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## **MILK PROTEIN POLYMORPHISM IN SUDANESE DAIRY CATTLE BREEDS**

(With 2 Tables and 2 Figures)

By

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**التعدد الجيني لبروتين اللبن في بعض سلالات أبقار اللبن السودانية**

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جورج إيرهارت**

استخدم في هذه الدراسة عدد ٢٢٨ رأس من الأبقار (البقارة-الكنانة-البطانة-هجين الفريزيان مع البطانة والكنانة) وذلك لمعرفة التنوع الوراثي في ٥ مواقع البنية لبروتين اللبن -كازين-  $\alpha$ 1-casein (*CSN1S1*),  $\alpha$ 2-casein (*CSN1S2*),  $\beta$ -casein (*CSN2*),  $\kappa$ -casein (*CSN3*)  $\beta$ -lactoglobulin (*LGB*) استخدمت طريقة التفريد الكهربائي لفرز الأليلات. اوضحت الدراسة ان كل المواقع الاليلية يوجد بها تحورات (polymorphism) جينية. كما اسفرت الدراسة عن وجود اليلين جديدين هما *CSN1S1\*1* و *CSN1S2\*X* وقد وجد الاول في كل من ابقار البطانة والبقارة بينما وجد الثاني في كل السلالات التي درست.

### **SUMMARY**

The genetic variation at five milk protein loci  $\alpha$ 1-casein (*CSN1S1*),  $\alpha$ 2-casein (*CSN1S2*),  $\beta$ -casein (*CSN2*),  $\kappa$ -casein (*CSN3*) and  $\beta$ -lactoglobulin (*LGB*) was investigated in 228 animals belonging to four dairy populations well adapted to prevailing climatic conditions of Sudan.. *Bos indicus* (Butana, Kenana and Baggara) and *Bos indicus* (Kenana or Butana) X Friesian (KBF) were studied using isoelectric focusing technique for loci characterization. All loci were polymorphic and two

new variants were detected at *CSNIS1* and *CSNIS2*. The *CSNIS1\*I* variant was shown by the Butana and Baggara cattle, while *CSNIS2\*X* variant was observed in all populations under the study. Milk protein loci, being positively selected loci, can also provide information about the occurrence of germplasm particularly useful for breeding strategies and production improvements.

**Key words:** *Milk proteins, casein, lactoglobulin, polymorphism, dairy cattle.*

## INTRODUCTION

Studies on milk protein genetic variability dated back almost 50 years ago by detecting bovine  $\beta$ -lactoglobulin main variants (Aschaffenburg and Drewry, 1957), and were intensively developed during the recent years. In the last 20 years, a new impulse has been given to investigations, not only for the well-known influence of milk protein variants on milk properties (Grosclaude, 1988; Di Stasio and Mariani, 2000). In fact, the bovine milk protein polymorphism have been investigated according to different molecular approaches allowing the DNA typing of known alleles (Medrano and Aguilar-Cordova, 1990; Damiani *et al.*, 1992; David and Deutch, 1992; Barroso *et al.*, 1999; Jann *et al.*, 2002b; Cerriotti *et al.*, 2004), the molecular characterization of some variants (Schlieben *et al.*, 1991; Rando *et al.*, 1998) and the identification of further alleles (Damiani, *et al.*, 1990; Prinzenberg, *et al.*, 1999; Jann *et al.*, 2002a; Ibeagha-Awemu, 2004).

It is known today that there are at least 39 genetic variants of the major six milk protein fractions. These variants occur as consequence of either substitution or deletion of amino acids within their polypeptide chain (Ng Kwai-Hang and Grosclaude, 1992). Interest in studies focusing on milk proteins involves both cosmopolitan and local bovine breeds, including some African populations, which could be better appreciated by a deeper knowledge of their genetic variability. Today, several 'niche' populations exist in Sudan, but they are often difficult to define because of their low productivity and to the marginal social and environmental context in which they have to produce (FAO, 1995). Their survival could be connected to the identification and conservation of peculiar traits of considerable interest in such social and environmental conditions.

During the recent years, the scientific community was attentive to the development of breeding strategies aiming to improve the different productive traits by preserving autochthonous germplasm particularly

fitted to the environmental conditions (Moazami-Goudarzi, *et al.*, 2001), and also by introducing specialized and well adaptable allochthonous germplasm (Syrstad, 1989; Ehui, *et al.*, 1996). The importance of taking into account milk protein genetic variability in breeding strategies is evident; because of the relationship with milk productive traits mentioned before and supported by recent quantitative trait loci (QTL) linkage analysis (Freyer, *et al.*, 1999; Velmala, *et al.*, 1999).

Milk protein polymorphism in cattle breeds is well characterized mainly in Europe and North America, including endangered populations (Formaggioni, *et al.* 1999). Data regarding milk protein polymorphism in Zebu populations are scarce (Grosclaude, *et al.*, 1974; Mahe *et al.*, 1999; Prinzenberg and Erhardt 1999; Prinzenberg *et al.*, 1999; Moazami-Goudarzi, *et al.*, 2001). An association of milk protein genotype with the composition and properties of milk could be exploited commercially by using these genotypes as an additional criterion in selecting bulls for artificial insemination. Sudan embraces wealth information on productivity of animals, based on phenotypic values and anthropocentric criteria. Our study aims to characterize genetic variability at protein level in milk protein loci of Butana, Kenana, Bagara ecotypes and (Kenana or Butana) X Friesian crosses. The animals involved among some important (in terms of number, productivity and special characteristics) breeds of Sudanese dairy cattle.

## MATERIALS and METHODS

**SAMPLING:** A total of 228 animals, 124 Zebu and 104 (zebu x taurus), were randomly chosen for milk collection from (20<sup>th</sup> December 2004 till 25<sup>th</sup> January 2005) from different locations. Sampling area and size of each ecotype are shown in Table (1).

**Table 1:** Breed and data collection information

Genus	Breed	Number	Sampling Area
Bos indicus	Butana	63	River Nile State
	Kenana	34	Blue Nile State
	Baggara	27	Kordofan State
Indicus X taurus	(Kenana or Butana) X Friesian	104	White Nile State

Animals selected for sampling should exhibit typical breed phenotypic characteristics for *Bos indicus*. Milk samples were taken

directly from the animals at the time of milking directly into 5 ml plastic containers containing 0.09 mg of sodium azid as preservative, and the samples were transferred directly for refrigerator storage.

#### **Milk Protein Variants Analysis:**

Phenotyping of milk samples was carried out by isoelectric focusing (IEF) technique in ultra-thin Polyacrylamide gels (265x115x0.03 mm) using the method of Erhardt (1991) with some modification. In detail, the screening gel with 8 M urea containing 0.81% (w/v) Servalyte pH 2.5-5.0; 0.648% (w/v) Pharmalyte pH 4.2-4.9 and 0.29 % Pharmalyte pH 5-7. Samples were then prepared by adding 6 µl skim milk to 50 µl sample mix [24 g urea + 1.5 Dithioreitol (3%)] in 50 ml distilled water and mixed well in shaker for 5 minutes. The samples were pepitted and applied (6µl in round slot applicator) by means of a multiple syringe mm micro-pepittes in front of the anode.

The phenotypes of all systems were identified based on the results of the different comparisons test organized by International Society for Animal genetics (ISAG).

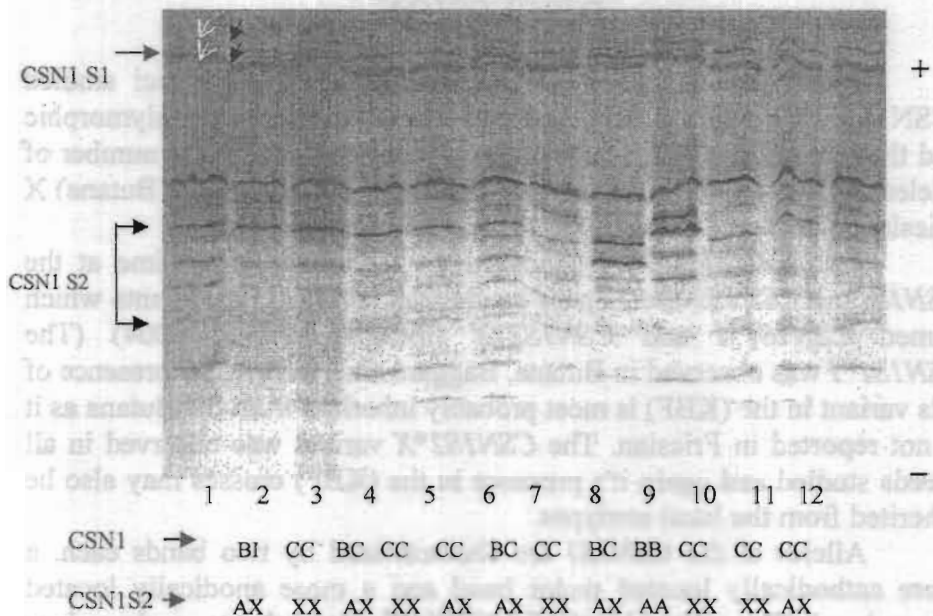
#### **Statistical Analysis:**

Allelic frequencies, observed and expected genotype frequencies and deviations from Hardy-Weinberg equilibrium were evaluated by POPGENE software (Raymond and Rousset, 1995).

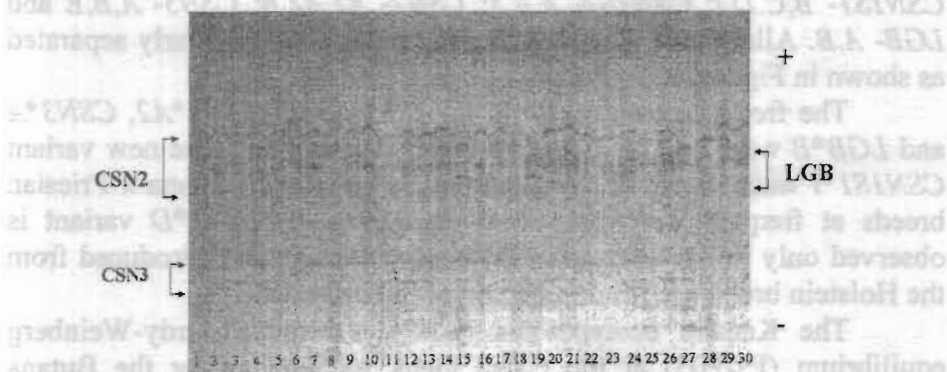
## **RESULTS**

**Table 2:** Allele frequencies in the different ecotypes

Locus	Allele	Butana	Kenana	Baggara	KBF
<i>CSN1S1</i>	<i>B</i>	0.2301	0.2429	0.3889	0.6358
	<i>C</i>	0.7540	0.7571	0.5557	0.3544
	<i>D</i>	0.0000	0.0000	0.0000	0.0049
	<i>I</i>	0.0159	0.0000	0.0554	0.0049
<i>CSN1S2</i>	<i>A</i>	0.7778	0.8088	0.8462	0.9167
	<i>B</i>	0.0159	0.0000	0.0000	0.0000
	<i>X</i>	0.2063	0.1912	0.1538	0.0833
<i>CSN2</i>	<i>A1</i>	0.1032	0.0286	0.0741	0.3301
	<i>A2</i>	0.8968	0.8714	0.9259	0.6311
	<i>B</i>	0.0000	0.1000	0.0000	0.0388
<i>CSN3</i>	<i>A</i>	0.8175	0.7714	0.6852	0.8750
	<i>B</i>	0.1825	0.2286	0.3148	0.1202
	<i>E</i>	0.0000	0.0000	0.0000	0.0048
<i>LGB</i>	<i>A</i>	0.1984	0.0429	0.0926	0.1490
	<i>B</i>	0.8016	0.9571	0.9074	0.8510



**Fig. 1:** Isoelectric focussing pattern of cattle milk samples for CSN1S1 and CSN1S2 loci. White arrows indicate the I variant.



**Figure 2:** Isoelectric focussing for cattle milk samples. Lanes 5&7 demonstrate homozygous (A2A2),lanes 9,21,24 & 26 demonstrate heterozygote (A1A2),while lanes 15 & 19 demonstrate A2B & A1B for CSN2. At CSN3 locus,lanes 2,3,6,12,20,26,27,&30 demonstrates the heterozygous (AB),while the remaining lanes demonstrate the homozygous (AA).For LGB, lanes 1& 8 demonstrate the genotype (AA), lanes 2,3,5,7,9,10,11,12,13,14,16,19,20,21,22,23,24,25,26,30 demonstrate the genotypes (BB) while lanes 4,6,15,17,18,22,27,28,29 are genotype (AB).

## DISCUSSION

Table (2) showed allelic frequencies at the five loci studied (*CSN1S1*, *CSN1S2*, *CSN2*, *CSN3* and *LGB*). All loci were polymorphic and they revealed a total of 15 alleles (2-4 allele/ locus). The number of alleles for Butana, Kenana, Baggara ecotypes and (Kenana or Butana) X Friesian (KBF) crosses were 12, 11, 11 and 14 respectively.

Two electrophoretic bands were seen for the first time at the *CSN1S1* and *CSN1S2* loci. These bands may be two new variants which named *CSN1S1\*I* and *CSN1S2\*X* (Ibeagha-Awemu, 2004) (The *CSN1S1\*I* was observed in Butana, Baggara and (KBF). The presence of this variant in the (KBF) is most probably inherited from the Butana as it is not reported in Friesian. The *CSN1S2\*X* variant was observed in all breeds studied and again it's presence in the (KBF) crosses may also be inherited from the local ecotypes.

Alleles at the *CSN1S1* are characterized by two bands each, a more cathodically located major band and a more anodically located minor band. The minor band of *CSN1S1\*I* occurs between the minor bands of B and C, likewise its major band occurs between the major bands of B and C (Figure, 1).

Three bands characterized alleles at *CSN1S2*. Bands of *CSN1S2\*X* are more cathodically located than bands of *CSN1S2\*A* (Figure, 1). Alleles observed at the five milk protein loci were therefore: *CSN1S1- B,C,D,I*; *CSN1S2- A,B,X*; *CSN2- A1,A2,B*; *CSN3- A,B,E* and *LGB- A,B*. Alleles at *CSN2*, *CSN3* and *LGB* loci were clearly separated as shown in Figure (2).

The frequencies of *CSN1S1\*C*, *CSN1S2\*A*, *CSN2\*A2*, *CSN3\*A* and *LGB\*B* were highest in all the population studied. The new variant *CSN1S1\*I* was present only in Butana, Baggara and Kenana x Friesian breeds at frequencies from 0.0049 to 0.0554. *CSN1S1\*D* variant is observed only in the cross (KF). This variant might be introduced from the Holstein breed used for upgrading of Kenana cattle.

The Kenana ecotype and (KBF), exhibited Hardy-Weinberg equilibrium ( $P < 0.05$ ) at the *CSN2* locus, as well as for the Butana ecotype at the *CSN1S1* locus. Kenana and Baggara ecotypes on the other hand showed equilibrium at the *CSN1S2* locus. *CSN3* and *LGB* loci do not conform Hardy-Weinberg equilibrium.

The present work enables to compare these results with previous data obtained by the traditional protein techniques in some African Bos genus (Mahé *et al.*, 1999; Ibeagha, 2004). The allele discrepancies

observed in this study can be interpreted, both by genetic drift and open breeding system effect. Sudanese cattle population are exposed to active pastoralism and to consequent uncontrolled crossing among populations, with a higher variability within breed and lower variability among breeds than expected.

The detection of milk protein variants through electrophoresis (e.g. PAG-IEF) of milk samples may be limiting in that, not all known variants can be demonstrated (Prinzenberg *et al.*, 1999) and only mature cows can be evaluated. It is however a quick and economic means to simultaneously investigate the presence or absence of already known alleles and the presence of new alleles in a population.

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