Running title: SNPs and meat traits in Piemontese breed

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

Claudio Lisa[1], Andrea Albera[2], Paolo Carnier[3], Liliana Di Stasio[1]

[1]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università di Torino, Italy

[2]Associazione Nazionale Allevatori Bovini di Razza Piemontese, Carrù, Italy

[3]Dipartimento di Scienze Animali, Università di Padova, Italy

Corresponding author: Prof. Liliana Di Stasio, DISAFA, Università di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy - Tel: +39.011.6708570 - Fax: +39.011.6708563 - Email: liliana.distasio@unito.it

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

To date a great number of candidate genes for production traits have been suggested in different livestock species, based on the knowledge of their position and/or function. For meat production, the interest has been mainly focused on genes involved in growth and meat quality, but only for a limited number of genes the effects of their polymorphisms have been investigated, often in a single breed.

On the other hand, the recent development of high-density SNP (single nucleotide polymorphism) genotyping microarrays has opened new selection perspectives for the possibility of estimating the breeding value of animals with no phenotypic records, with the potential advantages of increased genetic gain and lower costs (Meuwissen *et al*., 2001). However, as many of the genotyped SNPs are located in anonymous regions, the detection of associations with traits of interest does not directly lead to the identification of the underlying genes (Magee *et al*., 2010). For these reasons the candidate gene approach, which aims at identifying specific polymorphisms responsible for the observed effects in genes biologically related to the traits of interest, is still a valuable strategy (Ron and Weller, 2007).

On the basis of these considerations we carried out the present study in order to give a contribution to the analysis of genes possibly related to meat production. We focused on 12 genes, which were selected on the basis of their biological functions and for which effects on the traits of interest had been reported: *growth hormone* (*GH*), *growth hormone receptor* (*GHR*), *growth differentiation factor 8* (*GDF8*), *ghrelin* (*GHRL*), *insulin-like growth factor 2* (*IGF2*), *leptin* (*LEP*), *leptin receptor* (*LEPR*), *myogenic factor 5* (*MYF5*), *neuropeptide Y* (*NPY*), *proopiomelanocortin* (*POMC*), *uncoupling protein 2* (*UCP2*), *uncoupling protein 3* (*UCP3*). The products of most of these genes are involved in biologically-related processes regulating feed intake and growth. Circulating leptin, after binding to specific receptors in the brain, exerts its effects on feed intake and energy homeostasis *via* neurotransmitters such as neuropeptide Y and pro-opiomelanocortin (Houseknecht & Portocarrero, 1998). Leptin also

increases the expression of uncoupling protein 2 and 3, involved in energy expenditure (Scarpace *et al*., 1997), and modulates the secretion of growth hormone (Zieba *et al*., 2003), which binds to GH receptors on target tissues, activating the signal transduction culminating in GH biological effects (Kopchik & Andry, 2000).

Polymorphisms in the considered genes have been shown to affect growth, feed efficiency and carcass quality in different cattle breeds and crossbreds (Kim *et al*., 2004; Li *et al*., 2004; Buchanan *et al*., 2005; Nkrumah *et al*., 2005; Di Stasio *et al*., 2007; Goodall & Schmutz, 2007; DeVuyst *et al*., 2008; Sherman *et al*., 2008). It seems worth noting that very few studies considered breeds homozygous for the allele responsible for the muscular hypertrophy, which might interfere with genes affecting meat production as a consequence of its well known effects on growth and muscle development.

Therefore, the objective of this study was to estimate the variability of the above twelve genes and their associations with traits recorded during the performance testing of breeding candidates in the hypertrophic Piemontese breed.

Materials and methods

The study was carried out on 201 Piemontese male calves enrolled in the performance testing programme at the central Station of the Italian Association of Piemontese Cattle Breeders. The performance testing programme of the Piemontese breed is described in Albera *et al.* (2001).

Eight traits recorded during the performance testing were considered: average daily gain (DG), withers width (WW), shoulder muscularity (SM), loins width (LW), loins thickness (LT), thigh muscularity (TM), thigh profile (TP) and bone thinness (BT). The conformation traits were graded through a linear scoring of live animals using a 9-point scale, as reported

by Albera *et al*. (2001). Descriptive statistics for the investigated traits are presented in Table 1.

Blood samples were collected in tubes containing ethylenediaminetetraacetic acis as an anticoagulant and kept at 4°C until DNA isolation. Genomic DNA was extracted using the NucleoSpin® Blood kit (Macherey-Nagel, Düren, Germany). A total of 19 SNPs were investigated in 12 genes (Table 2). Genotyping was performed by a commercial company (http://www.kbioscience.co.uk).

Allele frequencies were estimated by simple counting. Tests for Hardy-Weinberg equilibrium at each SNP and for linkage disequilibrium between the SNP pairs were performed using the FSTAT software (Goudet, 2002). For the linked SNPs, haplotypes were constructed using the PHASE v.2.1 software (Stephens *et al*., 2001), which implements a Bayesian method for reconstructing haplotypes from population genotype data.

The association of the observed polymorphisms with phenotypes for the recorded traits was investigated using a statistical model similar to that used for the prediction of breeding values of Piemontese bulls, but also including the effect of the single SNP or haplotype.

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

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

where y is a vector of observations on the considered trait, b is a vector of systematic nongenetic effects, u is a vector of animal additive genetic effects, c is a vector of SNP genotype or haplotype effects, e is a vector of random residuals and X, Z and W are incidence matrices of proper order relating observations to b, u and c, respectively.

For all traits, nongenetic effects included in the linear model were the effect of the contemporary group of animals on test and of the parity of the dam. Additionally, the weight at the beginning of the test for growth and the weight at scoring for meat conformation traits were included as covariates. For the single SNPs analysis, the model included the effect of the

SNP genotype, whereas for the haplotypes the regression on the number of haplotype copies was included, as the accuracy of the haplotype reconstruction was very high (P: 0.938 to 0.998).

The effects of the observed polymorphisms were investigated using Bayesian procedures. The Bayesian analysis, performing numerical integration through Gibbs sampling, was used to estimate the marginal posterior distribution of parameters of concern (Legarra *et al*., 2008). Animal and residual effects were assumed to be normally distributed "a priori" as $u \sim N(0, A\sigma_a^2)$ and $e \sim N(0, I\sigma_e^2)$, respectively, where A was the numerator relationship matrix, σ_a^2 was the additive genetic variance, I was an identity matrix of proper order and σ_e^2 was the residual variance. Flat priors were assumed for systematic nongenetic and for SNP genotype or haplotype effects. As the number of animals included in the study was too limited to estimate variance components, estimates of additive genetic and residual variances obtained by Albera *et al.* (2001) were used. A single chain of 1,000,000 iterations with a burn-in of 200,000 was run for each trait/SNP analysis, saving samples every 400 iterations.

Inference on additive and dominance SNP effects, as defined by Falconer & Mackay (1996), was based on the estimated marginal posterior density of these effects. Haplotype effects were estimated as deviations from the effect of the 'reference' haplotype which was arbitrarily set to zero. The 'reference' haplotype was chosen randomly. The mean of the marginal posterior distribution of a SNP/haplotype effect was used as a point estimate of the effect.

On the basis of the realised response to selection for meat traits in the Piemontese population in the last ten years (ANABORAPI, 2010) and also considering the effectiveness of exploiting variation due to candidate genes, a SNP/haplotype effect was considered to be 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

GHR-3 with the slightly favourable *G* allele at GHR-2 raised the effect on DG to 34 g/d (nearly $0.45s_A$), with a probability for the effect of being relevant (greater than $0.1s_A$) as high as 95%, whereas it exerted a negative effect on muscularity and especially on BT.

As for the SNPs in the *LEP* gene (Table 4), the *C* allele at LEP-1 was consistently associated with negative values for all the traits, except BT; relevant dominance effects on the traits related to meat conformation were also observed. For LEP-2, results were comparable to those for LEP-1, with the *C* allele exerting negative additive effects on all traits with the exception of BT. The *C* allele at LEP-3 was associated with increased DG, with an estimated additive effect of 32.0 g/d (i.e., $0.42s_A$) and a probability of the effect being larger than $0.1s_A$ of 80%.

For the analysis of the combined effects of the three SNPs in the *LEP* gene, only the most frequent haplotypes were considered: *C*-*G*-*C* (0.45), *T*-*G*-*C* (0.37) and *C*-*C*-*T* (0.14). Four additional rare haplotypes were found, with a cumulative frequency lower than 0.04. Compared to haplotype *C*-*G*-*C*, the haplotype containing all the favourable alleles (*T*-*G*-*C*) confirmed the favourable association with DG and showed positive effects, although of little magnitude on the other traits (Table 5). The haplotype combining the less favourable alleles (*C*-*C*-*T*) showed trivial effects on DG, but surprisingly positively affected meat conformation traits, particularly those related to the muscularity of the fore part of the body (WW and SM).

A large positive effect of the *C* allele at IGF2 was detected for DG (24.14 g/d), whereas small negative additive effects were observed for meat conformation traits (Table 6). For LEPR (Table 6), a relevant additive effect was observed on DG, with the *C* allele associated to higher values (about 45 g/d); negative additive and dominance effects were observed for all the conformation traits.

Discussion

In the past decades an increasing number of associations between SNPs in candidate genes and several production traits have been reported in beef cattle, but very often no studies were performed to validate the results, or inconsistencies were observed across populations, so that the possibility to exploit the detected associations in selection programmes was limited.

The present study revealed absence of polymorphism at GDF8-1, GDF8-2, GH, GHR-1, and NPY-1 in the examined sample, and negligible effects of the SNPs in *GHRL*, *MYF5*, *NPY*, *POMC*, *UCP2* and *UCP3* genes. Therefore, it can be concluded that all these SNPs are not suitable as markers in the Piemontese breed for the traits recorded during the performance testing**.**

More promising results have been obtained for the remaining SNPs.

The *GHR* is one of the most investigated genes for relationships with growth, because evidences other than its physiological role in the expression of the trait suggest it as primary candidate for traits related to growth and meat production in many species (Blair and Savage, 2002; Tixier-Boichard, 2002; List *et al*., 2011).

Previous studies of *GHR* gene in cattle mainly focused on two polymorphisms in exon 10 which induce amino acid substitutions, but did not reveal any significant effect on growth traits in Angus cattle (Ge *et al*. 2003) nor in the Piemontese breed (Di Stasio *et al*., 2005), leading to the conclusion that *GHR* gene did not seem a useful marker for traits related to growth.

On the contrary, two of the SNPs here investigated (GHR-2 and GHR-3) showed relevant associations with daily gain, specially when the haplotypes at the two SNPs were considered. In addition, when the examined sample was subdivided into two groups, one including the individuals selected for artificial insemination and the other the culled candidates, on the basis of the selection index of the Piemontese breed which includes daily gain with a weight of 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-

eye area, which affects the economic return of the carcass. Effects on body weight, daily gain, feed conversion and rib eye area were also detected by Sherman *et al*. (2008), but for rib eye area they were in the opposite direction compared to findings of Goodall and Schmutz (2007).

Our results also revealed associations of IGF2 with growth, but indicated a positive effect of the *C* allele on daily gain, and indirectly on feed efficiency for the reasons previously mentioned, which is opposite to the results of Sherman *et al*. (2008), who found that *TT* animals had a greater daily gain and lower feed conversion ratio.

Few studies exist on *LEPR* gene in cattle. The SNP here considered was shown to be associated with leptin concentration during late pregnancy in Friesian breed (Liefers *et al*., 2004), while no relationships with daily gain were found in Aberdeen Angus and Charolais breeds (Almeida *et al*., 2008). In opposition to the findings in beef cattle, the present study revealed that the *LEPR* had the largest effect on daily gain. This result deserves further investigations, for the impact it can have for the genetic improvement of the breed.

Conclusions

The study investigated the variability of twelve candidate genes in the Piemontese breed, showing relevant associations of SNPs in *GHR*, *LEP*, *IGF2* and *LEPR* genes with traits recorded during the performance testing of Piemontese bulls. Although further studies would be useful to confirm the results for *IGF2* and *LEPR*, the associations observed for *GHR* and *LEP* were consistent with those of previous studies, providing additional evidence of their usefulness as markers.

Incorporating information of these markers in the breeding programme of the Piemontese cattle might increase the rate of genetic gain for some of the traits in the breeding goal of the population. Of course, before suggesting practical use of the investigated polymorphisms,

evaluation of costs, operational aspects and extra gain relative to traditional breeding programmes exploiting only polygenic effects need to be performed.

Acknowledgments

Research funding was provided by the Italian Ministry of Agricultural and Forestry Policy (SELMOL Project).

References

Albera, A., Mantovani, R., Bittante, G., Groen, A.F., Carnier, P., 2001 Genetic parameters for daily live-weight gain, live fleshiness and bone thinness in station-tested Piemontese young bulls. Anim. Sci.:72, 449-456.

Almeida, S.E.M., Santos, L.B.S., Passos, D.T., Corbellini, A.O., Lopes, B.M.T., Kirst, C., Terra, G., Neves, J.P., Gonçalves, P.B.D., Moraes, J.C.F., de Azevedo Weimer, T. 2008. Genetic polymprphisms at the leptin receptor gene in three beef cattle breeds. Genet. Mol. Biol. 31:680-685.

ANABORAPI (2010) Relazione tecnica e statistiche. ANABORAPI, Carrù, Italy. Available from: http://www.anaborapi.it/images/media/pdf/stat/AnaborapiRelazioneTecnica2010.

Barendse, W., Bunch, R.J. Harrison, B.E., 2005. The leptin C73T missense mutation is not associated with marbling and fatness traits in a large gene mapping experiment in Australian cattle. Anim. Genet. 36:86-88.

Blair, J.C., Savage, M.O., 2002. The GH-IGF-I axis in children with idiopathic short stature. Trends Endocrin. Met. 13:325-330.

Buchanan, F.C., Fitzsimmons, C.J., Van Kessel, A.G., Thue, T.D., Winkelman-Sim, D.C., Schmutz, S.M., 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genet. Sel. Evol. 34:105-116.

318 Buchanan, F.C., Van Kessel, A.G., Waldner, C., Christensen, D.A., Laarveld, B., Schmutz,
319 S.M., 2003, An association between a leptin single nucleotide polymorphism and milk and
320 protein yield. J. Dairy Sci. 86:3164-3166.
321 Buchanan, F.C.V., Thue, T.D., Yu, P., Winkelman-Sim, D.C., 2005 Single nuecleotide
322 polymorphisms in the corticotrophin-releasing hormone and pro-opiomelanocortin genes
323 are associated with growth and carcass yield in beef cattle. Anim. Genet. 36:127-131.
324 Crews, D.H., Nkrumah, J.D., Yu, J., Moore, S.S., 2004. Association of single nucleotide
325 polymorphisms in the bovine leptin gene with feedlot and carcass characteristics of
326 crossbred steers. Can. J. Anim. Sci. 84:749-750.
327 DeVuyst, E.A., Bauer, M.L., Cheng, F.C., Mitchell, J., Larson, D., 2008 The impact of a
328 leptin gene SNP on beef calf weaning weights. Anim. Genet. 39:284-286.
329 Di Stasio, L., Destefanis, G., Brugiapaglia, A., Albera, A., Rolando, A., 2005. Polymorphism
330 of GHR gene in cattle and relationships with meat production and quality. Anim. Genet.
331 36:138-140.
332 Di Stasio, L., Brugiapaglia, A., Galloni, M., Destefanis, G., Lisa, C., 2007. Effect of the *leptin*
333 c.73T>C mutation on carcass traits in beef cattle. Anim. Genet. 38:316-317.
334 Dindot, S.V., Kent, K.C., Evers, B., Loskutoff, N., Womack, J., Piedrahita, J.A., 2004.
335 Conservation of genomic imprinting at the XIST, IGF2 and GTL2 loci in the bovine.
336 Mamm. Genome 15:966-974.
337 Falconer, D.S., Mackey, T.F.C., 1996. Introduction to quantitative genetics. Longmans Green,
338 Harlow, Essex, UK.
339 Ge, W., Davis, M.E., Hines, H.C., Irvin, K.M., Simmen, R.C.M., 2003. Association of single
340 nucleotide polymorphisms in the growth hormone and growth hormone receptor genes
341 with blood serum insulin-like growth factor I concentration and growth traits in Angus
342 cattle. J. Anim. Sci. 81:641-648.

343 Goodall, J.J., Schmutz, S.M., 2007. IGF2 gene characterization and association with rib eye
344 area in beef cattle. Anim. Genet. 38:154-161.
345 Goudet, J., 2002 FSTAT, a program to estimate and test gene diversities and fization indices
346 (version 2.9.3.2). Available from: http://www.unil.ch/izea/software/fstat.html.
347 Houseknecht, K.L., Portocarrero, C.P., 1998. Leptin and its receptors: regulators of whole-
348 body energy homeostasis. Domest. Anim. Endocrin. 15:457-475.
349 Kim, N.K., Seo, Y.W., Kim, G.H., Joh, J.H., Kim, O.H., Chung, E.R., Lee, C.S., 2004. A
350 previously unreported *Dra*I polymorphism within the regulatory region of the bovine
351 *growth hormone* gene and its association with growth traits in Korean Hanwoo cattle.
352 Anim. Genet. 35:152-154.
353 Kopchick, J.J., Andry, J. M., 2000. Growth Hormone (GH), GH Receptor, and Signal
354 Transduction. Mol. Genet. Metab. 71:293-314.
355 Lagonigro, R., Wiener, P., Pilla, F., Woolliams, J.A., Williams, J.L., 2003. A new mutation in
356 the coding region of the bovine leptin gene associated with feed intake. Anim. Genet.
357 34:71-374.
358 Legarra, A., Varona, L., Lopez de Maturana, E., 2008. TM User Manual
359 Li, C., Basarab, J., Snelling, W.M., Benkel, B., Murdoch, B., Hansen, C., Moore, S.S., 2004.
360 Assessment of positional candidate genes *myf*5 and *igf*1 for growth on bovine
361 chromosome 5 in commercial lines of *Bos taurus*. J. Anim. Sci. 82:1-7.
362 Liefers, S.C., Veerkamp, R.F., te Pas, M.F.W., Delavaud, C., Chilliard, Y., van der Lende, T.,
363 2004. Amissense mutation in the bovine leptin receptor gene is associated with leptin
364 concentrations during late pregnancy. Anim. Genet. 35:138-141.
365 Lim, H.Y., Oh, J.D., Kong, H.S., Jeon, G.J., Lee, H.K., Lee, S.S., Yoon, D.H., Kim, C.D.,
366 Cho, B.W., 2004. Association of genetic missense mutation and economic traits of leptin
367 gene using PCR-RFLP in Korea cattle (Han-Woo). J. Anim. Sci. Tech. 46:295-300.

List, E.O., Sackmann-Sala, L., Berryman, D.E., Funk, K., Kelder, B., Gosney, E.S., Okada, S., Ding, J., Cruz-Topete, D., Kopchick J.J., 2011. Endocrine parameters and phenotypes of the Growth Hormone Receptor gene disrupted (GHR-/-) mouse. Endocr. Rev. 32:356–386.

Magee, D.A., Berkowicz, E.W., Sikora, K.M., Berry, D.P., Park, S.D.E., Kelly, A.K., Sweeney, T., Kenny, D.A., Evans, R.D., Wickham, B.W., Spillane, C., MacHugh, D.E., 2010. A catalogue of validated single nucleotide polymorphisms in bovine ortologs of mammalian imprinted genes and associations with beef production traits. Animal 12:1958-1970.

McPherron, A.C., Lee, S.J., 1997. Double muscling in cattle due to mutations in the myostatin gene. P Natl. Acad. Sci.-Biol. 94: 12457-12461.

Meuwissen, T.H.E., Hayes, B.J., Goddard, M.E., 2001. Prediction of total genetic value using genome wide dense marker maps. Genetics 157:1819-1829.

Nkrumah, J.D., Li, C., Yu, J., Hansen, C., Keisler, D.H., Moore, S.S., 2005. Polymorphisms in the bovine leptin promoter associted with serum leptin concentration, growth, feed intake, feeding behavior, and measures of carcass merit. J. Anim. Sci. 83: 20-28.

Ron, M., Weller, J.I., 2007. From QTL to QTN identification in livestock – winning by points rather than knock-out: a review. Anim. Genet. 38:429-439.

Scarpace, P.J., Matheny, M., Pollock, B.H., Tumer N., 1997. Leptin increases uncoupling protein expression and energy expenditure. Am. J. Physiol. 273:E226-230.

Schenkel, F.S., Miller, S.P., Ye, X., Moore, S.S., Nkrumah, J.D., Li, C., Yu, J., Mandel,l I.B., Wilton, J.W., Williams, J.L., 2005. Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. J. Anim. Sci. 83:2009-2020.

Schmutz, S., Goodall, J., 2005. Improving production characteristics of cattle. European Patent Office WO2005007881.

393 Sherman, E.L., Nkrumah, J.D., Murdoch, B.M., Li, C., Wang, Z., Fu, A., Moore, S.S., 2008.
394 Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor,
395 ghrelin, insulin-like growth factor 2, and uncoupling proteins 2 and 3 genes and their
396 associations with measures of growth, performance, feed efficiency, and carcass merit in
397 beef cattle. J. Anim. Sci. 86:1-16.
398 Stephens, M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype
399 reconstruction from population data. Am. J. Hum. Genet. 68:978-989.
400 Tixier-Boichard, M., 2002. From phenotype to genotype: Major genes in chickens. Worlds
401 Poultry Sci. J. 58:65-75.
402 Vankan, D.M., Wayne, D.R., Fortes, M.R.S., 2010. Real-time PCR genotyping and frequency
403 of the myostatin F94L mutation in beef cattle breeds. Animal 4:530-534.
404 Zieba, D.A., Amstalden, M., Morton, S., Gallino, J.L., Edwards, J.F., Harms, P.G., Williams,
405 G.L., 2003. Effects of leptin on basal and GHRH-stimulated GH secretion from the bovine
406 adenohypophysis are dependent upon nutritional status. J. Endocrinol. 178:83-99.
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

Table 3. Estimates of additive (a) and dominance (d) effects of the SNPs and haplotypes in the *GHR* gene and marginal posterior probability (P) of the estimate of being larger than 0.1 $s_{A.}$ Symbols of the traits as in table 1.

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

Table 4. Estimates of additive (a) and dominance (d) effects of the SNPs and haplotypes in the *LEP* gene and marginal posterior probability (P) of the estimate of being larger than 0.1 s_A. Symbols of the traits as in table 1.

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

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

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

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

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.