ISBN 978-1-84626-022-3
Proceedings of 2010 International Conference on Agricultural and Animal Science (CAAS 2010)
Singapore, 26-28 February, 2010

The effect of genotypic variants of growth hormone on growth performances in Podolica and Charolais young bulls

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Abstract. Growth hormone gene was examined as a candidate gene because of its essential role on growth processes becoming a perfect candidate marker associated with somatotropic axis. The aim of this work were to assess the genetic variability in young bulls of Charolais and Podolica breed, traditionally reared for meat production and to study the relationship between GH-*AluI* polymorphism and growth performance traits. Blood samples for DNA genotyping were obtained from 22 Charolais and 48 Podolica unrelated bulls belonging to different farms located in Southern Italy. Animals were genotyped for the Leu/Val polymorphism in the GH gene. Allelic frequencies of L and V were 0.72 and 0.28 and 0.85 and 0.15 respectively for Charolais and Podolica bulls. The weights were measured at different age (W180, W270 and W360) and average daily gains (ADG) were calculated in three different periods (ADG180-270, ADG270-360 and ADG180-360). Charolais LL homozygotes had higher live weights (248.47 kg, 368.13 kg, 490.46 kg *vs* 243.50 kg, 360.28 kg and 479.88 kg) compared to the LV heterozygotes; Podolica LV heterozygotes had higher live weights (219.45 kg *vs* 217.54 kg) compared to the LL homozygotes only in the first 180days while at the last check (BW360) the LL showed the higher live weight (323.96 kg *vs* 321.96 kg); nevertheless all these differences were found not significant. Finally ADG was higher in the Charolais and Podolica LL animals compared to the LV bulls.

Keywords: GH polymorphism, Charolais breed, Podolica breed, growth performances.

1. Introduction

The rationale for choosing growth hormone (GH) as a candidate gene includes its role in growth, lactation, carbohydrate metabolism and many other aspects of homeorhesis [1, 2, 3]. Synthesis and secretion of GH are regulated by hypothalamic releasing factors, somatotrophic transcription factors, as well as a plethora of endocrine feedback signals [4, 5,6]. The bovine growth ormone gene is approximately 1800 bp long and consists of five exons separated by four intervening sequences [7, 8].

In farm animals, many polymorphisms have been identified in the GH gene. In the bovine Chikuni *et al.* [9, 10] found two SNPs at codon positions 127 and 172 on exon 5 of the GH gene. In homozygote, SNP at position 127 codes for either leucine (L-allele; CTG) in the LL genotype or valine (V-allele; GTG) in the VV genotype. Another SNP in the V genotype at the position 172 is called the C genotype: threonine (Thr; ACG) is replaced with methionine (Met; ATG). This mutation was also reported in Japanese black [9, 11] and Angus cattle[12]. Yao *et al.* [13] identified an indel of three bp (TGC) in the promoter region and an A to C transversion in exon 5. Zhang *et al.* [14] detected a mutation located within intron 3 and identified two alleles, designated C and D. A further polymorphic site was reported in the 3'flanking region of the gene, with the detection of two alleles, named E and F [15]. Rodrigues *et al*. [16] found a polymorphism in the promoter region of the gene.

In order to determine whether the GH genotype is useful as an indicator for animal's performance, the relationship between the exon 5 GH polymorphism at residue 127 and animal production was analyzed; in fact the substitution of a citosine for a guanine at position 2141 [17] causes an amino acid change from

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leucine (*L* allele) to a valine (*V* allele) at the residue 127. This transversion enables the genotyping at this *locus* using the endonuclease *Alu*I. This enzyme does not recognize its target sequence when a G is present instead of a C. Growth hormone was shown to be polymorphic in many breeds, being the distribution of GH variants (LL, LV, VV) and their frequencies different among each breed. Recently Dario *et al*. [18] investigated the associations between genetic polymorphism at the bGH *locus* with production traits, namely to milk production showing that the GH-*Alu*I polymorphism in Italian Jersey cows may be related to a higher milk, fat, and protein yield; similarly it has been associated with the birth-weight of beef cattle [19]; Chrenek *et al*. [20] reported an association between the GH1-*Alu*I polymorphism and meat production traits in Slovak Simmental bulls. Unanian *et al*. [21] studied the association between GH polymorphisms and weight traits in Nelore breed.

Therefore, the aim of the present study is to evaluate the effects of the GH1-*Alu*I genotypes on growth traits in Charolais and Podolica bulls because of the great interest in these breeds as their main purpose is meat production.

2. Materials and methods

2.1. Animals

The sample group consisted of 70 bulls belonging to two different breeds: Podolica (n=48) and Charolais (n=22). The Podolica breed derives from Bos primigenius podolicus [www.anabic.it], and has spread throughout an area that covers the inland territories of southern Italy. The breed numbers 100,000 head, 25,000 of which are listed in the Italian Herd Book of ANABIC (National Association of Italian Beef-Cattle Breeders). One of the outstanding characteristics of this cattle is its exceptional ability to adapt to particularly difficult environments, as well as its extraordinary capacity to utilize food resources that would not otherwise be used. The Podolica was long used mainly in a work capacity and only secondarily for beef and dairy products: with the rise and spread of agricultural mechanization, the selective trend of this breed became geared more towards beef production and, to a lesser extent, towards dairy production. In fact, its milk is ideal for producing the famous “caciocavallo” cheese. Nowadays, the purpose of selection is to obtain subjects with a marked capacity to be raised in open-pasture or semi-open-pasture systems, particularly in difficult environments, yielding good-quality beef. Excellent maternal capacity and long-life are other important selection goals.

The Charolais is a well known breed reared for meat production that comes from the west central and southeastern parts of France. These animals display excellent qualities, in terms of precocity, choiceness and fattening aptitudes but also for maternal qualities**:** an abundant production of milk (the highest among meat breeds), a strong maternal instinct, with elaborate care given to the veal and also a 92% rate of easy calving. Today is highly recognized for cross-breeding [www.charolais.fr] to improve local breed. In southern Italy Charolais purebred animals are not yet widespread [www.anacli.it].

The considered bulls are not relatives, they have different fathers and mothers as well, and they are kept on different farms and housed at one local station from initial average age (IAA) of 150±12.61 d and initial average weight (IAW) of 208.16±16.85 kg for Charolais and IAA of 150±16.17 d and IAW of 90.02±14.94 kg for Podolica. Before the challenge start all bulls were examined by the herd veterinarian. Subsequently animals’ body weight at 180 (BW180), 270 (BW270) and at 360 days (BW360) were measured. For each breed average daily gains (ADG) in three intervals were calculated (ADG180-270, ADG270-360 and ADG180-360). Animals were all fed with the same feeding ration (maize silage and concentrate) offered in a total mix ration and they had free access to water.

2.2. Genotyping

Individual blood samples for DNA genotyping were collected on K-EDTA tubes and stored at -25 °C. Genomic DNA was isolated from whole blood using GFX Genomic Kit (Amersham, Germany). After genomic DNA isolation the animals were genotyped for the L/V polymorphism in the GH gene. Genotypes were identified with the PCR-RFLP protocol as described by Reis *et al*. [22]. The 281-bp bGH gene fragment covers a part of the fourth intron and part of the adjacent fifth exon. After amplification, the PCR product was digested with *Alu*I restriction endonuclease (Sigma; 3h, 10 units/20 μL, 37°C) and analysed on a 2% agarose gel, stained with ethidium bromide, in TBE buffer.

2.3. Statistical analysis

The GH allele frequencies were calculated by simple allele counting [23]. A Chi-square test was carried out in order to verify the Hardy–Weinberg equilibrium. The effects of polymorphic variants of the GH gene on growth performance traits were analysed using the GLM procedure of SAS [24] according to the following statistical model: $Y_{ijk}= \mu + G_i + B_j + e_{ijk}$; where Y_{ijk} is the analysed trait of the bull (BW180, BW270, BW360, ADG180-270, ADG270-360, ADG180-360), μ is the overall mean, G_i is the fixed effect of the i^{th} genotype (1, 2), B_j is the fixed effect of the j^{th} breed (Charolais, Podolica), e_{ijk} is the residual error. Due to low number, animals with VV genotype were excluded from statistical analysis.

3. Results and Discussion

Three patterns were produced as result of *Alu*I restriction. Two (LL), one (VV) and three (LV) band patterns could be distinguished on the gel, which are the products of two alleles (L and V). In Table 1 were reported the observed genotypic frequencies: 11 (50%) and 34 (71%) LL homozygotes, 1 (5%) and 1 (2%) VV homozygotes, 10 (45%) and 13 (27%) LV heterozygotes in Charolais and Podolica respectively. On the basis of the Hardy-Weinberg formulas, the observed frequencies of L and V alleles were 0.73 and 0.27, 0.84 and 0.16 for Charolais and Podolica breed respectively (see Table1). Genetic equilibrium was found in both populations (Table 1). The low frequency of the VV genotype may be due to the low number of samples or to low actual genotype frequency. In Podolica breed it may also due to the natural selection at this *locus*.

Data included in Table 2 show the effect of the polymorphism on live weights and average daily gain for each breed. In the group of Charolais bulls the LL homozygotes had higher live weights (248.47 kg, 368.13 kg, 490.46 kg *vs* 243.50 kg, 360.28 kg and 479.88 kg) compared with the LV heterozygotes; even if these differences were found not significant. At the same time ADG in the first 90days (ADG180-270) and in the latter period of the test (ADG270-360) was higher in the LL (1.33 kg/d *vs* 1.30 kg/d; 1.36 kg/d *vs* 1.33 kg/d) compared to the LV Charolais animals. Initially the group of Podolica bulls showed a BW180 almost like on both genotypes (120.02 kg *vs* 120.18 kg), subsequently (BW270) heterozygotes had higher live weights (219.45 kg *vs* 217.54 kg) compared with the LL homozygotes showing a ADG180-270 of 1.18 kg/d for LL and 1.14 kg/d for LV. At the last check (BW360) the LL showed the higher live weight (323.96 kg *vs* 321.96 kg), consequently ADG270-360 was higher in the LL compared with LV heterozygotes (1.18 kg/d *vs* 1.14 kg/d), however all these differences were found not significant.

Our preliminary results show no significant differences between LV and LL animals in both considered breeds; this seems to agree with as reported by Sirotkin *et al*. [25] who observed that bulls of VV genotype had lower body mass and daily gain compared to LL and LV genotypes but no differences in these indexes between LL and LV genotypes were found. Similarly Chrenek *et al*. [20] observed that Slovak Pied bulls with genotype VV had significantly lower ($P < 0.05$) body weight and average daily gain in comparison to bulls with genotypes LL or LV. However data obtained about the phenotypic effects of the GH gene polymorphism on productive traits are not concordant. In Friesian bulls Oprzadek *et al*. [26] observed a lower meat deposition in VV animals. In Japanese black cattle Katoh *et al.* [27] reported that animals with the L-allele have a greater body weight and rate of daily gain but a lower marbling score, while those with the V-allele have a higher marbling score. Furthermore the body weight for the VV genotype was significantly lower than those for the LL and LV genotypes. Tatsuda *et al*. [11], in the same breed, reported an high carcass weight and low beef marbling associated with haplotype A, whereas beef marbling was increased by haplotype C. Nevertheless Zwierzchowski *et al*. [28] showed that the VV beef bulls had higher daily weight gain and therefore were heavier than those of other genotypes. On the other hand, Di Stasio *et al*. [29] suggested a lack of association between GH gene polymorphism and meat production traits in Piemontese cattle. A dominance effect of the GH1 L-allele might be responsible for the differences in growth performances, both on Charolais and Podolica bulls, starting from the age of 270d to the final weight at the age of 360 days. The reports presented above make it possible to conclude that phenotypic effects of the GH gene polymorphism on growth are not concordant. Nevertheless there was evidence that polymorphisms in the GH gene was associated with several production traits, although the magnitude of effects tended to be modest in most cases. These observations are of economic interest even if our assumption is that other mutations within the GH gene or genes closely linked to the polymorphic GH could be responsible of its effect.

In order to determine whether the GH genotype is useful as an indicator for animal's performance, the relationship between this polymorphism of the GH gene and animal production needs to be further clarified.

4. References

[1] C. Ohlsson, B.A. Bengtsson, O.G. Isaksson, T.T. Andreassen, M.C. Slootweg. Growth hormone and bone *Endocr. Rev.* 1998, **19** (1): 55-79.

[2] R.M. Akers. Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. *J. Dairy Sci.* 2006, **89** (4): 1222-1234.

[3] J. Ayuk and M.C. Sheppard. Growth hormone and its disorders. *Postgrad. Med. J.* 2006, **82** (963): 24-30.

[4] A. Giustina and J.D. Veldhuis. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocr. Rev.* 1998, **19** (6): 717-797.

[5] R.W. Pfaffle, C. Kim, O. Blankenstein, H. Kentrup. GH transcription factors. *J. Pediatr. Endocr. Metab.* 1999, **12** (Suppl 1): 311-317.

[6] M. Fodor, C. Kordon, J. Epelbaum. Anatomy of the hypophysiotropic somatostatinergic and growth hormone-releasing hormone system minireview. *Neurochem. Res.* 2006, **31** (2): 137-143.

[7] R.P. Woychick, S.A. Camper, R.H. Lyons, S, Horovitz, E.C. Goodwin, F.M. Rottman. Cloning and nucleotide sequencing of the bovine growth hormone gene. *Nucleic Acids Res.* 1982, **10** (22): 7197-7210.

[8] D.F. Gordon, D.P. Quick, C.R. Erwin, J.E. Donelson, R.A. Maurer. Nucleotide sequence of the bovine growth hormone chromosomal gene. *Mol. Cell. Endocrinol.* 1983, **33** (1): 81-95.

[9] K. Chikuni, T. Nagatsuma, T. Tabata, S. Muroya, S. Ozawa. Genetic variants of the growth hormone gene in Japanese cattle. *Anim. Sci. Technol.* 1994, **65**: 340–346.

[10] K. Chikuni, F. Terada, S. Kageyama, T. Koishikawa, S. Kato, K. Ozutsumi. Identification of DNA sequence variants for amino acid residue 127 of bovine growth hormone using the polymerase chain reaction method. *Anim. Sci. Technol.* 1991, **62**: 660–666.

[11] K. Tatsuda, A. Oka, E. Iwamoto, Y. Kuroda, H. Takeshita, H. Kataoka, S. Kouno. Relationship of the Bovine Growth Hormone Gene to Carcass Traits in Japanese Black Cattle. *J. Anim. Breed. Genet.* 2008, **125** (1): 45-49.

[12] W. Ge, M.E. Davis, H.C. Hines, K.M. Irvin, R.C.M. Simmen. Association of single nucleotide polymorphisms in the growth hormone and growth hormone receptor genes with blood serum insulin-like growth factor I concentration and growth traits in Angus cattle. *J. Anim. Sci.* 2003, **81** (3): 641–648.

[13] J. Yao, S. E. Aggrey, D. Zadworny, J.F. Hayes, U. Kuhnlein. Sequence variations in the bovine growth hormone gene characterized by single-strand conformation polymorphism (SSCP) analysis and their association with milk production traits in Holsteins. *Genetics.* 1996, **144** (4): 1809–1816.

[14] H.M. Zhang, D.R. Brown, S.K. Denise, R.L. Ax Rapid communication: polymerase chain reaction–restriction fragment length polymorphism analysis of the bovine somatotropin gene. *J. Anim. Sci.* 1993, **71** (8): 2276.

[15] M.M. Unanian, S.K. Denise, H.M. Zhang, R.L. Ax. Rapid communication: polymerase chain reaction-restriction fragment length polymorphism in the bovine growth hormone gene. *J. Anim. Sci.* 1994, **72** (8): 2203.

[16] C.V. Rodrigues, S.E.F. Guimaraes, E.D. Neto, L.E.L. Pinheiro. Identification of a novel polymorphism in the promoter region of the bovine Growth Hormone gene. *Anim. Genet.* 1998, **29** (1), 65-66.

[17] H. M. Zhang, D. R. Brown, S. K. Denise, R. L. Ax. Nucleotide sequence determination of a bovine somatotropin allele. *Anim. Genet.* 1992, **23** (6): 578.

[18] C. Dario, D. Carnicella, F. Ciotola, V. Peretti, G. Bufano. Polymorphism of Growth hormone GH1-AluI in Jersey cows and its effect on milk yield and composition. *Asian-Austral. J. Anim. Sci.* 2008, **21** (1): 1-5.

[19] J.L. Rocha, J.F. Baker, J.E. Womack, J.O. Sanders, J.F. Taylor. Statistical Associations between restriction length polymorphisms and quantitative traits in beef cattle. *J. Anim. Sci.* 1992, **70** (11): 3360-3370.

[20] P. Chrenek, J. Kmet, V. Sakowski, V. Vasicek, J. Huba, V. Chrenek. Relationships of growth hormone genotypes with meat production traits of Slovak Pied bulls. *Czech J. Anim. Sci.* 1998, **43** (12): 541-544.

[21] M.M. Unanian, C.C. Barreto, A.R. Freitas, C.M.T. Cordeiro, L.A. Josahkian. Associação do polimorfismo do gene do hormônio de crescimento com a característica peso em bovinos da raça Nelore. *Rev. Bras. Zootec.* 2000, **29** (5): 1380-1386.

[22] Reis C., Navas D., Pereira M., Cravador A., 2001 - Growth hormone AluI polymorphism analysis in eight Portuguese bovine breeds. *Archivos de Zootecnia* 50, 41-48.

[23] Falconer D.S., Mackay T.F.C. Introduction to Quantitative Genetics. 4th ed 1996 Essex, UK: Longman Group Ltd.

[24] Sas User's Guide Statistics, Version 8.0 Edition SAS Inst., Inc., Cary, NC, 1998

[25] Sirotkin A.V., Chrenek P., Makarevich A.V., Huba J., Bulla J., 2000 - Interrelationships between breed, growth hormone genotype, plasma IGF-1 level and meat performance in bulls of different ages. *Archives of Animal Breeding* 43, 591-596

[26] Oprzadek J., Dymnicki E., Zwierzchowski L., Lukaszewicz M., 1999 - The effect of growth hormone (GH), k-casein (CASK) and b-lactoglobulin (BLG) genotype on carcass traits in Friesian bulls. *Animal Science Papers and Reports* 17, 85-92.

[27] Katoh K., Kouno S., Okazaki A., Suzuki K., Obara Y., 2008 - Interaction of GH polymorphism with body weight and endocrine functions in Japanese black calves. *Domestic Animal Endocrinology* 34, 25–30.

[28] Zwierzchowski L., Oprzadek J., Dymnicki E., Dzierzbicki P., 2001 - An association of growth hormone, k-casein, b-lactoglobulin, leptin and Pit-11 loci polymorphism with growth rate and carcass traits in beef cattle. *Animal Science Papers and Report*, 19, 65-78.

[29] Di Stasio L., Sartore S., Albera,A., 2002 - Lack of association of *GH1* and *POU1F1* gene variants with meat production traits in Piemontese cattle. *Animal Genetics* 33, 61-64.

Table1. Observed and expected numbers and percentages (in brackets) of GH1 genotypes detected by *Alu*I RFLP analysis and allele frequencies in the two samples

	GH1 genotype			Allele frequency	
Number	LL	LV	VV	*L*	*V*
		CHAROLAIS (n=22)			
OBSERVED	11 (50%)	10 (45%)	1 (5%)	0.73	0.27
EXPECTED	11.6 (52.9%)	8.7 (39.7%)	1.7 (7.4%)		
X^2 = 0.47	P>0.1				
		PODOLICA (n=48)			
OBSERVED	34 (71%)	13 (27%)	1 (2%)	0.84	0.16
EXPECTED	34.2 (71.2%)	12.6 (26.4%)	1.2 (2.4%)		
X^2 = 0.04	P>0.5				

Table 2. Body Weight and Average Daily Gain (Kg ± s.e.) per breed and GH1 genotype

BREED	LL	LV	BREED	LL	LV
	Kg ± s.e.	Kg ± s.e.		Kg ± s.e.	Kg ± s.e.
CHAROLAIS			CHAROLAIS		
Bw180	248.47±5.91	243.50±5.60	ADG180-270	1.33±0.03	1.30±0.03
Bw270	368.13±7.40	360.28±7.02	ADG270-360	1.36±0.06	1.33±0.06
Bw360	490.46±9.29	479.88±8.82	ADG180-360	1.34±0.04	1.31±0.04
PODOLICA			PODOLICA		
Bw180	120.02±3.62	120.18±5.12	ADG180-270	1.08±0.02	1.10±0.03
Bw270	217.54±4.53	219.45±6.41	ADG270-360	1.18±0.04	1.14±0.05
Bw360	323.96±5.69	321.96±8.05	ADG180-360	1.13±0.02	1.12±0.03