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

(Article begins on next page)

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22   **Abstract**

23   In the last years an increasing number of associations between SNPs in candidate genes and

24   several production traits have been reported in beef cattle, but very often the results were not

25   validated and few studies considered breeds homozygous for the allele responsible for the

26   muscular hypertrophy. Therefore, we analysed the variability of 19 previously reported SNPs

27   in 12 genes (*GH*, *GHR*, *GDF8*, *GHRL*, *IGF2*, *LEP*, *LEPR*, *MYF5*, *NPY*, *POMC*, *UCP2*,

28   *UCP3*) in the hypertrophic Piemontese breed and investigated the effects of the observed

29   polymorphisms on growth and conformation traits recorded during performance testing.

30   Fourteen SNPs were polymorphic and a significant linkage disequilibrium was observed

31   between SNPs in *GHR*, *LEP* and *NPY* genes, for which both single-SNP and haplotype effects

32   were estimated. Negligible effects on the investigated traits were observed for *GHRL*, *MYF5*,

33   *NPY*, *POMC*, *UCP2* and *UCP3* genes. The *GHR* gene significantly affected daily gain and its

34   effect was further increased when haplotypes were considered (*G-A vs G-G*: +34.04 g/d). The

35   *C* allele at LEP-1 and LEP-2 had moderate negative effects on the considered traits, whereas

36   the *C* allele at LEP-3 mostly had positive effects; relative to single SNPs, haplotypes in the

37   *LEP* gene showed weaker but favourable associations with all the traits. The *C* allele at *IGF2*

38   and *LEPR* had favourable effects on daily gain and negative effects on meat conformation

39   traits. The associations observed for *GHR* and *LEP* were consistent with those of previous

40   studies, providing additional evidence of their usefulness as markers. Practical aspects of the

41   applications to the breeding programme of the Piemontese breed need to be examined.

42

43   **Keywords:** Cattle, Piemontese breed, SNPs, Meat production

44

45   **Introduction**

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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**

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300 **References**

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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

20

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.