

HAPLOTIPOS DE α_{s1} Y κ -CASEÍNAS OVINAS EN LAS RAZAS CHURRA Y MANCHEGA

(OVINE α_{s1} AND κ -CASEIN HAPLOTYPES IN THE CHURRA AND MANCHEGA BREEDS)

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SUMMARY

By using the RFLP (restriction fragment length polymorphisms) technique and five endonucleases, the ovine α_{s1} and κ -casein haplotypes in forty-nine Spanish dairy sheep, 26 Churra and 23 Manchega, were analyzed. Four and five haplotypes were detected in the α_{s1} and κ -caseins, respectively. The A₄ and D₄ haplotypes were only found in Manchega. PIC (polymorphic information content) values were situated about 0.5 and 0.6 in Churra and Manchega, respectively.

RESUMEN

Se analizan los haplotipos de las α_{s1} y κ -caseínas ovinas, mediante la técnica de RFLP (polimorfismos de la longitud de los fragmentos de restricción) y cinco endonucleasas, en cuarenta y nueve ovejas españolas de aptitud lechera: 26 de Churra y 23 de Manchega. En las α_{s1} y κ -caseínas se detectaron 4 y 5 haplotipos, respectivamente. Los haplotipos A₄ y D₄ se hallaron solamente en la raza Manchega. Los valores de PIC (contenido de información polimórfica) se situaron en torno al 0.5 y 0.6 en Churra y Manchega, respectivamente.

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The DNA polymorphisms of the ovine caseins α_{S1} -Cn (CSN1S1) and k-Cn (CSN3) have been described by Di Gregorio *et al.*³ and Leveziel *et al.*⁵ Ovine caseins⁴ have been assigned to the LG2 linkage group of chromosome 6 or 4.

When only one enzyme per locus is considered the polymorphic information content (PIC) of the DNA polymorphisms of ovine caseins is relatively low¹². However, if various enzymes per locus are analyzed the restriction fragments can be grouped in haplotypes, which allows much higher PIC values to be estimated.

The haplotypes of the caseins α_{S1} -Cn and k-Cn, their frequencies and PIC values in two Spanish dairy sheep breeds are presented in this study.

Forty-nine sheep were analyzed: 26 Churra from the León and 23 Manchega from Ciudad Real. The sheep studied from each breed belonged to various families.

Samples were analyzed using the RFLP technique. This has proved to be very effective in detection DNA polymorphisms in ovine caseins and was chosen for this reason.

The DNA samples were obtained from leucocytes using the Goossens & Kan⁶ technique. The DNA (15 μ g) was digested with *Eco* RI, *Taq* I, *Hind* III, *Pvu* II and *Pst* I endonucleases, (Amersham & Boehringer). Electrophoresis and DNA transfer to nylon membranes (Hybond N) were carried out according to Masina *et al.*¹⁰ Filters were prehybridized at 42°C in plastic bags with 50 % deionized formamide, 5xSSC, 50 mM NA phosphate buffer pH 6.5, 1% glycine, 0.1 % Na DodSO₄, 5x Denhardt's and 250 g/ml sonicated and denatured herring sperm DNA. C184 and C371 plasmids containing cDNAs of the α_{S1} and κ -bovine caseins¹⁴, respectively, were used as probes. The plasmids were marked using the nick translation reaction with [α ³²P]dCTP, 800 Ci/mmol. Hybridization, filter washing and autoradiography were carried out as described by Masina *et al.*¹⁰ and Di Gregorio *et al.*³

Heterozygosity and PIC values were calculated according to Ott¹³ and Botstein *et al.*¹, respectively.

Enzymes *Eco* RI and *Taq* I showed polymorphisms of two and three alleles with fragments of 3.6+1.1 and 4.7 kb, and 5.0, 8.0 and 10.0 kb respectively, at locus α_{S1} -Cn. Family data reveal that the following haplotypes segregate as Mendelian alleles:

A₁=4.7 kb *Eco* RI - 8.0 kb *Taq* I
 A₂=3.6+1.1 kb *Eco* RI - 10.0 kb *Taq* I
 A₃=3.6+1.1 kb *Eco* RI - 8.0 kb *Taq* I
 A₄=4.7 kb *Eco* RI - 5.0 kb *Taq* I

Each of the endonucleases *Hind* III and *Pvu* II showed a polymorphism of two alleles with fragments of 2.3 and 3.2 kb, and 21.0+6.8 and 27.8 kb respectively, at locus k-Cn. The endonuclease *Pst* I showed a polymorphism of three alleles with fragments of 3.5, 4.6 and 5.8 kb with *Pst* I at locus k-Cn. Family data also indicate that the following haplotypes segregate as Mendelian alleles:

D₁=3.2 kb *Hind* III - 27.8 kb *Pvu* II - 4.6 kb *Pst* I
 D₂=2.3 kb *Hind* III - 27.8 kb *Pvu* II - 4.6 kb *Pst* I

D₃=2.3 kb *Hind* III - 27.8 kb *Pvu* II - 5.8 kb *Pst* I

D₄=2.3 kb *Hind* III - 21.0+6.8 kb *Pvu* II - 4.6 kb *Pst* I

D₅=2.3 kb *Hind* III - 27.8 kb *Pvu* II - 3.5 kb *Pst* I

The number, fragment size and haplotypes observed at loci α_{S1} -Cn and k-Cn in the Churra and Manchega breeds are identical to those found in other European sheep breeds³.

Table 1 shows the haplotype frequencies, degree of heterozygosity and PIC values for each locus and breed. Haplotypes A₂ and D₂ are the most frequently occurring in both breeds. Haplotypes A₄ and D₄ were only detected in the Manchega breed.

Table 1.- Haplotype frequencies, heterozygosity and PIC values for each locus and breed

Locus	Haplotype	CHURRA (n=26)			MANCHEGA (n=23)		
		Freq. ^a	H ^b	PIC ^c	Freq. ^a	H ^b	PIC ^c
α_{S1} -Cn	A ₁	0.08			0.13		
	A ₂	0.65			0.46		
	A ₃	0.27			0.39		
	A ₄	0.00	0.50	0.43	0.02	0.62	0.54
k-Cn	D ₁	0.40			0.13		
	D ₂	0.46			0.48		
	D ₃	0.12			0.26		
	D ₄	0.00			0.04		
	D ₅	0.02	0.61	0.53	0.09	0.68	0.65

^aHaplotype frequency, ^bEstimated heterozygosity, ^cPolymorphic information content.

In contrast to the low genetic variability that casein protein polymorphisms^{2,5,7,11} exhibit, the DNA haplotypes show considerable genetic variability. The applicability of a marker⁹ is measured by its PIC value. High PIC values (around 0.55) in the two loci and in both breeds were obtained in this study. As a result the α_{S1} and κ -casein haplotypes can be used as genetic markers in the Churra and Manchega breeds.

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**INVESTIGACIONES RELACIONADAS CON LA
ACUICULTURA EN LA UNIVERSIDAD DE LEÓN.
(RESEARCH RELATED TO AQUACULTURE AT THE
UNIVERSITY OF LEÓN).**

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Palabras clave: Acuicultura, investigación, León.
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SUMMARY

A general overview of European aquaculture, which includes a list of animal species, culture purposes, state of culture methods and geographic location, is reported. Afterwards, the world and European output production is given followed by data from Spain, Castille-León and the province of León, where the research activities developed at the University and compiled in this paper are established. This compilation is organized by zoological groups, species and subject matter. Also, the list of papers arranged by alphabetic senior surname and publication date is provided.

RESUMEN

Se comienza con una visión general de la acuicultura en Europa, acompañada de una relación de las especies animales utilizadas, así como el destino de la producción, niveles tecnológicos del cultivo y localización geográfica. A continuación, se aportan datos productivos mundiales y europeos, para considerar finalmente la situación en España, comunidad de Castilla y León y provincia de León, en cuya Universidad radica la actividad investigadora objeto de esta recopilación, estructurada según grupos zoológicos, especies y materias de estudio. También se aporta una relación de publicaciones por orden alfabético de primer autor y año de publicación.

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