

¹Federal University of São Carlos, São Carlos, ²Animal Science Institute of São Paulo, Nova Odessa, ³Embrapa Southeast Cattle Research Center, São Carlos and ⁴Animal Science Department – ESALQ/USP, Piracicaba, SP, Brazil

Candidate genes for growth traits in beef cattle crosses *Bos taurus* × *Bos indicus*

By D. D. TAMBASCO¹, C. C. P. PAZ², M. TAMBASCO-STUDART¹, A. P. PEREIRA¹,
M. M. ALENCAR³, A. R. FREITAS³, L. L. COUTINHO⁴, I. U. PACKER⁴ and L. C. A. REGITANO³

Summary

Candidate gene strategy has been proposed to direct the search for quantitative trait loci, and polymorphisms at these loci have been associated with several production traits in bovine. A total of 211 animals resulting from crosses between Nelore females with sires from either Aberdeen Angus, Canchim and Simmental breeds were analyzed for restriction fragment length polymorphisms (RFLPs) in the κ -casein, β -lactoglobulin (LGB) and growth hormone *GH* genes. Average daily gain from birth to weaning (GBW) and from weaning to yearling (GWY) were analyzed by a statistical model comprising RFLP genotypes and their interaction effects. Significant effects ($p < 0.05$) of growth hormone (*GH*) and of the interaction β -lactoglobulin × *GH* were verified for the GWY. Animals with LL genotypes for *GH* had higher GBW than LV animals but lower GWY ($p < 0.05$).

Zusammenfassung

Kandidatengene für Wachstum in Fleischrinderkreuzungen Bos taurus × Bos indicus

Für die direkte Suche nach QTLs ist der Kandidatengen-Ansatz eine Möglichkeit und Polymorphismen in diesen Genen zeigen Assoziationen zu Produktionsmerkmalen beim Rind auf. Insgesamt wurden 211 Tiere aus Kreuzungen zwischen Nelore-Kühen und Aberdeen Angus-, Canchim- oder Simmental-Bullen für die Analyse von RFLPs im κ -Kasein, β -Laktoglobulin (LGB) und Wachstumshormon (*GH*) verwendet. Die durchschnittliche tägliche Zunahme von der Geburt bis zum Absetzen (GBW) und vom Absetzen bis zum Alter von einem Jahr (GWY) wurden durch ein statistisches Modell unter Berücksichtigung der RFLP-Genotypen und deren Interaktionen analysiert. Signifikante Effekte ($p < 0,05$) von *GH* und die Interaktion β -LGB × *GH* wurden für GWY verifiziert. Tiere mit dem Genotyp *GH* × LL hatten höhere GBWs als LV-Tiere, jedoch niedrigere GWY ($p < 0,05$).

Introduction

Animal improvement has been achieved by selection based on either phenotype or predicted additive genetic merit of the superior animals for production traits. Molecular biology techniques allow the identification of genetic variation at specific loci and the association between variation at quantitative trait loci (QTL) and production traits. The final goal is to use marker assisted selection to improve the genetic gain achieved by selection as a result of higher accuracy on the estimation of an animal's genetic value. Candidate gene strategy has been proposed to direct the search for QTL, assuming that the genetic variation at genes affecting the physiological pathways related to a phenotype would be more likely to affect the quantitative variation in that phenotype than genes or chromosome regions chosen by chance. Polymorphisms at the growth hormone gene have been associated with several production traits in cattle such as milk production and quality (LAGZIEL et al. 1996), growth (ROCHA et al. 1992, UNANIAN et al. 2000) and carcass composition and quality (SCHLEE et al. 1994a, b; TAYLOR et al. 1998). Genes coding for

milk proteins have been associated with milk quality and yield (RON et al. 1994) as well as growth traits (LIN et al. 1992, MOODY et al. 1996). The objective of the present work was to investigate the effects of growth hormone (*GH*) – *AluI*, κ -casein-*HinfI* (*CSN3*) and β -lactoglobulin (*LGB*) – *HaeIII* polymorphism on growth traits of three beef cattle crosses: Aberdeen Angus \times Nelore (STAI), Canchim \times Nelore (SRCI) and Simmental \times Nelore (STSI).

Materials and methods

The animals resulted from the following crosses: Nelore females sired by five Aberdeen Angus ($n = 72$), five Canchim ($n = 74$) and four Simmental bulls ($n = 65$), totaling 211 progenies born in 1998 and 1999. The animals were maintained under intensive management on *Brachiaria brizantha* and *Panicum maximum* pastures for dry and rainy seasons, respectively. The birth weight means for 1998 and 1999 were 32.9 and 34.4 kg for STAI, 32.1 and 32.8 kg for SRCI, 34.1 and 33.2 kg for STSI, respectively. The age at weaning was approximately 240 days. Nelore females used in the crosses were a representative sample of the Brazilian herd comprising animals from different farms. The cows used in both reproduction periods were the same, with exception to some replacements. Their ages at calving ranged from 3.4 to 17.6 years, with a mean of 6.6 years.

The restriction fragment length polymorphisms κ -casein – *HinfI* (*CSN3*), (*LGB*) – *HaeIII* and (*GH*) – *AluI*, located on the chromosomes 6, 11 and 19, respectively, were determined by digestion of polymerase chain reaction (PCR) products. DNA samples were obtained from white blood cells using a standard salting out technique. PCR reactions consisted of 200 ng genomic DNA in a standard PCR buffer with 1.5 mM $MgCl_2$, 200 nM of each dNTP, 0.4 μM of each primer and 0.5 units *Taq* DNA polymerase in 25 μl reactions. Primers for *CSN3*, *LGB* and *GH* were those described by MEDRANO & CORDOVA (1990), RON et al. (1994) and SCHLEE et al. (1994a), respectively. PCR products of *CSN3*, *LGB* and *GH* were digested with *HinfI*, *HaeIII* and *AluI*, respectively and separated the A and B alleles for the first two loci and the L and V alleles for *GH*.

Statistical analyses

Average daily gain from birth to weaning (GBW) and from weaning to yearling age (GWY) were analyzed by SAS PROGRAM (1999) using the GLM (General Linear Models) procedure and at first the following statistical model:

$$y_{ijklmno} = \mu + GG_i + S(GG)_j + CG_k + GH_l + LGB_m + CSN3_n + GH \times LGB_{lm} + GH \times CSN3_{ln} + LGB \times CSN3_{mn} + e_{ijklmno}$$

where, $y_{ijklmno}$ = trait (GBW and GWY) evaluated on the $ijklmno$ th animal, μ = overall mean for each trait, GG_i , $S(GG)_j$, CG_k , GH_l , LGB_m , $CSN3_n$, $GH \times LGB_{lm}$, $GH \times CSN3_{ln}$, $LGB \times CSN3_{mn}$ = fixed effects associated with the i th genetic group, the j th sire nested within genetic group, the k th contemporary group, the l th *GH* genotype (LL, LV), the m th *LGB* genotype (AA, AB, AB), the n th *CSN3* genotype, the interaction between respective genotypes. $e_{ijklmno}$ = random error associated with $ijklmno$ th observation.

This model was used as a preliminary analysis and the non-significant effects for *CSN3* and corresponding interactions were discarded for the final analysis.

Results and discussion

The three loci were polymorphic in all genetic groups. The genotypes AA/AB/BB for the milk proteins polymorphisms and LL/LV for the *GH* locus were observed in all genetic

groups. Despite other results suggesting effects of *CSN3* genotype on growth traits (MOODY et al. 1996; LIN et al. 1992), the present data did not show any influence of *CSN3* genotypes on the traits studied. Therefore, this main effect and its respective interactions were excluded from the statistical model. Significant differences ($p < 0.01$) on GBW trait were observed for GG, CG, sire (GG) and *GH* effects: for the GG effects these differences were Aberdeen Angus \times Nelore vs. Canchim \times Nelore and Canchim \times Nelore vs. Simmental \times Nelore (data not shown). For the second period of growth (GWY) CG, *GH* and the interaction *LGB* \times *GH* contributed significantly to the variation (Table 1). *LGB* alone did not influence GBW and GWY but there was a significant interaction ($p < 0.05$) of this locus with *GH* for GWY and an approximate significance for GBW (Table 1). Animals with LL genotypes for *GH* had higher GBW ($p < 0.05$) than LV animals but lower GWY (Table 2). As all Nelore females were homozygotes LL, no VV genotype was observed in the progenies of these crosses, so it was not possible to determine the type of gene action. Previous studies have found influence of this polymorphism on growth traits. Genotype LV was associated with a higher carcass gain than LL and VV in Simmental sires (SCHLEE et al. 1994a, b) whereas homozygotes VV had better carcass classification scores (SCHLEE et al. 1994a). Working on Piemontese \times Chianina crosses, PILLA et al. (1994) found the VV genotype to be associated with the highest chest girth and the lowest chest length. SARTORI and DI STASIO (2000) suggested indirect selection for the V allele, associated with preselection after a performance test in Piemontese calves. However, in a later work (DI STASIO et al. 2002), the authors did not provide evidence of associations of *GH* polymorphisms with meat production traits in Piemontese cattle.

Table 1. ANOVA summary for GBW and GWY

Source	DF	GBW Mean square	GWY Mean square
GG	2	0.080**	0.011
CG	10	0.061**	0.456**
Sire (GG)	11	0.026**	0.025
<i>GH</i>	1	0.051*	0.139**
<i>LGB</i>	2	0.003	0.009
<i>GH</i> \times <i>LGB</i>	2	0.027	0.041*
Error	182	0.009	—
	167	—	0.013

** $p \leq 0.01$; * $p \leq 0.05$; GG, CG = genetic group, contemporary group, respectively.

Table 2. GBW and GWY means and standard error (SE) according to *GH* genotype and *GH* \times *LGB* combinations

<i>GH</i>	<i>LGB</i>	GBW (kg) Mean \pm SE	GWY (kg) Mean \pm SE
LL	—	0.835 \pm 0.014	0.651 \pm 0.016
LV	—	0.784 \pm 0.020	0.735 \pm 0.024
LL	AA	0.832 \pm 0.017	0.631 \pm 0.035
LL	AB	0.809 \pm 0.017	0.671 \pm 0.019
LL	BB	0.862 \pm 0.014	0.649 \pm 0.017
LV	AA	0.764 \pm 0.043	0.792 \pm 0.051
LV	AB	0.809 \pm 0.024	0.678 \pm 0.029
LV	BB	0.777 \pm 0.025	0.735 \pm 0.030

GBW, GWY = average daily gain from birth to weaning and from weaning to yearling, respectively.

In the present work, differences for GWY between LL and LV genotypes were significant only when animals were homozygotes AA or BB for *LGB* (Fig. 1). Figure 2 shows the interactions between *GH* and *LGB* genotypes for GBW. For this period the interaction was not significant, although statistical significance is barely missed. Means of heterozygotes for *GH* with respect to *LGB* genotypes were 0.79, 0.68 and 0.73 kg for AA, BB and AB genotypes, respectively. The only significant difference ($p < 0.05$) was observed between the first two means. Among LL individuals there were no differences with respect to *LGB* genotype.

The same analysis was conducted to verify the effects of *GH* and *LGB* genotypes on the weight gain from birth to yearling, as a sum of the two growth periods. The results demonstrated the non-significance of these genotypes, as well of their interaction. This fact could be related to the opposite effects of *GH* genotypes on the GBW and GWY means.

Interactions between loci (*epistasis*) have not been considered for quantitative traits analysis for practical reasons, including mathematical modeling and computational power. The lack of practical evidences of its existence has long justified this simplification. This scenario is being changed by recent findings. PICCOLI et al. (2002) observed epistatic effects on pre-weaning gain of Hereford \times Nelore calves. Similar effects were verified on the post-weaning weight gain of Nelore calves (CARDOSO et al. 2002). With the advances in molecular markers studies, individual contributions of QTLs can be isolated, and interactions may be discovered. Recently, CASAS et al. (2000) identified an interaction between myostatin and two QTLs, on chromosome 5 and for fat depth on chromosome 14 for Warner-Bratzler shear force. CHEVERUD (2002), searching for QTLs affecting growth in inbred mice strains, found many instances of epistatic interaction among loci affecting this trait. Using mouse models to dissect the genetics of obesity, BROCKMANN and BEVOVA (2002) observed that interactions between QTLs contributed considerably to the phenotypic variance of growth and obesity.

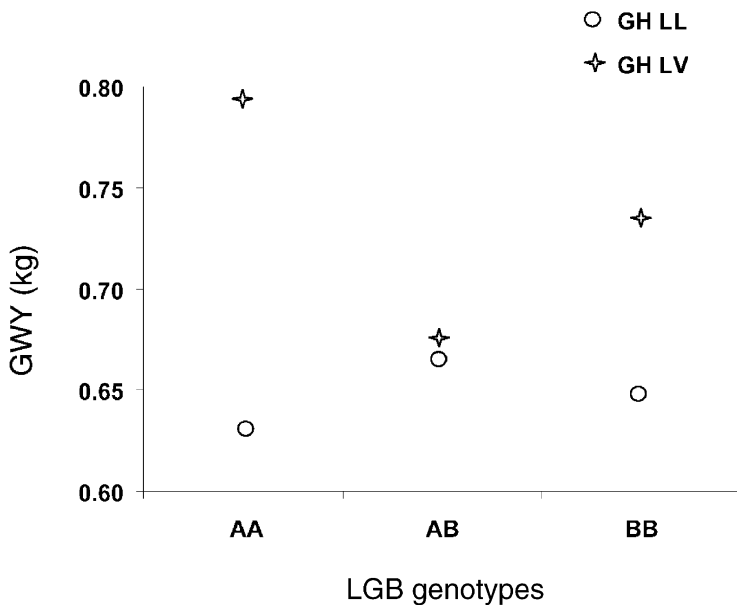


Fig. 1. Interaction of *LGB* and *GH* genotypes for GWY. (Axis X = *LGB* genotypes, Axis Y = GWY (kg))

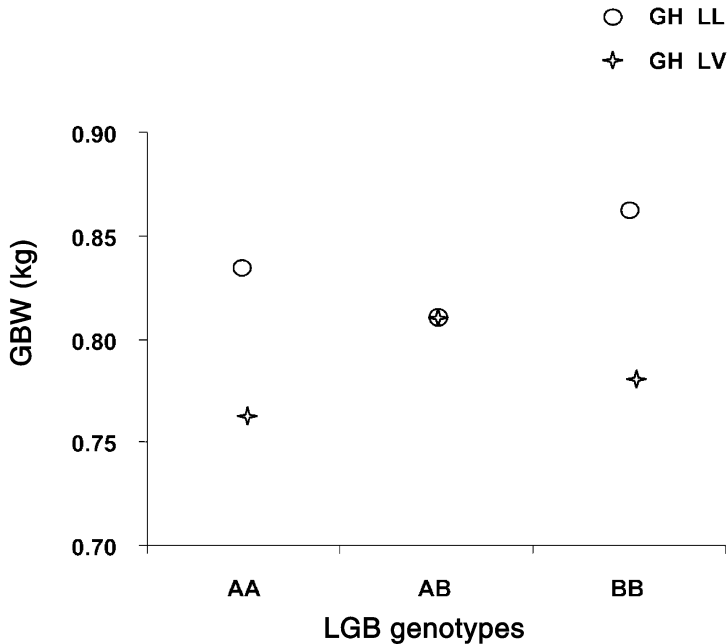


Fig. 2. Interaction of *LGB* and *GH* genotypes for GBW. (Axis X = *LGB* genotypes, Axis Y = GBW (kg))

In most QTL studies, statistical analyses have routinely considered each locus separately. Thus, they do not account for interaction between different loci. The present study indicates that interactions between marker loci should be more carefully investigated as such interactions may account for differences in genotype responses across populations and genetic backgrounds. The growth hormone LV genotype was positively related to gain at later ages (GWY), which is advantageous for beef cattle improvement, however, the influence of the *LGB* genotype should be taken into consideration.

Acknowledgments

This work had financial support from FAPESP, Embrapa and CNPq. LL Coutinho is a recipient of a productivity scholarship from CNPq.

References

- BROCKMANN, G. A.; BEVOVA, M. R., 2002: Using mouse models to dissect the genetics of obesity. *Trends Genet.* **18**: 367–376.
- CARDOSO, V.; QUEIROZ, S. A.; BRITO, F. V.; FRIES, L. A., 2002: Evidence of heterotic and epistatic effects on postweaning weight gain of Nelore calves. In: 7th World Congress on Genetics Applied to Livestock production.
- CASAS, E.; SHACKELFORD, S. D.; KEELE, J. W.; STONE, R. T.; KAPPES, S. M.; KOOHMARAIE, M., 2000: Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. *J. Anim. Sci.* **78**: 560–569.
- CHEVERUD, J. M., 2002: Quantitative trait loci affecting postnatal growth in mice. In: 7th World Congress on Genetics Applied to Livestock production.
- DI STASIO, L.; SARTORE, S.; ALBERA, A., 2002: Lack of association of GH1 and POU1F1 gene variants with meat production traits in Piemontese cattle. *Anim. Genet.* **33**: 61–64.

- LAGZIEL, A.; LIPKIN, E.; SOLLER, M., 1996: Association between SSCP haplotypes at the bovine growth hormone gene and milk protein percentage. *Genetics* **142**: 945–951.
- LIN, C. Y.; SABOUR, M. P.; LEE, A. J., 1992: Direct typing of milk proteins as an aid for genetic improvement of dairy bulls and cows: a review. *Anim. Breed. Abst.* **60**: 1–10.
- MEDRANO, J. F.; AGUILAR-CORDOVA, E., 1990: Genotyping of bovine kappa-casein loci following DNA sequence amplification. *Bio/Technology* **8**: 144–146.
- MOODY, D. E.; POMP, D.; NEWMAN, S.; MCNEIL, M. D., 1996: Characterization of DNA polymorphisms in three populations of Hereford cattle and their associations with growth and maternal EPD in line 1 Herefords. *J. Anim. Sci.* **74**: 1784–1793.
- PICCOLI, M. L.; ROSO, V. M.; BRITO, F. V.; SEVERO, J. L. P.; SCHENKEL, F. S.; FRIES, L. A., 2002. Additive, complementarity, (additive*additive), dominance, and epistatic effects on preweaning weight gain of Hereford × Nelore calves. In: 7th World Congress on Genetics Applied to Livestock production.
- PILLA, A. M.; NAPOLITANO, F.; MOIOLI, B. M.; PUPPO, S.; PILLA, F.; CARRETTA, A., 1994: Association between restriction fragment length polymorphisms and quantitative traits in Piemontese × Chianina crossbred. In: Proceedings of the 5th World Congress on Genetics Applied to Livestock Production **21**, pp. 284–287.
- ROCHA, J. L.; BAKER, J. F.; WOMACK, J. E.; SANDERS, J. O.; TAYLOR, J. F., 1992: Statistical associations between restriction fragment length polymorphisms and quantitative traits in beef cattle. *J. Anim. Sci.* **70**: 3360–3370.
- RON, M.; YOFFE, O.; EZRA, E.; MEDRANO, J. F.; WELLER, J. I., 1994: Determination of effects of milk protein genotyping on production traits of Israeli Holstein. *J. Dairy Sci.* **77**: 1106–1113.
- SARTORI, S.; DI STASIO, L., 2000: Analisi genetica del locus GH (ormone della crescita) nella razza bovina Piemontesa. In: Proceedings of the 54th Convegno Società Italiana delle Scienze Veterinarie LIV. pp. 411–412.
- SAS Institute Inc., 1999. SAS/STAT User's Guide: SAS Institute Inc., Cary, NC, USA.
- SCHLEE, P.; GRAML, R.; ROTTMANN, O.; PIRCHNER, F., 1994a: Influence of growth – hormone genotypes on breeding values of Simmental bulls. *J. Anim. Breed. Genet.* **111**: 253–256.
- SCHLEE, P.; GRAML, R.; SCHALLENBERGER, E.; SCHAMS, D.; ROTTMANN, O.; OLBRICH-BLUDAU, A.; PIRCHNER, F., 1994b: Growth hormone and insulin-like growth factor-I concentrations in bulls of various growth hormone genotypes. *Theor. Appl. Genet.* **88**: 497–500.
- TAYLOR, J. F.; COUTINHO, L. L.; HERRING, K. L.; GALLAGHER Jr, D. S.; BRENNEMAN, R. A.; BURNEY, N.; SANDERS, J. O.; TURNER, J. W.; SMITH, S. B.; MILLER, R. K.; SAVELL, J. W.; DAVIS, S. K., 1998: Candidate Gene Analysis of Gh1 for effects on growth and carcass composition of cattle. *Anim. Genet.* **29**: 194–201.
- UNANIAN, M. M.; BARRETO, C. C.; FREITAS, A. R.; CORDEIRO, C. M. T.; JOSAHKIAN, L. A., 2000: Associação do polimorfismo do gene do hormônio de crescimento com a característica peso em bovinos da raça Nelore. *Rev. Bras. Zootec.* **29**: 1380–1386.

Authors' addresses: D. D. TAMBASCO, M. TAMBASCO-STUDART and A. P. PEREIRA, Federal University of São Carlos, Box 678, 13565-905, São Carlos, SP, Brazil;
 C. C. P. PAZ, Animal Science Institute of São Paulo, Box 60, 13400-000, Nova Odessa, SP, Brazil;
 M. M. ALENCAR, A. R. FREITAS and L. C. A. REGITANO (for correspondence), Embrapa Southeast Cattle Research Center, Box 339, 13560-970 São Carlos, SP, Brazil;
 L. L. COUTINHO, I. U. PACKER, Animal Science Department – ESALQ/USP, Box 09, 13418-900, Piracicaba, SP, Brazil. E-mail: luciana@cpps.embrapa.br