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Genetic variability of Senepol cattle in Colombia using molecular markers¤

Variabilidad genética de bovinos Senepol en Colombia por marcadores moleculares

Variabilidade genética de gado Senepol na Colômbia por marcadores moleculares

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Summary

The Senepol beef cattle breed was introduced into Colombia through the use of artificial insemination and embryo transfer from a small nucleus of animals. Objective: to estimate the genetic variability of Senepol cattle in Colombia by heterologous microsatellites and to estimate gene and genotypic frequencies of single nucleotide polymorphic markers through calpastatin (CAST1), calpain (CALP316), and leptin (PB) genes. Methods: 412 blood samples from 28 herds were genotyped for population genetic structure with the STR: INRA32, BM2113, ETH10, BM1824, INRA037, ETH225, INRA064, SPS115, TGLA126, and TGLA122 microsatellite markers. Three SNPs of calpastatin, calpain, and leptin genes were used. Results: all microsatellites and SNP markers were polymorphic. The number of alleles ranged from 4 (BM1824) to 11 (INRA37), and the observed heterozygosity varied between 0.21 (INRA64) and 0.89 (BM2113). Combined probability of exclusion for the microsatellites was higher than 99.99%, indicating the usefulness of this set of markers for parentage testing in Senepol. Conclusions: despite being a small and closed population, this nucleus presents high genetic variability and low inbreeding.

Key words: *beef cattle, genetic diversity, genetic structure, microsatellite marker.*

Resumen

El ganado Senepol fue introducido en Colombia mediante el uso de la inseminación artificial y transferencia de embriones de un pequeño núcleo de los animales. Objetivo: estimar la variabilidad genética del ganado Senepol de Colombia por medio de marcadores microsatélites y estimar las frecuencias alélicas y genotípicas de SNPs en los genes que codifican para la calpastatina (CAST1), calpaína (CALP316) y

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leptina (PB). Métodos: 412 muestras de sangre de animales pertenecientes a 28 fincas fueron analizados para los STRs: INRA32, BM2113, ETH10, BM1824, INRA037, ETH225, INRA064, SPS115, TGLA126 y TGLA122 y los tres SNPs. Resultados: los microsatélites y los SNPs fueron polimórficos. El número de alelos de los microsatélites variaron entre 4 (BM1824) y 11 (INRA37), la heterocigosidad observada varió entre 0.21 (INRA64) y 0.89 (BM2113). La probabilidad de exclusión para el total de microsatélites fue mayor que 99.99%, indicando que el conjunto de microsatélites pueden ser usados para pruebas de filiación. Conclusiones: a pesar de ser una población pequeña y cerrada, este núcleo presenta una alta variabilidad genética y baja consanguinidad.

Palabras clave: *diversidad genética, estructura genética, ganado carne, marcadores microsatélites.*

Resumo

O gado Senepol foi introduzido na Colômbia mediante o uso da inseminação artificial e a transferência de embriões de um núcleo pequeno de animais. Objetivo: estimar a variabilidade genética do gado Senepol da Colômbia mediante marcadores microsatélites e estimar as frequências alélicas e genotípicas dos SNPs dos genes de calpastatina (CAST1), calpaina (CALP316) e leptina (PB). Métodos: 412 amostras de sangue de animais pertencentes a 28 rebanhos foram analisadas para os STRs INRA32, BM2113, ETH10, BM1824, INRA037, ETH225, INRA064, SPS115, TGLA126 e TGLA122 e os três SNPs. Resultados: os microsatélites e os SNPs foram polimórficos. O número de alelos dos microsatélites variaram entre 4 (BM1824) e 11 (INRA37), a heterocigosidade observada variou entre 0,21 (INRA64) e 0,89 (BM2113). A probabilidade de exclusão para o total de microsatélites polimórficos foi maior que 99.99%, indicando que o conjunto de microsatélites podem ser usados para testes de filiação. Conclusões: embora seja uma população pequena e fechada, o núcleo apresenta uma alta variabilidade genética e baixa consanguinidade.

Palavras chave: *diversidade genética, estrutura genética, gado de corte, marcadores microsatélites.*

Introduction

The Senepol beef cattle breed was first developed in 1918 in St Croix, US Virgin Islands located in the central Caribbean region, by crossing Red Poll and N'Dama cattle. The purpose of this crossbreed was to satisfy specific needs such as adaptation to tropical conditions and to meet meat quality and production objectives (Thrift *et al.*, 1986). Only two Red Poll sires were used to start this crossbred (López, 2002). Senepol entered Colombia from Brazil, The Virgin Islands, and Venezuela, becoming dispersed throughout the territory by means of embryo transfer from a small number of donor cows as well as artificial insemination (Sánchez, 2010).

Due to the small group of sires and cows used for breeding, it is probable that inbreeding would be found in the population. The use of microsatellite markers (STRs) allows for the study of the genetic diversity and population structure as well as the estimation of inbreeding coefficients (Parker *et al.*, 1998).

The STR markers are characterized by having a highly variable DNA sequence, which allows for establishing differences among individuals within and between populations (Parker *et al.*, 1998; Aranguren Méndez *et al.*, 2005; Bowling, 2001). They are also useful for genetic population studies, individual identification, genetic diversity analysis, marker-assisted selection, and detection of genes responsible for economically important characteristics, such as calpain, calpastatin, and leptin (Motter *et al.*, 2009; Casas, 2006; Giblin *et al.*, 2010).

A variety of studies report that the calpastatin protein is an endogenous inhibitor that plays an important role in regulating calpains. These studies, conducted in beef cattle, aimed to determine the physiological role played by calpastatin in tenderness in addition to the genetic implications of this gene (Killefer and Koohmaraie, 1994; Koohmaraie, 1994; Lonergan *et al.*, 1995; Boehm *et al.*, 1998). Calpastatin is found throughout the tissues where calpains are expressed, including

skeletal muscle. Its expression is even higher than that of calpains (Emori *et al.*, 1987).

Previous mapping and association studies (Smith *et al.*, 2000; Motter *et al.*, 2009; Parra-Bracamonte *et al.*, 2007) have shown changes in meat tenderness in *Bos taurus* associated to a cistein-specific calcium-dependent protein within the photolytic system of calpains (ì-calpain-CAPN1) as the underlying mechanism of postmortem proteolysis in association with its calpastatin inhibitor (Koohmaraie, 1996; Page *et al.*, 2004).

On the other hand, leptin regulates energy intake in mammals (Hossner, 1998; Geary *et al.*, 2003). It is released from fat tissue and can also regulate appetite, energy balance, and body mass in animals (Houseknecht *et al.*, 1998). Several studies have reported an association between leptin and carcass quality in cattle (Wegner *et al.*, 2001; Geary *et al.*, 2003; Giblin *et al.*, 2010; Ruiz *et al.*, 2009).

The aim of this study was to conduct a genetic characterization of Senepol cattle by using molecular STR markers and determine the allelic and genotypic frequencies for calpain, calpastatin, and leptin SNPs.

Materials and methods

Population sample

Blood samples (B) from 412 cows and sires from 28 herds (H) located in Antioquia (B=81, H=9), Atlántico (B=6, H=1), Boyacá (B=8, H=1), Casanare (B=22, H=2), Córdoba (B=114, H=2), Cundinamarca (B=85, H=6), Meta (B=59, H=3), Santander (B=16, H=1), Sucre (B=4, H=1), and Tolima (B=17, H=2) provinces were analyzed. The DNA isolation was conducted using a salting-out protocol (Miller *et al.*, 1998) and preserved at -20 °C in Buffer TE and pH 8.0 (Tris-EDTA). Procedures took place in the Animal Genetics Laboratory, University of Antioquia, Colombia.

Genotyping

The INRA32, BM2113, ETH10, BM1824, INRA037, ETH225, INRA064, SPS115, TGLA126, and TGLA122 microsatellite markers were used in this study. They were taken from the International Society for Animal Genetic (ISAG) recommended list for studies on genetic variability (Table 1), and were used applying multiplex systems (duplex or triplex) according to each marker's amplification conditions in 6% polyacrylamide gels followed by silver nitrate staining (Budowle *et al.*, 1991). The SNP's products were separated and visualized on 2% low-melting point agarose gels for calpain and calpastatin, while 3% low-melting point agarose gels were used for leptin. All amplification and restriction reactions were conducted in a Thermocycler BioRAd C 1000®. The results were verified by sequencing at Macrogen® labs (Rockville, MD, USA).

Table 1. Primer sequences, chromosome (Chr), and annealing temperature (AT) of markers used, and microsatellites in Senepol cattle.

Locus	Primer sequences 5´ - 3´ (FW-RW)	Chr	AT °C	Enzyme
CAST1 (Calpastin)	FW: AGC AGC CAC CAT CAG AGA AA	7	57	PdmI
	RW: TCA GCT GGT TCG GCA GAT			
CALP316 (Calpain)	FW: CCC CTC GCA CAC ATT ACT CCA AC	29	57.9	HhaI
	RW: ATA CGG CCT GCC ACT TTT TGA TG			
PB (Leptin)	FW: ATG CGC TGT GGA CCC CTG TAT C	4	65	Kpn21
	RW: TGG TGT CAT CCT GGA CCT TCC			
TGLA126	FW: CTA ATT TAG AAT GAG AGA GGC TTC T	20	56	
	RW: TTG GTC TCT ATT CTC TGA ATA TTC C			
INRA037	FW: GAT CCT GCT TAT ATT TAA CCA C	10	56	
	RW: AAA ATT CCA TGG AGA GAG AAA C			
BM2113	FW: GCT GCC TTC TAC CAA ATA CCC	2	58	
	RW: CTT CCT GAG AGA AGC AAC ACC			
ETH225	FW: GAT CAC CTT GCC ACT ATT TCC T	9	56	
	RW: ACA TGA CAG CCA GCT GCT ACT			
TGLA122	FW: CCC TCC TCC AGG TAA ATC AGC	21	56	
	RW: AAT CAC ATG GCA AAT AAG TAC ATA C			
INRA032	FW: AAA CTG TAT TCT CTA ATA GCA C	11	58	
	RW: GCA AGA CAT ATC TCC ATT CCT TT			
INRA064	FW: GCC CAC AGC GCT CTC TAC	23	56	
	RW: CTG AAA GCA GAA TGA GGT GC			
BM1824	FW: GAG CAA GGT GTT TTT CCA ATC	1	56	
	RW: CAT TCT CCA ACT GCT TCC TTG			
SPS115	FW: AAA GTG ACA CAA CAG CTT CTC CAG	15	56	
	RW: AAC GCG TGT CCT AGT TTG GCT GTG			
ETH10	FW: GTT CAG GAC TGG CCC TGC TAA CA	5	56	
	RW: CCT CCA GCC CAC TTT CTC TTC TC			

Statistical analysis

The genetic diversity, mean number of alleles per locus (na), effective number of alleles per locus (ne),

observed heterozygosity (Ho), expected heterozygosity (He), and Hardy-Weinberg equilibrium test (HWE) were estimated by the Popgene simulation program for population genetics (Yeh and Yang, 1999).

The percentage of polymorphic loci (Kallinowski *et al.*, 2007), the polymorphic information content (PIC) for each marker (Botstein *et al.*, 1980), and the non-exclusion parentage probabilities of first, second, and both parents (Ne-1p, Ne-2p, and Ne-pp, respectively) by each microsatellite and by the total were estimated using Cervus 3.0 software (Kallinowski *et al.*, 2007). The population structure was obtained with the R_{ST} index (Slatkin, 1995; modified by Goodman, 1997) assuming stepwise mutations -regarded as a more suitable index for microsatellites- and by using the Fstat software (Goudet, 2001). The above procedures were conducted to determine if these microsatellites are informative in studies to characterize population genetics and paternity testing.

Results

The SNP's favorable allelic and genotypic frequencies were CAST1 (A=0.69 and AA=0.47), CALP316 (A= 0.30 and AA=0.10) and PB (T = 0.57 and TT = 0.23). Genotype AA was lower than heterozygote AB in the calpain gene. A higher frequency for the favorable genotypes AA and AB was observed for calpastatin gene. Leptin SNP showed Hardy Weinberg disequilibrium and had the highest heterozygote excess (Table 2).

Regarding STR markers, the allele frequencies per locus, number of alleles, expected and observed heterozygosities (He and Ho, respectively), R_{ST} index, polymorphic information content (PIC), and non-exclusion pattern probabilities are presented in tables 3 and 4. The number of alleles varied between 4 and 11 (BM1824 and INRA37, respectively). The INRA64 and BM2113 STR showed the lowest and highest Ho (0.21 and 0.89, respectively). According to the Hardy Weinberg test results, seven microsatellites were in equilibrium ($p>0.05$), suggesting that these alleles are uniformly segregated in the studied population. Negligible differences were observed between allele frequencies, both with and without null alleles for nine microsatellites (-0.05 to 0.05), and a moderate to negligible difference (0.06) for TGLA122, which did not affect the analyses.

High He and Ho were observed (0.51 to 0.89) for nine microsatellites (except INRA64). The polymorphic information content indexes were greater than 0.50 (0.57 to 0.79) for eight microsatellites (except for INRA64: 0.21 and TGLA126: 0.49). The R_{ST} indexes obtained were close to zero (<0.04) in nine microsatellites, while it reached 0.13 in ETH10, meaning that there are no differences between subpopulations (herds). The RST index for the microsatellite sets was 0.03 (Table 4).

Table 2. Equilibrium test, genotypic, and allele frequencies of calpastatin, calpain, and leptin SNP in Colombian Senepol cattle.

Markers	Allele	Frequency	Genotype	Frequency	HWE	FIS
Calpastatin (CAST1)	A	0.69	AA	0.47	$0.63^{n.s.}$	-0.03
			AB	0.44		
	B	0.31	BB	0.09		
Calpain (CALP136)	A	0.30	AA	0.10	$0.57^{n.s.}$	-0.23
			AB	0.41		
	B	0.70	BB	0.49		
Leptin (PB)	C	0.43	CC	0.09	<0.01*	-0.57*
			CT	0.69		
	T	0.57	TT	0.22		

HWE: Hardy Weinberg test by Levene (1949). / F_{IS}: Inbreeding coefficient by heterozygosity disequilibrium.
n.s.: Equilibrium. / *: Disequilibrium.

Table 3. Frequency alleles, molecular weight (parenthesis values), expected (He) and observed (Ho) heterozygosities, polymorphic information content (PIC), R_{ST} index, and non-exclusion probabilities for the microsatellites in Colombian Senepol cattle.

Allele	BM1824	ETH225	ETH10	INRA64	TGLA126	SPS115	INRA32	BM2113	TGLA122	INRA37
1	0.14 (174)	<0.01 (140)	0.21 (211)	<0.01 (175)	0.09 (117)	0.31 (246)	<0.01 (160)	0.08 (128)	0.01 (137)	<0.01 (120)
2	0.27 (176)	0.15 (144)	0.24 (213)	<0.01 (177)	0.03 (119)	0.10 (248)	0.12 (174)	0.20 (130)	0.04 (141)	0.03 (122)
3	0.53* (178)	0.50 (148)	0.03 (215)	0.88* (179)	0.05 (121)	0.29 (250)	0.17 (176)	0.05 (132)	0.31* (143)	<0.01 (124)
4	0.06 (184)	0.10 (150)	0.13 (219)	0.09 (181)	0.01 (123)	0.16 (252)	0.23 (178)	0.13 (134)	0.48* (151)	0.08 (126)
5		0.24 (158)	0.15 (221)	0.02 (183)	0.67* (125)	0.04 (254)	0.17 (180)	<0.01 (136)	0.11 (153)	0.30 (128)
6		<0.01 (160)	0.24 (223)	<0.01 (180)	0.07 (127)	0.02 (256)	0.25* (182)	0.11 (138)	0.07 (155)	0.03 (130)
7					0.07 (129)	0.07 (258)	0.05 (184)	0.10 (140)	0.05* (161)	0.25 (132)
8								0.32* (142)	<0.01 (173)	0.21 (134)
9										<0.01 (142)
10										<0.01 (144)
11										0.07 (146)
He	0.63	0.66	0.80	0.22	0.51	0.78	0.81	0.81	0.71	0.76
Ho	0.68	0.66	0.81	0.21	0.48	0.77	0.83	0.89	0.78	0.75
PIC	0.57	0.61	0.77	0.21	0.49	0.74	0.78	0.79	0.66	0.72
Na	-0.05	0.00	0.00	0.03	0.02	0.00	-0.01	-0.05	-0.06	0.00
NE1-P	0.79	0.76	0.58	0.98	0.85	0.61	0.56	0.54	0.70	0.64
NE-2P	0.63	0.59	0.40	0.89	0.68	0.43	0.38	0.37	0.53	0.46
NE-PP	0.46	0.41	0.22	0.80	0.49	0.25	0.21	0.18	0.33	0.28
EHW	Si	Si	Si	Si	Si	Si	Si	No	No	No
R_{ST}	<0.01	0.02	0.13	0.04	0.04	0.02	0.02	0.01	0.03	0.02

Na= Allele frequency without null alleles minus least allele frequency with null alleles, where "*" indicates a slight difference for those alleles.

NE1-P= Combined non-exclusion probability (first parent).

NE-2P= Combined non-exclusion probability (second parent).

NE-PP=Combined non-exclusion probability (both parents).

RST= Slatkin´s fixation index, considering the stepwise mutation model.

The probability for not excluding a false candidate parent of a tested offspring without genotype information of any true parent (NE-1P) varied between 0.54 (BM2113) and 0.98 (INRA64). The non-exclusion probability for one candidate parent, given the genotype of a known parent of the opposite sex (NE-2P), varied between 0.37 (BM2113) and 0.89 (INRA64), and the non-exclusion probability for both parents (NE-PP) varied between 0.18 (BM2113) and 0.80 (INRA64). The total non-exclusion probability for NE-1P, NE-2P, and NE-PP microsatellites was close to zero (Table 4). Therefore, a 98, 99, and 99% probability to exclude a first, second, and both false parents, respectively, could be expected by using the entire set of ten microsatellite loci.

Table 4. Non-exclusion probability and total R_{ST} index using the set of 10 microsatellites for parentage assessment in Colombian Senepol cattle.

Mean number of alleles	7.1
Mean expected heterozygosity (He)	0.67
Mean polymorphic information content (PIC)	0.63
Combined non-exclusion probability (first parent)	0.02
Combined non-exclusion probability (second parent)	<0.01
Combined non-exclusion probability (both parents)	<0.01
Rst index (Goodman, 1997)	0.03

Discussion

The heterozygote (CT) excess and the high frequency of the TT genotype for leptin in the analyzed population showed Hardy Weinberg deviations ($p<0.05$). The high frequency of the T allele is possibly due to selection procedures focused on reaching high body fat accretion. These results are similar to those from association studies conducted in *Bos Taurus,* where carcasses from individuals having the T allele are fattier than animals with CC genotype (Buchanan *et al.*, 2002, Kononoff *et al.*, 2005).

The heterozygote's excess (CT) of the leptin gene offers the opportunity to design Senepol breeding programs using genotyped dams and sires intended for increasing the frequency of TT genotypes when the goals are selection for high feed efficiency and weight gain (Buchanan *et al.*, 2002; Cerón-Muñoz *et al.*, 2009; Giblin *et al.*, 2010).

Calpain and calpastatin genes exhibited HW equilibrium ($p>0.05$) in the analyzed population, which indicates a possible lack of selection for meat tenderness. Nevertheless, the high frequency of animals carrying the favorable allele (A) for both genes could be useful for designing selection and breeding programs directed towards meat quality improvement (Lara *et al.*, 2005; Chung *et al.*, 2001) due to the fact that both enzymes are responsible for the meat tenderization process (Page *et al.*, 2002; Delgado *et al.*, 2001). The calpain gene in Charolais, Senepol, and Senepol x Charolais was associated with daily weight gain at 205 and 240 d, estimated live weight at 205 d, weaning age, trimming weight, individual muscle weight, and Warner Bratzler force (measured at 0 d post mortem), among other measurements (Bosques-Mendez, 2008).

Results from the microsatellite panel (Table 3) showed that Colombia's Senepol population has high genetic diversity. It is worth noticing that this basic panel (average He=0.67, Ho=0.69 and PIC=0.63) is informative and adequate for genetic population studies (Botstein *et al.*, 1980; Bowcock *et al.*, 1994).

The exclusion probability of the whole polymorphic microsatellite markers was higher than 98% (with NE-1P=0.02, N-2P<0.01, and N-PP <0.01), indicating the usefulness of this set of markers for parentage testing of Senepol breeds in Colombia.

The R_{ST} average for the ten microsatellites evaluated was 0.03 by the Goodman (1972) method, indicating that 3% of the genetic variability could be due to genetic differences among herds, and to the fact that there is a high genetic flux between herds (genetic material exchange). The reason for this variability could be the high heterozygosis in the Colombian Senepol population, despite being conformed by a small nucleus and a small number of breeders.

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