Retinol binding protein 4 gene and reproductive traits in pigs

Abstract
The aim of the study was to use the DNA mutations in the RBP4 gene to determine associations between the different genotypes and litter size in Polish Large White sows. Reproductive traits investigated were: number of piglets born alive (NBA) and number of piglets weaned (NW). The polymorphism in RBP4 gene was detected using the PCR-RFLP method, with specific primers and the restriction enzymes MspI. Two different alleles of RBP4 gene were identified: allele A (0.65) and B (0.35) and three genotypes: AA (0.46), AB (0.37) and BB (0.17). The relationship between the RBP4 genotypes NBA and NW were analyzed. The analysis showed in first parity sows statistically significant (P ≤ 0.05) differences between sows carrying different RBP4 genotypes. In later parities sows with the BB genotype still had the largest litter size compared to AA and AB sows, but the difference was statistically not significant.

Key Words: RBP4 gene, pigs, polymorphism, reproductive traits

Zusammenfassung
Titel der Arbeit: Das RBP4-Gen und Wurfleistungsmerkmale beim Schwein
Ziel der Arbeit war die Untersuchung von DNA Mutationen im RBP 4-Gen und deren Zusammenhänge mit Wurfleistungsmerkmalen wie der Anzahl lebend geborener Ferkel (NBA) und Anzahl abgesetzter Ferkel (NW) bei Sauen der Großen Polnischen Rasse. Der Polymorphismus des RBP4-Gens wurde bei Anwendung der PCR-RFLP- Methode unter Heranziehung spezifischer Starter und des MspI-Restriktionsenzmys bestimmt. Es wurden die zwei Allele des RBP4-Gens A-Allel (0,65) und B-Allel (0,35) sowie die drei Genotypen AA (0,46) AB (0,37) und BB (0,17) identifiziert. Die Zusammenhänge zwischen den einzelnen Genotypen des RBP4-Gens und den Wurfleistungsmerkmalen konnten analysiert und diese bei den Erstlingswürfen der Sauen signifikant nachgewiesen werden. Diese Zusammenhänge fanden sich tendenziell auch bei späteren Würfen aber sie waren nicht signifikant.

Schlüsselwörter: Schwein, RBP4-Gen, Polymorphismus, Wurfleistungsmerkmale

The advancement in research on swine genome enabled identification of polymorphic loci of individual genes that control the level of reproductive traits, which are know to have influence on reproductive performance in sows (WANG et al., 2006; TERMAN, 2005; LINVILLE et al., 2001; DROGEMULLER et al., 1999; ROTHSCILD et al., 1996) and boars (TERMAN et al., 2006; MAĆKOWSKI et al., 2004; SCHLINGMANN et al., 2002; KMIEĆ et al., 2001). Molecular techniques can now be used to increase rate of response to selection. It has been proposed that “candidate gene” analyses be used to identify individual genes responsible for traits of economic importance (ROTHSCHILD and SOLLER, 1997). One of these genes can be retinol binding protein 4 gene which was localized in chromosome 14 in pigs. HARNEY et al., (1993) have shown that there is an increasing RBP4 gene expression in gravid porcine endometrium from d 10 to 12. Their results support an important role for this vitamin A transport protein in uterine and conceptus
physiology during the establishment of pregnancy. Therefore, RBP4 was investigated as a candidate gene for litter size owing to its role at the time of high embryonic mortality rate.

A SacI polymorphism was detected in pig genomic DNA by hybridization of Southern blots with a pig retinol-binding protein 4 (RBP4) probe (MESSER et al., 1996). This polymorphism was significantly associated with litter size in pigs (DROEGEMULLER et al., 2001; ROTHSCCHILD et al., 1996, 2000).

The purpose of this study was conducted to examine the RBP4 gene polymorphism and its effects on reproductive traits in Polish Large White sows. Reproductive traits investigated were: number of piglets born alive (NBA) and number of piglets weaned (NW).

Materials and Methods

The experimental populations included in total 101 Polish Large White sows. The animals were bred and raised at a farm in Western Pomerania (Poland). Rearing and feeding conditions were equalized for all animals. Genomic DNA was extracted from blood sample using Master Pure kit of Epicentre Technologies.

Genotypes of the RBP4 gene were determined by the PCR-RFLP method, but only for the sows that had farrowed successfully. The RBP4 gene fragment was amplified from genomic template using the PCR with designed primers in exons 2 and 4 of sequences reported by ROTHSCCHILD et al., (2000). The region of the RBP4 gene containing the polymorphic MspI site was amplified using primers: forward – GAGCAAGATGGAATGGGTT and reverse – CTCGGTGCTCTGTAAGGTTG in a 15 µl PCR containing using 90 ng porcine genomic DNA, 200 µM of each dNTP, 1.5 mM MgCl₂ and 0.6 units of Taq DNA polymerase (MBI Fermentas) in a standard 1x PCR buffer. Primers were used at a concentration of 10 pmol with a thermal cycling regime of 93°C for 3 minutes followed by 40 cycles of 93°C from 30 second, 56°C for 45 second, 72°C for 45 second and ending with a final step of 72°C for 5 minutes. Digestion of PCR product (550 bp) was performed with 3 I.U. of appropriate restriction endonuclease MspI at 37°C overnight. PCR product was then examined by electrophoresis on a 2% agarose gel stained with ethidium bromide. After that the gels were analyzed in UV rays. Performance traits data were collected from farm documentation and they contained: number piglets born alive (NBA) and numbers of piglets weaned (NW). The relations between RBP4 genotypes and studied reproductive traits were analyzed with one-way analysis of variance and the significance of differences was verified using Duncan test with computer program Statistica’99.

The following model was used:

\[ y_{ijklm} = \mu + G_i + Y_j + M_k + P_1 + S_m + e_{ijklm} \]

where:

- \( y_{ijklm} \) – observed value,
- \( \mu \) – overall mean,
- \( G_i \) – effect of i-th genotype (i = AA, AB, BB),
- \( Y_j \) – effect of j-th year of farrowing (j = 1, 2, 3, …, 6),
- \( M_k \) – effect of k-th month (k = 1, 2, 3, …, 12),
$P_l$ – effect of l-th parity ($l = 1, 2, 3, \geq 4$),
$S_m$ – effect of m-th sire,
$e_{ijklm}$ – random residual.

**Results**

Two different $RBP4$ alleles were identified in the sows herd under study: allele A and allele B that controlled three genotypes, namely AA, AB and BB. The lengths of restriction fragments detected during the experiment are given in Table 1. The allele and genotype frequency were distributions in Hardy-Weinberg equilibrium.

<table>
<thead>
<tr>
<th>Candidate Gene</th>
<th>PCR product size (bp)</th>
<th>Endonuclease</th>
<th>Allele size (bp)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$RBP4$</td>
<td>550</td>
<td>$MspI$</td>
<td>A – 190, 154, 136, 70</td>
<td>ROTHSCCHILD et al. (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B – 190, 136, 125, 70, 29</td>
<td></td>
</tr>
</tbody>
</table>

In the analyzed sows herd the allele A occurred with the frequency 0.65, whereas the allele B – with the frequency 0.35. The AA genotype occurred with the frequency 0.46, AB with frequency 0.37 and BB with 0.17 – Table 2.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>Large White</td>
<td>number</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>frequency</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Table 1

Endonuclease and allele sizes of $RBP4$ gene

Table 2

The frequency of $RBP4$ genotypes and alleles of Polish Large White sows

<table>
<thead>
<tr>
<th>RBP4 genotype</th>
<th>Parity</th>
<th>n</th>
<th>NBA$^i$</th>
<th>NW$^i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AA$</td>
<td>I</td>
<td>43</td>
<td>9.07$^a \pm 2.27$</td>
<td>8.12$^A \pm 2.24$</td>
</tr>
<tr>
<td>$AB$</td>
<td></td>
<td>37</td>
<td>9.05$^a \pm 2.43$</td>
<td>7.92$^A \pm 2.38$</td>
</tr>
<tr>
<td>$BB$</td>
<td></td>
<td>17</td>
<td>9.41$^b \pm 2.42$</td>
<td>9.00$^B \pm 1.84$</td>
</tr>
<tr>
<td>$AA$</td>
<td>II</td>
<td>37</td>
<td>8.73$^a \pm 2.61$</td>
<td>7.59$^a \pm 2.01$</td>
</tr>
<tr>
<td>$AB$</td>
<td></td>
<td>29</td>
<td>9.17$^a \pm 2.83$</td>
<td>8.07$^a \pm 2.68$</td>
</tr>
<tr>
<td>$BB$</td>
<td></td>
<td>9</td>
<td>10.67$^b \pm 3.00$</td>
<td>9.78$^b \pm 2.63$</td>
</tr>
<tr>
<td>$AA$</td>
<td>III</td>
<td>27</td>
<td>8.98$^a \pm 3.10$</td>
<td>8.07$^a \pm 2.37$</td>
</tr>
<tr>
<td>$AB$</td>
<td></td>
<td>22</td>
<td>9.68$^a \pm 2.85$</td>
<td>8.59$^a \pm 2.65$</td>
</tr>
<tr>
<td>$BB$</td>
<td></td>
<td>5</td>
<td>10.00$^b \pm 2.45$</td>
<td>9.40$^b \pm 2.30$</td>
</tr>
<tr>
<td>$AA$</td>
<td></td>
<td>76</td>
<td>9.35$^a \pm 2.70$</td>
<td>7.84$^a \pm 2.10$</td>
</tr>
<tr>
<td>$AB$</td>
<td>≥ IV</td>
<td>111</td>
<td>9.68$^a \pm 2.64$</td>
<td>8.23$^a \pm 2.55$</td>
</tr>
<tr>
<td>$BB$</td>
<td></td>
<td>13</td>
<td>10.38$^a \pm 2.10$</td>
<td>8.69$^a \pm 1.75$</td>
</tr>
</tbody>
</table>

Small letters (a, b) denoted significance difference (P$\leq 0.05$); capital letters (A, B) denoted significance difference (P$\leq 0.01$).

Table 3

Effects of $RBP4$ genotypes on reproductive traits of sows

$^i$ NBA – number of piglets born alive; NW – number of piglets weaned,
n – number of sows within parities.

The relationship between the $RBP4$ genotypes and litter size were analyzed – Table 3. The analysis of the number born piglets alive (NBA), showed in first and second parity sows statistically significant (P$\leq 0.05$) differences between sows carrying BB
genotypes compared to AA and AB genotypes. The sows with BB genotypes had also larger number of piglets weaned (NW) than sows carrying AA and AB genotypes and this differences were statistically significant (P≤0.01) in first parity and (P≤0.05) in second parity – Table 3. In later parities sows with the BB genotype still had the largest litter size compared to AA and AB sows, but the difference was statistically not significant. Analysis of the interaction PARITY x RBP4 showed small and non-significant differences.

Discussion
A similar frequency of allele A (0.62) was observed in German Landrace x Duroc crossbreed sows (DROEGEMÜLLER et al. 2001; ROTHSCCHILD et al., 2000). A higher frequency of allele A (0.67) was observed by DROEGEMÜLLER et al. (2001), who studied the breed German Landrace. A lower frequency of allele A compared to the present study was revealed in Landrace (0.59), Large White (0.55) and Landrace x Large White crossbreed (0.42) pigs (ROTHSCCHILD et al., 2000; WANG et al., 2006; LINVILLE et al., 2001).

In the current study, many candidate gene markers for litter size was found including alleles at the ESR gene locus, the PRLR locus, and the CYP21 locus (ROTHSCCHILD et al., 1996, 2000; SHORT et al., 1997; VINCENT et al., 1998; TERNMAN, 2005; ZIEMAK and GRZESIAK, 2006). ROTHSCCHILD et al. (2000), using data of nearly 2,800 litters of 1,300 RBP4 genotyped sows of six commercial lines, reported a significant additive effect associated with RBP4 of +0.15 NBA. ROTHSCCHILD et al. (2000) stated that it is difficult to determine whether it is linked to one or more genes having this effect. WANG et al. (2006), showed that sows with BB genotype of RBP4 locus had more piglets per litter than sows with AA and AB genotypes. These results are comparable to the results reported in this study.

The analysis of the total number born (TNB) and number born alive (NBA) reported by DROEGEMÜLLER et al. 2001, showed no significant effect RBP4 locus on litter size in pigs.

Conclusion
The preliminary study of RBP4 gene showed that the sows with the BB genotype produced more piglet than sows with the AA and AB genotypes. This result was confirmed statistically (P≤0.05) in first and second parity. The present study showed that the RBP4 gene is strongly associated with litter size in sows. The analysis of the number piglets born alive (NBA), numbers piglets weaned (NW) in later parities showed small and statistically not significant differences between sows carrying different RBP4 genotypes. The obtained results suggest a possibility of utilisation of the polymorphism in breeding that aims at an improvement of some reproductive performance traits of sows.

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