Variability in candidate genes revealed associations with meat traits in the Piemontese cattle breed

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4   **Variability in candidate genes revealed associations with meat traits in the Piemontese**

5   **cattle breed**

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Abstract

In the last years an increasing number of associations between SNPs in candidate genes and several production traits have been reported in beef cattle, but very often the results were not validated and few studies considered breeds homozygous for the allele responsible for the muscular hypertrophy. Therefore, we analysed the variability of 19 previously reported SNPs in 12 genes (*GH*, *GHR*, *GDF8*, *GHRL*, *IGF2*, *LEP*, *LEPR*, *MYF5*, *NPY*, *POMC*, *UCP2*, *UCP3*) in the hypertrophic Piemontese breed and investigated the effects of the observed polymorphisms on growth and conformation traits recorded during performance testing. Fourteen SNPs were polymorphic and a significant linkage disequilibrium was observed between SNPs in *GHR*, *LEP* and *NPY* genes, for which both single-SNP and haplotype effects were estimated. Negligible effects on the investigated traits were observed for *GHRL*, *MYF5*, *NPY*, *POMC*, *UCP2* and *UCP3* genes. The *GHR* gene significantly affected daily gain and its effect was further increased when haplotypes were considered (*G-A vs G-G*: +34.04 g/d). The *C* allele at LEP-1 and LEP-2 had moderate negative effects on the considered traits, whereas the *C* allele at LEP-3 mostly had positive effects; relative to single SNPs, haplotypes in the *LEP* gene showed weaker but favourable associations with all the traits. The *C* allele at *IGF2* and *LEPR* had favourable effects on daily gain and negative effects on meat conformation traits. The associations observed for *GHR* and *LEP* were consistent with those of previous studies, providing additional evidence of their usefulness as markers. Practical aspects of the applications to the breeding programme of the Piemontese breed need to be examined.

Keywords: Cattle, Piemontese breed, SNPs, Meat production

Introduction

46   To date a great number of candidate genes for production traits have been suggested in

47   different livestock species, based on the knowledge of their position and/or function. For meat

48   production, the interest has been mainly focused on genes involved in growth and meat

49   quality, but only for a limited number of genes the effects of their polymorphisms have been

50   investigated, often in a single breed.

51   On the other hand, the recent development of high-density SNP (single nucleotide

52   polymorphism) genotyping microarrays has opened new selection perspectives for the

53   possibility of estimating the breeding value of animals with no phenotypic records, with the

54   potential advantages of increased genetic gain and lower costs (Meuwissen *et al*., 2001).

55   However, as many of the genotyped SNPs are located in anonymous regions, the detection of

56   associations with traits of interest does not directly lead to the identification of the underlying

57   genes (Magee *et al*., 2010). For these reasons the candidate gene approach, which aims at

58   identifying specific polymorphisms responsible for the observed effects in genes biologically

59   related to the traits of interest, is still a valuable strategy (Ron and Weller, 2007).

60   On the basis of these considerations we carried out the present study in order to give a

61   contribution to the analysis of genes possibly related to meat production. We focused on 12

62   genes, which were selected on the basis of their biological functions and for which effects on

63   the traits of interest had been reported: *growth hormone* (*GH*), *growth hormone receptor*

64   (*GHR*), *growth differentiation factor 8* (*GDF8*), *ghrelin* (*GHRL*), *insulin-like growth factor 2*

65   (*IGF2*), *leptin* (*LEP*), *leptin receptor* (*LEPR*), *myogenic factor 5* (*MYF5*), *neuropeptide Y*

66   (*NPY*), *proopiomelanocortin* (*POMC*), *uncoupling protein 2* (*UCP2*), *uncoupling protein 3*

67   (*UCP3*). The products of most of these genes are involved in biologically-related processes

68   regulating feed intake and growth. Circulating leptin, after binding to specific receptors in the

69   brain, exerts its effects on feed intake and energy homeostasis *via* neurotransmitters such as

70   neuropeptide Y and pro-opiomelanocortin (Houseknecht & Portocarrero, 1998). Leptin also

71   increases the expression of uncoupling protein 2 and 3, involved in energy expenditure

72   (Scarpace *et al*., 1997), and modulates the secretion of growth hormone (Zieba *et al*., 2003),

73   which binds to GH receptors on target tissues, activating the signal transduction culminating

74   in GH biological effects (Kopchik & Andry, 2000).

75   Polymorphisms in the considered genes have been shown to affect growth, feed efficiency

76   and carcass quality in different cattle breeds and crossbreds (Kim *et al*., 2004; Li *et al*., 2004;

77   Buchanan *et al*., 2005; Nkrumah *et al*., 2005; Di Stasio *et al*., 2007; Goodall & Schmutz,

78   2007; DeVuyst *et al*., 2008; Sherman *et al*., 2008). It seems worth noting that very few studies

79   considered breeds homozygous for the allele responsible for the muscular hypertrophy, which

80   might interfere with genes affecting meat production as a consequence of its well known

81   effects on growth and muscle development.

82   Therefore, the objective of this study was to estimate the variability of the above twelve

83   genes and their associations with traits recorded during the performance testing of breeding

84   candidates in the hypertrophic Piemontese breed.

85

86   **Materials and methods**

87   The study was carried out on 201 Piemontese male calves enrolled in the performance testing

88   programme at the central Station of the Italian Association of Piemontese Cattle Breeders.

89   The performance testing programme of the Piemontese breed is described in Albera *et al*.

90   (2001).

91   Eight traits recorded during the performance testing were considered: average daily gain

92   (DG), withers width (WW), shoulder muscularity (SM), loins width (LW), loins thickness

93   (LT), thigh muscularity (TM), thigh profile (TP) and bone thinness (BT). The conformation

94   traits were graded through a linear scoring of live animals using a 9-point scale, as reported

95 by Albera *et al*. (2001). Descriptive statistics for the investigated traits are presented in Table

96 1.

97 Blood samples were collected in tubes containing ethylenediaminetetraacetic acis as an

98 anticoagulant and kept at 4°C until DNA isolation. Genomic DNA was extracted using the

99 NucleoSpin[®] Blood kit (Macherey-Nagel, Düren, Germany). A total of 19 SNPs were

100 investigated in 12 genes (Table 2). Genotyping was performed by a commercial company

101 (http://www.kbioscience.co.uk).

102 Allele frequencies were estimated by simple counting. Tests for Hardy-Weinberg

103 equilibrium at each SNP and for linkage disequilibrium between the SNP pairs were

104 performed using the FSTAT software (Goudet, 2002). For the linked SNPs, haplotypes were

105 constructed using the PHASE v.2.1 software (Stephens *et al*., 2001), which implements a

106 Bayesian method for reconstructing haplotypes from population genotype data.

107 The association of the observed polymorphisms with phenotypes for the recorded traits

108 was investigated using a statistical model similar to that used for the prediction of breeding

109 values of Piemontese bulls, but also including the effect of the single SNP or haplotype.

110 The general univariate linear model, in matrix notation, was:

111 $y = Xb + Zu + Wc + e$

112 where y is a vector of observations on the considered trait, b is a vector of systematic

113 nongenetic effects, u is a vector of animal additive genetic effects, c is a vector of SNP

114 genotype or haplotype effects, e is a vector of random residuals and X, Z and W are incidence

115 matrices of proper order relating observations to b, u and c, respectively.

116 For all traits, nongenetic effects included in the linear model were the effect of the

117 contemporary group of animals on test and of the parity of the dam. Additionally, the weight

118 at the beginning of the test for growth and the weight at scoring for meat conformation traits

119 were included as covariates. For the single SNPs analysis, the model included the effect of the

120 SNP genotype, whereas for the haplotypes the regression on the number of haplotype copies

121 was included, as the accuracy of the haplotype reconstruction was very high (P: 0.938 to

122 0.998).

123   The effects of the observed polymorphisms were investigated using Bayesian procedures.

124 The Bayesian analysis, performing numerical integration through Gibbs sampling, was used

125 to estimate the marginal posterior distribution of parameters of concern (Legarra *et al.*, 2008).

126 Animal and residual effects were assumed to be normally distributed "a priori" as

127 $u \sim N(0, A\sigma_a^2)$ and $e \sim N(0, I\sigma_e^2)$, respectively, where A was the numerator relationship

128 matrix, σ_a^2 was the additive genetic variance, I was an identity matrix of proper order and σ_e^2

129 was the residual variance. Flat priors were assumed for systematic nongenetic and for SNP

130 genotype or haplotype effects. As the number of animals included in the study was too limited

131 to estimate variance components, estimates of additive genetic and residual variances

132 obtained by Albera *et al.* (2001) were used. A single chain of 1,000,000 iterations with a

133 burn-in of 200,000 was run for each trait/SNP analysis, saving samples every 400 iterations.

134   Inference on additive and dominance SNP effects, as defined by Falconer & Mackay

135 (1996), was based on the estimated marginal posterior density of these effects. Haplotype

136 effects were estimated as deviations from the effect of the 'reference' haplotype which was

137 arbitrarily set to zero. The 'reference' haplotype was chosen randomly. The mean of the

138 marginal posterior distribution of a SNP/haplotype effect was used as a point estimate of the

139 effect.

140   On the basis of the realised response to selection for meat traits in the Piemontese

141 population in the last ten years (ANABORAPI, 2010) and also considering the effectiveness

142 of exploiting variation due to candidate genes, a SNP/haplotype effect was considered to be

143 relevant when its absolute value was greater than 10% of the additive genetic standard

144   deviation of the trait. For a given effect, the probability of being relevant was calculated from

145   the estimated marginal posterior distribution.

146

147   **Results**

148   Genotyping revealed that GDF8-1, GDF8-2, GH, GHR-1 and NPY-1 were monomorphic in

149   the examined sample (Table 2). The absence of variability for GDF8-2, in the exon 1 of

150   *GDF8* gene, seems noteworthy, because previous studies had reported the presence of the *A*

151   allele in the Piemontese breed (McPherron & Lee, 1997), although at a very low frequency

152   (0.02; Vankan *et al*., 2010).

153   The polymorphic SNPs showed a different degree of variability, with the minor allele

154   frequency ranging from 0.08 (LEPR) to 0.45 (GHR-2). For all the SNPs, the genotype

155   frequencies were in agreement with Hardy-Weinberg equilibrium frequencies ($P>0.05$).

156   A linkage disequilibrium significant at the 5% nominal level was observed for SNPs within

157   a gene: GHR-2 – GHR-3, LEP-1 – LEP-2 – LEP-3, NPY-1 – NPY-2. For these SNPs, both

158   single SNPs and haplotype effects were investigated.

159   Seven SNPs, which in other breeds showed associations with growth, feed efficiency, and

160   carcass traits (Li *et al*., 2004; Buchanan *et al*., 2005; Sherman *et al*., 2008), in the Piemontese

161   breed exhibited negligible effects on the investigated traits. These effects were of small

162   magnitude (MYF5, NPY-2, NPY-3) or showed a very wide posterior distribution (GHRL,

163   POMC, UCP2 and UCP3) and, therefore, will be not further discussed.

164   In the *GHR* gene (Table 3), the *A* allele of GHR-2 had a general unfavourable additive

165   effect on meat conformation traits and especially on BT, as well as relevant dominance

166   effects, particularly on WW and LT. A large favourable additive effect, associated to the *A*

167   allele, on DG and BT was observed for GHR-3. The effect on DG greatly increased when

168   haplotypes of the two SNPs were considered: the association of the favourable *A* allele at

169 GHR-3 with the slightly favourable *G* allele at GHR-2 raised the effect on DG to 34 g/d

170 (nearly 0.45s_A), with a probability for the effect of being relevant (greater than 0.1s_A) as high

171 as 95%, whereas it exerted a negative effect on muscularity and especially on BT.

172   As for the SNPs in the *LEP* gene (Table 4), the *C* allele at LEP-1 was consistently

173 associated with negative values for all the traits, except BT; relevant dominance effects on the

174 traits related to meat conformation were also observed. For LEP-2, results were comparable to

175 those for LEP-1, with the *C* allele exerting negative additive effects on all traits with the

176 exception of BT. The *C* allele at LEP-3 was associated with increased DG, with an estimated

177 additive effect of 32.0 g/d (i.e., 0.42s_A) and a probability of the effect being larger than 0.1s_A

178 of 80%.

179   For the analysis of the combined effects of the three SNPs in the *LEP* gene, only the most

180 frequent haplotypes were considered: *C-G-C* (0.45), *T-G-C* (0.37) and *C-C-T* (0.14). Four

181 additional rare haplotypes were found, with a cumulative frequency lower than 0.04.

182 Compared to haplotype *C-G-C*, the haplotype containing all the favourable alleles (*T-G-C*)

183 confirmed the favourable association with DG and showed positive effects, although of little

184 magnitude on the other traits (Table 5). The haplotype combining the less favourable alleles

185 (*C-C-T*) showed trivial effects on DG, but surprisingly positively affected meat conformation

186 traits, particularly those related to the muscularity of the fore part of the body (WW and SM).

187   A large positive effect of the *C* allele at IGF2 was detected for DG (24.14 g/d), whereas

188 small negative additive effects were observed for meat conformation traits (Table 6). For

189 LEPR (Table 6), a relevant additive effect was observed on DG, with the *C* allele associated

190 to higher values (about 45 g/d); negative additive and dominance effects were observed for all

191 the conformation traits.

192

193 **Discussion**

194 In the past decades an increasing number of associations between SNPs in candidate genes

195 and several production traits have been reported in beef cattle, but very often no studies were

196 performed to validate the results, or inconsistencies were observed across populations, so that

197 the possibility to exploit the detected associations in selection programmes was limited.

198 The present study revealed absence of polymorphism at GDF8-1, GDF8-2, GH, GHR-1,

199 and NPY-1 in the examined sample, and negligible effects of the SNPs in *GHRL*, *MYF5*,

200 *NPY*, *POMC, UCP2* and *UCP3* genes. Therefore, it can be concluded that all these SNPs are

201 not suitable as markers in the Piemontese breed for the traits recorded during the performance

202 testing**.**

203 More promising results have been obtained for the remaining SNPs.

204 The *GHR* is one of the most investigated genes for relationships with growth, because

205 evidences other than its physiological role in the expression of the trait suggest it as primary

206 candidate for traits related to growth and meat production in many species (Blair and Savage,

207 2002; Tixier-Boichard, 2002; List *et al*., 2011).

208 Previous studies of *GHR* gene in cattle mainly focused on two polymorphisms in exon

209 10 which induce amino acid substitutions, but did not reveal any significant effect on growth

210 traits in Angus cattle (Ge *et al*. 2003) nor in the Piemontese breed (Di Stasio *et al*., 2005),

211 leading to the conclusion that *GHR* gene did not seem a useful marker for traits related to

212 growth.

213 On the contrary, two of the SNPs here investigated (GHR-2 and GHR-3) showed relevant

214 associations with daily gain, specially when the haplotypes at the two SNPs were considered.

215 In addition, when the examined sample was subdivided into two groups, one including the

216 individuals selected for artificial insemination and the other the culled candidates, on the basis

217 of the selection index of the Piemontese breed which includes daily gain with a weight of

218 14%, a significantly higher frequency of the favourable *G* allele at GHR-2 in the selected

219   group was observed (0.63 *vs* 0.51; P = 0.01). As changes in allele frequencies of a SNP in the

220   direction expected because of the selection could contribute to validate a putative marker

221   (Ron and Weller, 2007), the finding provides further evidence that these SNPs at the *GHR*

222   gene affect daily gain.

223   The favourable effect of the *A* allele at GHR-3 on daily gain was previously observed by

224   Sherman *et al*. (2008) in experimental animals of composite breeds, even if, in opposition to

225   our results, the effects were reduced when haplotypes were considered.

226   Together with the genes of the somatotropic axis, the *LEP* gene is one of the most

227   intensively studied for relationships with feed intake and fat-related traits in cattle, whereas

228   fewer data exist on its effects on growth (Nkrumah *et al*., 2005; Di Stasio *et al*., 2007).

229   Associations of the *TT* genotype at LEP-1 with increased leptin concentration, backfat

230   thickness and marbling score, as well as with greater feed intake, growth rate and live weight

231   at slaughter were reported in crossbred animals (Nkrumah *et al*., 2005). The increased daily

232   gain associated to the *T* allele was confirmed by the present data. As during the performance

233   testing the animals were fed the same diet under restricted conditions, the association with

234   growth indirectly suggests an improved feed conversion, in agreement with Crews *et al*.

235   (2004) and Nkrumah *et al*. (2005). This could have a relevant practical impact because

236   improvement in feed efficiency could contribute to reduce the feed costs, thus increasing the

237   profitability of beef production.

238   A greater frequency of the favourable *T* allele (0.42) was observed in Piemontese animals

239   relative to the frequency reported for other populations (Nkrumah *et al*., 2005; Schenkel *et al*.,

240   2005).

241   The favourable effects of the *G* allele at LEP-2 on most traits was not unexpected,

242   considering the marked linkage disequilibrium with LEP-1, previously detected in other

243   breeds also (Nkrumah *et al*., 2005; Schenkel *et al*., 2005). The associations found are in

244 agreement with those described by Nkrumah *et al*. (2005), who reported increased feed

245 intake, growth rate and body weight associated to *GG* genotype at this SNP.

246   As for LEP-3, the results of previous investigations on the relationships with meat

247 production traits were rather inconsistent, showing either association with carcass fatness

248 (Buchanan *et al*., 2002; Lim *et al*., 2004; Schenkel *et al*., 2005), or no effect on feed intake

249 and fatness traits (Lagonigro *et al*., 2003; Barendse *et al*., 2005).

250   The present data revealed a highly favourable effect of the *C* allele at LEP-3 on daily gain,

251 consistently with results obtained in another hypertrophic breed, the Blonde d'Aquitaine,

252 where the *C* allele positively affected daily gain, with a large and significant effect

253 corresponding to 0.66 phenotypic standard deviation (Di Stasio *et al*., 2007). Other studies

254 showed that the *T* allele was associated with increased milk production (Buchanan *et al*.,

255 2003), whereas crossbred CT and TT cows were reported to wean heavier calves (DeVuyst *et*

256 *al.*, 2008).

257   Insulin-like growth factors belong to the class of polypeptides involved in the regulation of

258 cell development, and therefore the coding genes have been proposed as candidates for

259 growth and production in livestock. One of these genes, *IGF2*, is imprinted in cattle (Dindot

260 *et al*., 2004), as in other mammalian species, but undergoes a postnatal loss of imprinting

261 (Goodall and Schmutz, 2007), so that only the paternal allele is expressed during the foetal

262 life, while both alleles are expressed after birth. Recently, imprinted genes, including *IGF2*,

263 were confirmed as candidates for beef production traits in Limousin breed, supporting their

264 role in animal growth and development (Magee *et al*., 2010).

265   Associations of the *IGF2* polymorphism here considered with birth weight were reported

266 in different beef populations and crossbreds, and selection for *CC* sires was proposed to

267 ensure lower birth weight in order to reduce dystocia risks (Schmutz and Goodall, 2005;

268 Goodall and Schmutz, 2007). The same Authors also found that *CC* animals had larger rib-

269 eye area, which affects the economic return of the carcass. Effects on body weight, daily gain,

270 feed conversion and rib eye area were also detected by Sherman *et al*. (2008), but for rib eye

271 area they were in the opposite direction compared to findings of Goodall and Schmutz (2007).

272 Our results also revealed associations of IGF2 with growth, but indicated a positive effect

273 of the *C* allele on daily gain, and indirectly on feed efficiency for the reasons previously

274 mentioned, which is opposite to the results of Sherman *et al*. (2008), who found that *TT*

275 animals had a greater daily gain and lower feed conversion ratio.

276 Few studies exist on *LEPR* gene in cattle. The SNP here considered was shown to be

277 associated with leptin concentration during late pregnancy in Friesian breed (Liefers *et al*.,

278 2004), while no relationships with daily gain were found in Aberdeen Angus and Charolais

279 breeds (Almeida *et al*., 2008). In opposition to the findings in beef cattle, the present study

280 revealed that the *LEPR* had the largest effect on daily gain. This result deserves further

281 investigations, for the impact it can have for the genetic improvement of the breed.

282

283 **Conclusions**

284 The study investigated the variability of twelve candidate genes in the Piemontese breed,

285 showing relevant associations of SNPs in *GHR*, *LEP*, *IGF2* and *LEPR* genes with traits

286 recorded during the performance testing of Piemontese bulls. Although further studies would

287 be useful to confirm the results for *IGF2* and *LEPR*, the associations observed for *GHR* and

288 *LEP* were consistent with those of previous studies, providing additional evidence of their

289 usefulness as markers.

290 Incorporating information of these markers in the breeding programme of the Piemontese

291 cattle might increase the rate of genetic gain for some of the traits in the breeding goal of the

292 population. Of course, before suggesting practical use of the investigated polymorphisms,

293 evaluation of costs, operational aspects and extra gain relative to traditional breeding

294 programmes exploiting only polygenic effects need to be performed.

295

296 **Acknowledgments**

299

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407

408   Table 1. Descriptive statistics of the traits in the analysed sample (s_P= phenotypic standard

409   deviation; s_A = additive genetic standard deviation).

410

Trait	mean	s_P	minimun	maximum	s_A
Average daily gain, DG (g/day)	1353.10	125.42	953.00	1705.00	76.1
Withers width,WW	7.04	1.01	4.67	9.00	0.56
Shoulder muscularity, SM	7.00	0.99	4.00	9.00	0.52
Loin width, LW	6.85	0.87	4.67	9.00	0.44
Loin thickness, LT	7.08	0.95	4.67	9.00	0.44
Thigh muscularity, TM	7.43	1.04	4.67	9.00	0.75
Thigh profile, TP	7.20	1.05	4.33	9.00	0.76
Bone thickness, BT	6.05	0.94	5.03	8.00	0.51

411

412

413   Table 2. SNP information.

Gene	Bovine chromosome	SNP name	SNP location	SNP description	Frequency of the first allele in the SNP description
GDF8	BTA2	GDF8-1	promoter	AJ438578 g.843T>A	1.00
		GDF8-2	exon 1	AF320998:g.433C>A	1.00
GH	BTA19	GH	promoter	AY445811:g.358C>T	1.00
GHR	BTA20	GHR-1	promoter	U15731:g.9371C>T	1.00
		GHR-2	promoter	AF126288:g.149A>G	0.45
		GHR-3	intron 4	AY643807:g.300A>G	0.65
GHRL	BTA22	GHRL	intron 3	AY455980:g.446A>G	0.80
IGF2	BTA29	IGF2	exon 2	AY237543:g.150C>T	0.75
LEP	BTA4	LEP-1	promoter	AB070368:g.528C>T	0.58
		LEP-2	promoter	AB070368:g.1759G>C	0.86
		LEP-3	exon 2	AY138588:g.305T>C	0.17
LEPR	BTA3	LEPR	exon 20	AJ580801:g.115C>T	0.92
MYF5	BTA5	MYF5	intron 2	M95684:g.1948A>G	0.42
NPY	BTA4	NPY-1	intron 2	AY4911054:g.284A>G	1.00
		NPY-2	intron 2	AY4911054:g.666A>G	0.23
		NPY-3	intron 2	AY4911054:g.3032C>T	0.32
POMC	BTA11	POMC	exon 3	J00021:g.254C>T	0.83
UCP2	BTA15	UCP2	intron 2	AY147821:g.380G>C	0.18
UCP3	BTA15	UCP3	intron 3	AF127030:g.1099G>A	0.23

414

415

416 Table 3. Estimates of additive (a) and dominance (d) effects of the SNPs and haplotypes in the *GHR* gene and marginal posterior probability (P)

417 of the estimate of being larger than 0.1 s_A. Symbols of the traits as in table 1.

418

Trait	GHR-2				GHR-3				haplotype effect			
	a (*A vs G*)		d		a (*A vs G*)		d		*A-A vs G-G*		*G-A vs G-G*	
	mean	P	mean	P	mean	P	mean	P	mean	P	mean	P
DG	-2.03	0.33	2.31	0.38	18.59	0.81	-6.45	0.48	0.82	0.53	34.04	0.95
WW	-0.05	0.47	-0.28	0.95	-0.11	0.70	-0.06	0.50	-0.13	0.73	-0.15	0.76
SM	-0.10	0.69	-0.10	0.65	-0.10	0.69	0.02	0.40	-0.13	0.76	-0.12	0.71
LW	0.00	0.32	-0.18	0.88	-0.03	0.44	0.04	0.49	-0.04	0.48	-0.11	0.73
LT	0.02	0.38	-0.21	0.92	-0.07	0.63	-0.06	0.54	-0.04	0.46	-0.14	0.79
TM	-0.11	0.60	-0.19	0.74	-0.02	0.35	0.14	0.65	-0.15	0.70	-0.13	0.64
TP	-0.09	0.54	-0.22	0.80	-0.04	0.37	0.06	0.46	-0.14	0.68	-0.10	0.56
BT	-0.14	0.82	0.10	0.67	-0.30	0.99	-0.16	0.80	-0.24	0.97	-0.26	0.97

419

420

421   Table 4. Estimates of additive (a) and dominance (d) effects of the SNPs and haplotypes in the *LEP* gene and marginal posterior probability (P)

422   of the estimate of being larger than 0.1 s_A. Symbols of the traits as in table 1.

423

Trait	LEP-1				LEP-2				LEP-3			
	a (*C vs T*)		d		a (*C vs G*)		d		a (*C vs T*)		d	
	mean	P	mean	P	mean	P	mean	P	mean	P	mean	P
DG	-13.53	0.67	-5.19	0.44	-24.84	0.66	19.93	0.61	32.00	0.80	34.53	0.79
WW	-0.12	0.73	-0.26	0.93	0.02	0.46	0.31	0.75	-0.23	0.78	-0.03	0.46
SM	-0.15	0.82	-0.31	0.97	-0.01	0.45	0.24	0.70	-0.20	0.75	-0.03	0.46
LW	-0.05	0.51	-0.17	0.86	-0.19	0.69	0.36	0.84	0.00	0.42	0.05	0.52
LT	-0.09	0.71	-0.24	0.94	-0.15	0.64	0.31	0.80	-0.07	0.56	-0.01	0.44
TM	-0.09	0.54	-0.41	0.98	-0.21	0.63	0.33	0.72	0.09	0.52	0.06	0.48
TP	-0.13	0.67	-0.45	0.98	-0.32	0.74	0.51	0.86	0.06	0.48	0.10	0.53
BT	0.03	0.41	-0.20	0.89	0.35	0.86	-0.04	0.49	-0.21	0.76	-0.03	0.47

424

425

426 Table 5. Estimates of the *LEP* haplotype effects and marginal posterior probability (P) of the estimate of being larger than 0.1 s_A. Symbols of the

427 traits as in table 1.

428

Trait	LEP-1 – LEP-2 – LEP-3			
	T-G-C vs C-G-C		*C-C-T vs C-G-C*	
	mean	P	mean	P
DG	16.21	0.73	4.09	0.43
WW	0.12	0.70	0.31	0.94
SM	0.14	0.79	0.30	0.93
LW	0.05	0.54	0.16	0.78
LT	0.07	0.63	0.18	0.81
TM	0.03	0.35	0.07	0.49
TP	0.05	0.43	0.12	0.59
BT	0.03	0.42	0.36	0.98

429

430

431

432 Table 6. Estimates of additive (a) and dominance (d) effects of the SNPs in the *IGF2* and *LEPR* genes and marginal posterior probability (P) of

433 the estimate of being larger than $0.1 \, s_A$. Symbols of the traits as in table 1

434

Trait	IGF2				LEPR			
	a (*C vs T*)		d		a (*C vs T*)		d	
	mean	P	mean	P	mean	P	mean	P
DG	24.14	0.86	15.59	0.67	44.80	0.74	-3.42	0.47
WW	-0.06	0.52	-0.34	0.96	-0.35	0.75	-0.50	0.82
SM	-0.03	0.41	-0.22	0.86	-0.11	0.55	-0.39	0.76
LW	-0.02	0.40	-0.15	0.78	-0.13	0.59	-0.39	0.79
LT	-0.09	0.66	-0.22	0.90	-0.29	0.75	-0.69	0.95
TM	-0.08	0.52	-0.10	0.56	-0.42	0.76	-0.81	0.92
TP	0.02	0.35	-0.03	0.40	-0.43	0.77	-0.88	0.94
BT	-0.06	0.53	-0.09	0.62	-0.34	0.76	-0.34	0.76

435

Answers to the reviewers

Reviewer A:

Page 4/line 14: Candidate genes and selected puntual mutations within those genes is an important aspect of the material and methods. The authors should consider to refer to Table 2 also in material and methods.

Table 2 was already mentioned in M&M (page 5/line 100)

Page 4/line18: A short description of the distribution of animals accross the main non-genetic effects ((sex, age, diet and weight at slaughtering) could be of interest

There is no need for such a description: the analysed animals were candidates to the performance test (page 4/line 87), therefore all males, fed the same diet, and, of course, not evaluated for slaughtering performances.

Page 6/line 2-9: I consider this paragraph lack of relevance in the context of the paper.

Deleted

Page 6/line 23: Is the analysis carried out by a software developed ad hoc by the authors?, or they used a previously developed software by other authors?

Added

Page 7/line 14: Is not this a surprise result?. It is supposed that, at least for any SNPs, selection is acting, so H-W equilibrium should not match

In fact, it is not so surprising, considering that our sample is quite large in this respect and the tests for H-W proportions are not very sensitive to deviations from the expected genotype proportions.

Table 1: Did you realize about the low heritability values for traits which traditionally have higher values?

We have made clear that the additive standard deviation refers to the population value.

Reviewer B:

The authors respected the guidelines. Only one revision is needed: lines should be left numbered in continuum.

Done

The abstract lacks a brief introduction.

Added

All first letters of key words should be in capital letters.

Done

In the material and method section (page 5, line 1), it could be better to indicate how was blood taken and handled till DNA isolation.

Added

In the result section (page 7, line 13), if 0.08 is the MAF value for the LEPR SNP it could be better to write "data not shown". Please verify this value.

The value is correct: we don't understand the comment.

The discussion section is very strong to read. It could be better to reduce it.

Reduced

Reference section: some citations are in the references but not in the paper. E.g.: page 15 Ge et al., 2000, Guo et al., 2008, page 16 Legarra et al., 2008, page 17 Maj et al., 2005, page 18 Stephens et al., 2003.

Revised

Table 1: it could be better to remove the acronym of the trait. In the table in fact is reported the whole name of each trait

We would prefer to maintain the acronyms, which are used in the following tables.