm)	13.66±3.69	14.00±3.94	14.27±3.40
Composition of colostrum and milk from 21-day lactation			
fat (%)			
colostrum	**3.93**^{B}±2.70	**4.14**^{b}±1.85	**5.33**^{aA}±2.49
milk	6.99±0.92	7.30±1.08	7.33±1.54
protein (%)			
colostrum	**13.51**^{aA}±3.09	**14.75**^{b}±3.14	**15.81**^{B}±3.16
milk	5.05±1.38	4.65±0.56	4.97±1.51
lactose (%)			
colostrum	2.53±0.87	2.22±0.73	2.04±2.58
milk	5.45±0.63	5.68±0.40	5.41±0.73
solids (%)			
colostrum	**20.71**±3.07^{aA}	**22.02**±3.13^{b}	**23.45**±3.17^{cB}
milk	17.90±1.26	17.94±1.40	18.05±1.89
Rearing of piglets during 21-day lactation			
no. of piglets at days of age (head)			
1	11.69±1.14	11.40±1.32	11.54±1.21
7	11.01±1.33	10.90±1.26	10.81±1.38
21	10.21±1.69	10.26±1.34	10.11±1.59
weight of piglets at days of age (kg)			
1	1.46±0.14	1.47±0.14	1.50±0.17
7	2.68±0.31	2.67±0.31	2.80±0.39
21	5.70±0.56	5.66±0.62	5.78±0.61
weight gain of piglet until day (kg)			
7	1.22±0.27	1.20±0.31	1.30±0.36
21	4.24±0.54	4.19±0.57	4.28±0.60
% reared until day			
7	94.35±7.67	95.91±6.08	93.81±8.37
21	87.41±12.10	90.44±9.45	87.85±11.68

$^{aA...}$In rows means bearing different superscripts differ significantly at: small letters – P≤0.05; capitals P≤0.01.

protein, and 1.31% (P≤0.05) and 2.74% (P≤0.01) for solids. Analysis of the chemical composition of milk from the 21-day of lactation revealed similar trends for fat and solids, but differences between the groups were not significant. Also for piglet rearing performance differences between the polymorphic groups of the *MC4R* gene were not significant. However, homozygous sows (*MC4R*^{GG}), which produced colostrum and milk containing more nutrients, gave birth to slightly heavier piglets with quicker weight gains at 7 and 21 days of age compared to sows of the *MC4R*^{AG} and *MC4R*^{GG} genotypes (without statistical significance). Similarly, studies analyzing the effect of

Table 2. Means and standard deviations for sows' condition on the day of mating, chemical composition of colostrum and milk from 21-day lactation, and rearing performance of piglets from sows of dam breeds with different *LEPR* genotypes

Item	PLW and PL		
	LEPR		
	AA (n=14)	*AB* (n=110)	*BB* (n=106)
Condition			
body weight at mating (kg)	197.1±28.5	188.0±27.1	190.0±24.
backfat thickness (P2) (mm)	**12.50**^{a}±2.18	13.73±3.61	**14.36**^{b} ±3.81
Composition of colostrum and milk from 21-day lactation			
fat (%)			
colostrum	**6.00**^{aA}±3.93	**4.25**^{B}±2.10	**4.62**^{b}±2.17
milk	7.50±1.54	7.32±1.45	7.05±0.82
protein (%)			
colostrum	**15.91**^{a}±2.71	15.47±4.10	**14.26**^{b}±2.61
milk	**4.54**^{b}±0.50	**5.22**^{a}±1.58	**4.60**^{b}±0.68
lactose (%)			
colostrum	1.59±0.85	2.02±1.00	2.45±2.01
milk	5.54±0.69	5.38±0.72	5.64±0.42
solids (%)			
colostrum	**24.31**^{aA}±3.61	**22.55**^{b}±3.70	**16.97**^{B}±2.13
milk	17.88±2.15	18.32±1.73	17.60±1.08
Rearing of piglets during 21-day lactation			
no. of piglets at days of age (head)			
1	11.57±0.76	11.47±1.56	11.63±0.81
7	11.07±0.73	10.82±1.58	10.99±1.04
21	10.29±0.91	10.11±1.84	10.27±1.22
weight of piglets at days of age (kg)			
1	1.49±0.14	1.49±0.17	1.47±0.14
7	2.70±0.22	2.75±0.37	2.69±0.32
21	5.54±0.62	5.78±0.64	5.66±0.55
weight gain of piglet until day (kg)			
7	1.21±0.21	1.26±0.35	1.23±0.30
21	4.06±0.61	4.29±0.62	4.19±0.52
% reared until day			
7	95.77±4.40	94.56±7.61	94.60±7.60
21	89.21±9.34	88.54±12.65	88.44±9.63

$^{aA...}$In rows means bearing different superscripts differ significantly at: small letters – P≤0.05; capitals P≤0.01.

milk protein polymorphism in sows [Skrzypczak *et al.* 2012] and in goats [Schmidely *et al*. 2002, Barłowska *et al*. 2007] on rearing performance of the offspring showed that animals of the *CSN1S1*^{AA} and *CSN2*^{BB} genotypes produced milk with higher concentrations of protein and fat, thus ensuring better rearing performance of their offspring in the form of higher body weight and rapid weight gains. Also, Babicz *et al*. [2011] and Szyndler-Nędza *et al*. [2013a] demonstrated that a higher content of basic milk nutrients, in particular protein and solids, may be one of the factors contributing to increased weight gains and body weight of piglets during 21-day lactation.

Analysis of the effect of the leptin receptor gene polymorphism on the body weight of sows on the day of mating (Tab. 2) did not show any significant differences among the genotype groups. On the other hand, the $LEPR^{BB}$ homozygotes were characterized by significantly thicker backfat compared to animals of the $LEPR^{AA}$ genotype. Differences between the analysed polymorphic groups (*BB>AB, BB>AA*) were 0.63 and 1.86 mm ($P \leq 0.05$). This is consistent with the findings of Óvilo et al. [2002] and Tyra and Ropka-Molik [2011], who showed that pigs with the $LEPR^{BB}$ genotype have greater contents of intramuscular and subcutaneous fat compared to the *AB* animals.

When analysing the chemical composition of sow colostrum, it was observed that $LEPR^{AA}$ homozygotes had significantly higher contents of fat, protein and solids in colostrum compared to sows with the $LEPR^{AB}$ and $LEPR^{BB}$ genotypes. Differences in the content of individual components between the above genetic groups were 1.74% (*AA>AB*, $P \leq 0.01$) and 1.38% (*AA>BB*, $P \leq 0.05$) for fat, 0.44% (*AA>AB*) and 1.65% (*AA>BB*, $P \leq 0.05$) for protein, and 1.76% (*AA>AB*, $P \leq 0.05$) and 7.34% (*AA>BB*, $P \leq 0.01$) for solids. Analysis of the content of milk components over 21 days of lactation only revealed a statistically significant difference for protein content. $LEPR^{AB}$ heterozygotes were characterized by the highest protein content of milk ($P \leq 0.05$). Furthermore, as regards rearing of piglets during 21-day lactation, the *LEPR* gene had no effect on the number of piglets born or on their rearing performance. Different results for the number of piglets born were reported by Sun *et al*. [2009], who found that the polymorphism of this gene located in exon 2 had an effect on the number of piglets born in litters 1-4 from Luchuan sows. Luchuan sows of the $LEPR^{AA}$ genotype gave birth to significantly more live piglets compared to sows of the $LEPR^{AB}$ and $LEPR^{BB}$ genotypes. When studying the impact of the *LEPR* polymorphism on the composition of milk from cows of different breeds, Banos *et al*. [2008] also showed that this mutation had no noticeable effect on the content of individual nutrients. Suchocki *et al*. [2010] noted that in the Holstein-Friesian breed different alleles of the *LEPR* gene were associated with increasing milk fat content compared to the Jersey breed. On this basis the authors excluded the causal nature of the investigated *LEPR* mutation in cows.

It is concluded from the present results and the review of the literature that *MC4R* and *LEPR* polymorphisms affect carcass fat deposition in sows and production of nutrient-rich colostrum and milk. Sows of the $MC4R^{AA}$ genotype, characterized by higher feed intake and greater fatness [Piórkowska *et al*. 2010], and sows of the $LEPR^{BB}$ genotype, characterized by greater backfat thickness, produced colostrum with a lower content of fat, protein and solids ($LEPR^{BB}$ and $MC4R^{AA}$) as well as milk with lower fat and solids contents ($MC4R^{AA}$). Tummaruk et al. [2004] showed that an excessive reduction in backfat thickness, or the lack of body fat reserves in sows, has a negative effect on subsequent reproductive performance, and often contributes to their earlier culling due to excessive condition loss and disruption of lactation. On the other hand, Revell *et al*. [1998] demonstrated that overfat sows produced 15% less milk

compared to thinner sows, and these differences were more evident in early lactation. Szulc *et al*. [2013] also showed that PLW sows with backfat thickness exceeding 15 mm produced fewer piglets than sows with backfat thickness of 11-15 mm and less than 10 mm. The components of milk produced by the sows are derived not only from the components of consumed feed, but also from the dam's body fat reserves. Therefore, the optimal condition of pregnant sows and feeding level in advanced pregnancy and during nursing are important elements of lactation, during which colostrum and milk components are synthesized [Valros 2003, Beyga and Rekiel 2009]. Thus, it is thought that dams of the $MC4R^{GG}$ and $LEPR^{AA}$ genotypes, which were in good condition and probably had no tendency for excessive fat deposition, used the feed consumed during lactation in particular to enrich the composition of both their colostrum and milk. The present study showed no effect of the studied polymorphisms on rearing performance of the piglets; however, some trends were observed in the association between the *LEPR* and *MC4R* genes and piglet weight at birth and weight gains (at 7 and 21 days). Moreover, the direction of the influence of the investigated genes on colostrum and milk composition and sows' condition suggests that these markers could be used in selecting pigs of maternal breeds for decreased backfat thickness, without fear of reducing the quality of milk and thereby piglet rearing performance.

REFERENCES

1. ALSTON-MILLS B., IVERSON S.J., THOMPSON M.P., 2000 – A comparison of the composition of milks from Meishan and crossbred pigs. ***Livestock Production Science*** 63, 85-91.
2. ARIZA-NIETO C., BANDRICK M., BAIDOO S.K., ANIL L., MOLITOR T.W., HATHAWAY M.R., 2011 – Effect of dietary supplementation of oregano essential oils to sows on colostrum and milk composition, growth pattern and immune status of suckling pigs. ***Journal of Animal Science*** 89, 1079-1089.
3. BABICZ M., CICHOCKI R., KASPRZAK K., KROPIWIEC K., SZUBA K., 2011 – Analysis of relationship between sows colostrum and milk somatic cell count and reproductive performance traits. ***Annales Universitatis Mariae Curie-Skłodowska***, Sectio EE 3, 1-13.
4. BABICZ M., SKRZYPCZAK E., REJDUCH B., KOZUBSKA-SOBOCIŃSKA A., CHMIELOWIEC-KORZENIOWSKA A., KASPRZAK K., 2012 – Effect of thermal stress on reproductive performance parameters of sows with defined genotype at the RYR1 locus. ***Annals of Animal Science*** 12, 323-333.
5. BANOS G., WOOLLIAMS J.A., WOODWARD B.W., FORBES A.B., COFFEY M.P., 2008 – Impact of single nucleotide polymorphisms in leptin, leptin receptor, growth hormone receptor, and diacylglycerol acyltransferase (DGAT1) gene loci on milk production, feed, and body energy traits of UK dairy cows. ***Journal of Dairy Science*** 91, 3190-3200.
6. BARB C.R., HAUSMAN G.J., CZAJA K., 2005 – Leptin: a metabolic signal affecting central regulation of reproduction in the pig. ***Domestic Animal Endocrinology*** 29, 186-192.
7. BARŁOWSKA J., LITWIŃCZUK Z., FLOREK M., KĘDZIERSKA-MATYSEK M., 2007 – Yield and composition of goat milk from 4 Polish breeds differing in αs1-casein genotype. ***Medycyna Weterynaryjna*** 63, 1600-1603.
8. BEYER M., JENTSCH W., KUHLA S., WITTENBURG H., KREIENBRING F., SCHOLZE H., RUDOLPH P.E., METGES C.C., 2007 – Effects of dietary energy intake during gestation and lactation on milk yield and composition of first, second and fourth parity sows. ***Archives of Animal Nutrition*** 61, 452-468.

9. BEYGA K., REKIEL A., 2009 – Effect of the backfat thickness of sows in late pregnancy on the composition of colostrum and milk. ***Archiv Tierzucht*** 52, 593-602.
10. BURGOS C., CARRODEGUAS J.A., MORENO C., ALTARRIBA J., TARRAFETA L., BARCELONA J.A., LÓPEZ-BUESA P., 2006 – Allelic incidence in several pig breeds of a missense variant of pig melanocortin-4 receptor (*MC4R*) gene associated with carcass and productive traits; its relation to IGF2 genotype. ***Meat Science*** 73, 144-150.
11. GALVE A., BURGOS C., SILIÓ L., VARONA L., RODRIGUEZ C., OVILIO C., LÓPEZ-BUESA P., 2012 – The effects of leptin receptor (LEPR) and melanocortin-4 receptor (MC4R) polymorphisms on fat distribution and fat composition in a Duroc x Landrace/ Large White cross. ***Livestock Science*** 145, 145-152.
12. GOURDINE J.L., BIDANEL J.P., NOBLET J., RENAUDEAU D., 2006 - Effects of breed and season on performance of lactating sows in a tropical humid climate. ***Journal of Animal Science*** 84, 360-369.
13. HAUSMAN G.J., BARB C.R., 2010 – Adipose tissue and the reproductive axis: biological aspects. ***Endocrine Development*** 19, 31-44.
14. KIM K.S., LEE J.J., SHIN H.Y., CHOI B.H., LEE C.K., KIM J.J., CHO B.W., KIM T.H., 2006 – Association of melanocortin 4 receptor (MC4R) and high mobility group AT-hook 1 (HMGA1) polymorphisms with pig growth and fat deposition traits. ***Animal Genetics*** 37, 419-421.
15. LI X., KIM S.W., CHOI J.S., LEE Y.M., LEE C.K., CHOI B.H., KIM T.H., CHOI Y.I., KIM J.J., KIM K.S., 2010 – Investigation of porcine FABP3 and LEPR gene polymorphisms and mRNA expression for variation in intramuscular fat content. ***Molecular Biology Reports*** 37, 8, 3931-3939.
16. MEIDTNER K., WERMTER A.K., HINNEY A., REMSCHMIDT H., HEBEBRAND J., FRIES R., 2006 – Association of the melanocortin 4 receptor with feed intake and daily gain in F2 Mangalitsa x Piétrain pigs. ***Animal Genetics*** 37, 245-247.
17. ÓVILO C., OLIVER A., NOGUERA J.L., CLOP A., BARRAGÁN C., VARONA L., RODRIGUEZ C., TORO M., SÁNCHEZ A., PÉREZ-ENCISO M., SILIÓ L., 2002 – Test for positional candidate genes for body composition on pig chromosome 6. ***Genetics Selection Evolution*** 34, 465-79.
18. PIÓRKOWSKA K., TYRA M., ROGOZ M., ROPKA-MOLIK K., OCZKOWICZ M., RÓŻYCKI M., 2010 – Association of the melanocortin-4 receptor (MC4R) with feed intake, growth, fatness and carcass composition in pigs raised in Poland. ***Meat Science*** 85, 297-301.
19. PIERZCHAŁA M., PAREEK C.S., URBAŃSKI P., GOŁUCH D., KAMYCZEK M., RÓŻYCKI M., SMOCZYNSKI R., HORBAŃCZUK J.O., KURYŁ J., 2012 – Study of the differential transcription in liver of growth hormone receptor (GHR), insulin-like growth factors (IGF1, IGF2) and insulin-like growth factor receptor (IGF1R) genes at different postnatal developmental ages in pig breeds. ***Molecular Biology Reports*** 39, 3055-3066.
20. REVELL D.K., WILLIAMS I.H., MULLAN B.P., RANFORD J.L., SMITS R.J., 1998 – Body composition at farrowing and nutrition during lactation affect the performance of primiparous sows: II. Milk composition, milk yield and pig growth. ***Journal of Animal Science*** 76, 1738-43.
21. SCHMIDELY P.H., MESCHY F., TESSIER J., SAUVANT D., 2002 – Lactation response and nitrogen, calcium, and phosphorus utilization of dairy goats differing by the genotype for αs1-casein in milk, and fed diets varying in crude protein concentration. ***Journal of Dairy Science*** 85, 2299-2307.
22. SKRZYPCZAK E., BABICZ M., SZULC K., WALENDOWSKA A., PANEK A., 2012 – Impact of sow milk protein polymorphism on piglet rearing. ***Annals of Animal Science*** 12, 335-347.
23. STACHOWIAK M., SZYDŁOWSKI M., OBARZANEK-FOJT M., ŚWITOŃSKI M., 2006 – An effect of a missense mutation in the porcine melanocortin-4 receptor (MC4R) gene on production traits in Polish pig breeds is doubtful. ***Animal Genetics*** 37, 55–57.

24. STRATIL A., KOPECNY M., MOSER G., SCHROFFEL J., CEPICA S., 1998 – HpaII and RsaI PCRRFLPs within an intron of the porcine leptin receptor gene (*LEPR*) and its linkage mapping. Animal Genetics 29, 398-413.
25. SUCHOCKI T., KOMISAREK J., SZYDA J., 2010 – Testing candidate gene effects on milk production traits in dairy cattle under various parameterizations and modes of inheritance. ***Journal of Dairy Science*** 93, 2703-2717.
26. SUN C., WANG L., JIANG D.F., ZHANG B., 2009 – Missense mutations in exon 2 of the porcine leptin receptor gene and their associations with litter size and body weight. ***Czech Journal of Animal Science*** 54, 210-216.
27. SZULC K., KNECHT D., JANKOWSKA-MAKOSA A., SKRZYPCZAK E., NOWACZEWSKI S., 2013 - The influence of fattening and slaughter traits on reproduction in Polish Large White sows. ***Italian Journal of Animal Science*** 12, 16-20.
28. SZYNDLER-NĘDZA M., RÓŻYCKI M., ECKERT R., MUCHA A., KOSKA M., SZULC T., 2013a – Relationships between chemical composition of colostrum and milk and rearing performance of piglets during a 21 day lactation. ***Annals of Animal Science*** 4, 771-781.
29. SZYNDLER-NĘDZA M., TYRA M., ROPKA-MOLIK K., PIÓRKOWSKA K., MUCHA A., RÓŻYCKI M., KOSKA M., SZULC K., 2013b – Association between LEPR and MC4R genes polymorphism and composition of milk from sows of dam line. ***Molecular Biology Reports*** 40, 4339-4347.
30. TUMMARUK P., TANTASUPARUK W., TECHAKUMPHU M., KUNAVONGKRIT A., 2004 – Effect of season and outdoor climate on litter size at birth in purebred landrace and Yorkshire sows in Thailand. ***Journal of Veterinary Medical Science*** 66, 477-482.
31. TYRA M., ROPKA-MOLIK K., 2011 – Effect of the FABP3 and LEPR gene polymorphisms and expression levels on intramuscular fat (IMF) content and fat cover degree in pigs. ***Livestock Science*** 142, 114-120.
32. VALROS A., 2003 – Behaviour and physiology of lactating sows – associations with piglet performance and sow postweaning reproductive success. PhD thesis, Helsinki University, Finland. http://ethesis.helsinki.fi/julkaisut/ela/kliin/vk/valros/behaviou.pdf
33. VAN DEN MAAGDENBERG K., STINCKENS A., CLAEYS E., SEYNAEVE M., CLINQUART A., GEORGES M., BUYS N., DE SMET S., 2007 – The Asp298Asn missense mutation in the porcine melanocortin-4 receptor (MC4R) gene can be used to affect growth and carcass traits without an effect on meat quality. ***Animal*** 1, 1089-1098.
34. WANG W., HUE W., JIN B., ZHANG X., MA F., XU X., 2013 – Candidate gene expression affects intramuscular fat content and fatty acid composition in pigs. ***Journal of Applied Genetics*** 5, 113-118.
35. ZHANG C.Y., WANG Z., BRUCE H.L., JANZ J., GODDARD E., MOORE S., PLASTOW G.S., 2014 – Associations between single nucleotide polymorphisms in 33 candidate genes and meat quality traits in commercial pigs. ***Animal Genetics*** 45, 508-516.