A further look on genetic basis of carcass fat deposition in pigs of 'Casertana' ancient autochthonous genetic type

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A further look on genetic basis of carcass fat deposition in pigs of 'Casertana' ancient autochthonous genetic type


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Abstract. 'Casertana' pig ancient autochthonous genetic type (AAGT) has been considered in the years within wide programme of safeguard and rescue promoted by ConSDABI. One of the most relevant aims of this program is to search for gene variation related to quantity and technological, nutritive and organoleptic quality of fat in order to satisfy consumer's request. In the present work, 100 AAGT Casertana pigs were genotyped for 8 SNP at loci involved in the regulation of adipose tissue deposition [DECR1, FASN, MC4R, SCD and H-FABP haplotype (H-FABP1, H-FABP2, H-FABP3)] in order to carry out an association study. With some fatness characteristics of carcass. Statistical analysis was performed using the GLM procedure of the SAS package. The results showed association between HFABP haplotype and the majority of considered phenotypic traits. In detail, the effect of this haplotype was significant on the total weight of separable fat in carcass (P=0.002), total weight of adipose cuts (P=0.006) and back fat thickness measured at level of 1st thoracic vertebra (Th1) (P=0.056), 15th thoracic (Th15) (P=0.020) and between the 6th lumbar vertebra and sacrum (L6 – S) (P=0.007). FASN showed an effect on belly and jowl fat (P \leq 0.05). For DECR1 CC genotype influenced the weight of belly cut. Further investigations are ongoing for an operative utilisation of the H-FABP haplotype, FASN and DECR1 genes as molecular markers (candidates) in proper molecular assisted selection (MAS) plans.

Keywords. Casertana 'AAGT' – Fat traits – SNPs – Haplotype.
I – Introduction

'Casertana' (CT) ancient autochthonous genetic type (AAGT) is a black pig of ancient origins. It is object of considerable interest for its many peculiarities, like: good aptitude to grazing with ability to utilize poor feed; appreciable organoleptic and healthy quality of meat which is particularly suitable to obtain valuable local products (Matassino et al., 1968; Colatruglio et al., 1994; Girolami et al., 1996; Matassino et al., 2006; Barone et al., 2008). In the last years, the awareness of the nutritional issue has increased: healthy quality is the element that more concerns the consumer. In this context, the study of quanti-qualitative characteristics of the adipose tissue as well as of the factors influencing them in farm animals, especially of pig, has become increasingly important. Furthermore ongoing research at ConSDABI SUB NFP.I - FAO on pig AAGTs is corroborating the hypothesis to consider pig as a valid model to study human obesity. These pigs, characterized by a thicker back fat than cosmopolite breeds, can constitute an interesting resource to contribute to the knowledge of genetic factors involved in obesity; moreover, AAGTs can constitute a genetic reserve suitable to rescue organoleptic properties penalized in cosmopolite breeds. Different approaches are used to identify molecular markers linked to traits associated to adipose tissue deposition in pig: (i) candidate gene; (ii) QTL identification; (iii) combination of (i) and (ii); (iv) transcriptome analysis (Davoli et al., 2009).

Various genes involved in quali-quantitative characteristics of muscle and adipose tissue have been identified. The aim of the present contribute was to evaluate, in CT pig, possible associations between 8 SNPs at loci involved in regulation of adipose tissue deposition [DECR1, FASN, MC4R, SCD and H-FABP haplotype (H-FABP1, H-FABP2, H-FABP3)] and some fatness characteristics of carcass; these loci are known in literature for their significant association with some fatness traits (Gerbens et al., 1997; Kim et al., 2000; Wimmers et al., 2002; Munoz et al., 2003; Amills et al., 2005; Matassino et al., 2007 and 2009).

II – Materials and methods

DNA was extracted from blood and muscle samples of 100 subjects of CT AAGT, reared at experimental Farm of ConSDABI SUB NFP.I - FAO. Genotyping for SNPs considered at DECR1, FASN, H-FABP, MC4R and SCD loci was carried out by PCR-RFLP method according to literature protocols (Table 1).

Table 1. SNPs investigated in 'candidate' loci object of study

<table>
<thead>
<tr>
<th>SNP</th>
<th>ACRONYM DENOMINATION</th>
<th>LOCUS</th>
<th>FUNCTION</th>
<th>CHROMOSOME</th>
<th>BIBLIOGRAPHIC REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(163)G</td>
<td>DECR1</td>
<td>2,4 - Dienoyl reductase 1 mitochondrial</td>
<td>Encodes for an enzyme involved in unsaturated fatty acid beta-oxidation</td>
<td>4</td>
<td>Davoli et al., 2002</td>
</tr>
<tr>
<td>T265C</td>
<td>FASN</td>
<td>Fatty acid synthase</td>
<td>Enzyme key in the conversion of acetyl-CoA and malonyl-CoA into long-chain saturated fatty acids</td>
<td>12</td>
<td>Munoz et al., 2003</td>
</tr>
<tr>
<td>C(1811)G</td>
<td>H-FABP1 (D/d)</td>
<td>Heart fatty acid - binding protein</td>
<td>Trafficking of fatty acids at level of cardiac and striate muscle as well as lactating mammary gland</td>
<td>6</td>
<td>Gerbens et al., 1997</td>
</tr>
<tr>
<td>T(1324)C</td>
<td>H-FABP2 (H/h)</td>
<td>H-FABP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G(298)A</td>
<td>MC4R</td>
<td>Melanocortin 4 receptor</td>
<td>Control of uptake capacity and growth rate</td>
<td>1</td>
<td>Kim et al., 2000</td>
</tr>
<tr>
<td>T(233)C</td>
<td>SCD1</td>
<td>SCD</td>
<td>Stearoyl CoA Desaturase</td>
<td>14</td>
<td>Ren et al., 2004</td>
</tr>
<tr>
<td>C(641)T</td>
<td>SCD3</td>
<td>SCD</td>
<td>Stearoyl CoA Desaturase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For each SNP the allelic and genotypic frequencies were estimated. Concerning H-FABP, 12 out of 27 possible haplotypes were individuated, only 6 of which (HHddAa, HHddAA, HHDdAa, HHDdAA, HHdAa, HhDdAa) were used for statistical elaboration because of the insufficient
numerousness of the others; for each fatness indicator 15 possible pair-wise comparisons were carried out. In the present contribute the following parameters at carcass dissection were considered as adiposity indicators: (i) total weight of adipose cuts (with and without 'jowl'); (ii) total weight of separable fat; (iii) thickness (mm) of back fat measured at level of: 1st thoracic vertebra (Th1), 15th thoracic vertebra (Th15) and between the 6th lumbar vertebra and sacrum (L6 - S).

Statistical elaboration, concerning the association between genotypic and phenotypic data, was performed with the following factorial model of covariance analysis with fixed factors utilizing SAS 9.1v software:

\[ Y_{ijk} = \mu + b_1x_1 + b_2x_2 + b_3x_3 + \alpha_i + \text{sex}_j + e_{ijk} \]

where:

- \( \mu \) = constant common to all observations (overall mean);
- \( x_1 \) = weight of refrigerated carcass after 72 hours of refrigeration (covariate);
- \( x_2 \) = date of slaughtering (covariate);
- \( x_3 \) = age of the pig at slaughtering (covariate);
- \( \alpha_i \) = fixed effect common to all observation relative to \( i \)th genotype (\( i = 1,2,3 \)/haplotype (\( i=1, 2, 3, 4, 5, 6 \));
- \( \text{sex}_j \) = fixed effect common to all observations related to \( j \)th sex (\( j = 1,2 \));
- \( e_{ijk} \) = random error.

III – Results and discussion

From statistical elaboration it emerged a significant effect of some loci on carcass fatness. H-FABP. The comparison among the considered haplotypes highlighted that the pig with HHddAA or HHddAa haplotype, when compared with a subject with HHddAA or HHddAa or HHddaa haplotype, gives a higher adiposity estimated through any detected parameters (Table 2). In particular, the difference of: (i) back fat thickness can vary from about 9 (HHddAa vs HHddAA; \( P=0.020 \)) to about 17 mm (HHDdAA vs HHddAA; \( P=0.002 \)); (ii) total fat cuts (without belly) can range from 6.5 (HHddAA vs HHddAa; \( P=0.075 \)) to about 16 kg (HHDdAa vs HHddaa; \( P=0.002 \)); (iii) of separable cuts can vary from about 6.0 (HHDdAA vs HHddAa; \( P=0.073 \)) to 13.5 kg (HHDdAa vs HHddAA; \( P=0.0001 \)).

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Parameter</th>
<th>‘Fat cuts’, kg</th>
<th>‘Total separable fat’, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference (I)</td>
<td>Comparison (J)</td>
<td>‘Th1’ ( P )-value</td>
<td>‘Th15’ ( P )-value</td>
</tr>
<tr>
<td>HHddAA</td>
<td>12.443</td>
<td>0.031</td>
<td>15.113</td>
</tr>
<tr>
<td>HHddAa</td>
<td>10.958</td>
<td>0.035</td>
<td>11.934</td>
</tr>
<tr>
<td>HHddaa</td>
<td>15.412</td>
<td>0.043</td>
<td>15.277</td>
</tr>
<tr>
<td>HHddAA</td>
<td>11.321</td>
<td>0.031</td>
<td>11.640</td>
</tr>
<tr>
<td>HHddAa</td>
<td>9.836</td>
<td>0.022</td>
<td>8.461</td>
</tr>
<tr>
<td>HHddaa</td>
<td>14.290</td>
<td>0.043</td>
<td>11.804</td>
</tr>
</tbody>
</table>

The biological and operative importance to consider the effect of a haplotype on a qual-quantitative trait has been widely debated (Matassino et al., 1993; Zullo et al., 1994). Indeed, according to these authors, the global genotype has a semantic value for marker assisted selection (MAS). Within H-FABP, it was believed convenient to individuate a possible effect of
genotype at single locus on adiposity using single nucleotide polymorphism. The effect was significant only for D/d polymorphism: the subject with dd genotype gave a significant lower adiposity in comparison with heterozygote for the majority of the parameters considered, with a decrease equal to: (i) about 10 mm for back fat thickness (P= 0.003 at Th1 region; P= 0.001 at Th15 and L6-S regions); (ii) about 8 kg for total fat cuts and total separable fat (P<0.001).

FASN. The effect was significant on the weight of belly fat cut (P=0.051) and on that of separable fat from jowl (P=0.048). It is interesting to observe that the subject with TT genotype, in comparison with that with CC or CT genotype, gives a significant (P<0.05): (i) lower weight of belly cut [the difference is equal to 0.895 kg and 1.072 kg for CC vs TT and CT vs TT comparisons, respectively]; (ii) higher weight of separable fat from jowl (difference equal to -0.439 kg and - 0.454 for CC vs TT and CT vs TT comparisons, respectively).

DECR1. The effect of this gene was near to critical limit of significance (P= 0.072) on jowl; from the comparison between genotypes it emerges that the pig with CC genotype gives a lighter belly than the subject with CG or GG genotype; the comparison was significant (P= 0.022) for CC vs CG with a difference of -0.902 kg and tendentially significant (P=0.135) for CC vs GG with a difference of - 0.608 kg.

SCD. This gene tended to influence the weight of separable fat from coppa adipose cut (P= 0.148), back fat (P= 0.144) and total separable fat (P= 0.132).

MC4R. A tendency to significance (P= 0.179) was evidenced for separable fat from jowl.

**IV – Conclusions**

In the limits of the observation field, the results suggest that H-FABP haplotype significantly affects the parameters used for the estimation of carcass adiposity, determining differences among subjects with different haplotypes. The pig with HHddAA would be the less physiological obese for the total of adipose cuts without jowl and of separable fat. FASN locus would cause a differential fat deposition probably associated to the effect of TT genotype showing a variable lipogenetic aptitude in different anatomical regions of the body. For DECR1 locus, CC genotype would influence the weight of belly fat. Further investigations are ongoing for an operative utilisation of the H-FABP haplotype and FASN and DECR1 genes as molecular markers (candidates) in proper MAS plans.

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**References**


