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DNA Polymorphisms of Casein Genes in Local Goat's Population in the Southern Tunisia

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Abstract: The type and frequency of local goat milk casein αS1 and αS2 alleles was studied using about 150 individual milk and goat blood samples. The study starts the establishment of the local goat population genetic molecular characterization through the casein gene polymorphism. Genotypic and allelic individual DNA composition was realized by molecular analysis procedures. Results highlighted the local population large variability either for casein allele and variants. The most represented casein phenotype in the population was AB with about 38% as frequency; the BB and AA phenotypes have, respectively 36 and 17%. The genetic determinism of the local goat illustrates some specificity towards Mediterranean caprine breeds. Also, a large polymorphism seems to exist among local goat pigment types showing an intra population genetic diversity. For the allele A, the most variant identified was Ab. The high presence of the allele O within the local population (12%) distinguishes the indigenous population among caprine breeds. The traditional consanguine mating in the pastoral mode explains the O presence. The DNA polymorphisms of the caseins showed a high genetic variability and the possibility to select high productive local goats. Genetic improvement plans should account with the population specificities, especially at the level of the milk quality.

Key words: Local goat population, arid zone, allele, casein αS1 and αS2, polymorphism, Tunisia

INTRODUCTION

The local goat population represents an animal group raised since centuries in the Tunisian arid zone. The natural and human selection process oriented, during long period, this population to acquire a gene pool adapted towards range lands harsh environments and resources scarcities (Najari *et al*., 2007a; Gaddour *et al*., 2007a). Among the Tunisian national goat herd, more than 60% of livestock was actually raised in arid zone where pastoral breeding contributes to regional economy (Ouni *et al*., 2006a; Gaddour *et al*., 2007b). In fact, local goat remains as a rare genetic group able to valorise pastoral resources when other domestic species strive to survive (Najari, 2005; Najari and *et al*., 2007a).

The local goat pastoral herds have reduced production due, mainly, to the resources scarcities and the climatic negative impacts (Najari, 2005). Notwithstanding this relatively small overall impact, caprine productions play an essential part in certain difficult and marginal environments, where they often represent one of the rare sources of high quality proteins (Boyazoglua *et al*., 2001). The main local breeds' production, under extensive mode, is the kid's meat and milk is usually considered with secondary importance, especially in the herd cash flow (Barillet, 2007).

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After a long marginalization period, goat production interest has recently increased in many countries (Morand- Fehr *et al*., 2001). In the Mediterranean zone, goat milk is either produced in intensive modes when specialized caprine breeds were used and also, in southern marginal zones by exploiting local breeds and populations (Boyazoglua *et al*., 2001).

Both for kids growing and for milk industrialisation, the milk production quantity and quality were determining. Milk protein composition differs quantitatively as well as qualitatively among goat breeds and genetic groups (Chilliard *et al*., 2006). The protein and fat contents variability is genetically controlled, especially by the as-casein locus, which exhibits a high degree of polymorphism (Ricordeau *et al*., 1999). Also, the casein polymorphism is related with various allele mutations (Martin, 1993). As for selected breeds, for an animal population a large variability is currently expected at all biological level due to the large genotypic diversity. So, understanding the polymorphism of caseins contributes to characterize every population genetic structure and evolution (Naves, 2003). Such knowledge can, equally, offers some precious improving ways for local populations especially when the milk quality and cheese output is considered as a selection criteria.

A stochastic approach is proposed to predict responses to selection when using α_{s1}-casein genotype information in a selection scheme of a local goat's population when two independent selection objectives were considered: protein yield and protein content. Inclusion of the casein genotype as an additional selection criterion will improve gains for protein traits (Sánchez *et al*., 2005).

The gene of casein alpha-S1 presents many alleles. One can gather them in four categories according to their influence on the rate of casein alpha-S1 in milk: the alleles A, B and C are strong, the allele E is average, the alleles D and F are "weak" and the allele O is null. A polymorphism with multiple alleles was found in most Mediterranean breeds (Martin, 1993; Jordana, 1996; Moioli *et al*., 1998).

The Tunisian local goat population was subjected to a genetic characterization in the Arid Land Institute laboratories. Since a decade, pastoral husbandry and animal performances was studied to define local goat productive behaviour under arid conditions. Actually, many scientific results and parameters were established (Ouni, 2006; Ouni *et al*., 2006b; Najari, 2005; Najari *et al*., 2007a; Najari *et al*., 2007b; Gaddour *et al*., 2007a; Gaddour *et al*., 2007b ; Gaddour *et al*., 2007c). To diagnose deeply the local goat genetic characteristics, the study of the DNA polymorphism of some gene is started in the aim to valorise genetic specificities and to build selection criteria for our local population. Note that the type and frequency of goat milk protein alleles have not yet been studied for the local goat's population. The present research reports the initial and partial characterization of allelic polymorphism of casein alpha s1 of local goat. Thus, the aim of the present study is to present the local goat polymorphism regarding the casein s1 as a step to characterize this rustic population under arid harsh environment.

MATERIALS AND METHODS

Study Zone

The experiment was carried out in arid region of Tunisia as ecologically defined by Ferchichi (1996). The climate is arid Mediterranean, hard and precarious (Ouled Belgacem, 2006). With an annual average of 140 mm, precipitation presents a large spatial and seasonal variation. The arid zone is actually the most important of rangelands of the country and is mainly used for extensive camel and small ruminants grazing (Nasr *et al*., 2000).

Animal materialThe indigenous goat population constitutes an animal group adapted to the arid rangelands harsh conditions (Najari *et al*., 2006). The complex adaptation criteria were acquired by a long natural and human selection process under local hard conditions (Najari, 2005). The indigenous

Table 1: Blood and milk samples repartition by morphologic characters of local goat

Morphologic character		Samples no.
Pigment type	Lada	36
	Light belly	22
	No pattern	38
	pinto	7
	Head spotted	25
	Gelé	19
Horn	with	7
	Without	140
Wattles	with	131
	Without	16
Total		147

goat population shows a large variability both in morphology and performances (Najari, 2005). Characteristics of the population include the ability to walk long distances, water deprivation resistance and good kidding ability. Fertility rate is about 87% and prolificacy rate varies between 110 and 130% (Najari, 2005). Kidding season begins in October and continues till February with a concentration in November and December when 69.2% of kids are born.

Samples Collection and Analysis

The determination of the allele's casein α was limited to a subset of 150 goats raised in pastoral mode in different regions of arid zone. Samples collection and analysis were done during 2000. Table 1 presents the data distribution by morphologic criteria. Samples were collected in three herds raised in the Tunisian arid zone and the individual goat characteristics were registered to diagnose possible relation between genetic polymorphism and visible variability.

The frozen milk and blood samples were analyzed by electrophoresis to typify caseins αS1 and αS2 either at phenotypic and genotypic levels. Samples of fresh blood were treated, for extraction of the DNA, for different typing thanks to techniques developed at Institut National Recherche Agronomique (France) laboratory (Ricordeau *et al*., 1999).

RESULTS AND DISCUSSION

Genotypic Frequencies of the Casein Polymorphism for the Local Goats

Table 2 shows the phenotypic frequencies of milk α S1casein polymorphism observed in the local goat population in Tunisian arid zone. The phenotypes AB, B and A seem to be, respectively, the more represented within the population. So, the milk quality can be considered high due to the relation of these phenotypes with milk production quantity and quality in the caprine breeds (Chilliard *et al*., 2006; Angulo *et al*., 1994). Milk from homozygote phenotype A goats has higher protein, casein and fat contents and a higher casein/protein ratio than milk from FF goats (Vassal *et al*., 1994; Delacroix-Buchet *et al*., 2000). So, the local population genetic profile is specific and it illustrates, in parts, its dairy characteristics towards other caprine breeds and populations.

In other respects, the genetic polymorphism at the alpha-s1 casein locus has important effects on the true protein, the casein and the fat content of milk, the maximum gel strength and the gel strengthening rate (Remeuf, 1993).

Rather than this local population specificity, important intra population variability is show on Table 2. This large variability confirms the marked differences between breeds and also intra populations and goat genetic groups (Moatsou *et al*., 2004; Najari, 2005). The large caprine variability, concerning the casein polymorphism, was observed in the Mediterranean area and within the same regions and countries (Enne *et al*., 1997; Grosclaude and Martin, 1997). Hence, among our indigenous

Table 2: Frequencies at the S1casein genotypes for 150 samples of local goat population

Pigment type	AA	AC	AE	AEB	BB	BC	EB	AB
Lada	0.06	0.01	0.01	0.00	0.09	0.00	0.01	0.08
Light belly	0.00	0.00	0.00	0.01	0.08	0.00	0.01	0.06
No pattern	0.03	0.00	0.00	0.00	0.08	0.00	0.01	0.12
Pinto	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.02
Head spotted	0.04	0.00	0.00	0.00	0.07	0.01	0.01	0.04
Gelé	0.04	0.00	0.00	0.00	0.04	0.00	0.01	0.06
Total	0.17	0.01	0.01	0.01	0.36	0.01	0.06	0.38

Table 3: Genotypic frequencies of the casein αS2 with respect to the local goat pigment type

Pigment type	AA	AC	BB	CC
Lada	0.12	0.09	0.01	0.04
Light belly	0.08	0.07	0.00	0.01
No pattern	0.12	0.09	0.00	0.03
Pinto	0.03	0.02	0.00	0.00
Head spotted	0.12	0.05	0.00	0.00
Gelé	0.07	0.05	0.00	0.01
Total	0.54	0.37	0.01	0.09

pigment types, a large variability of phenotypes frequencies exists in studied morphological goat types. All phenotypes vary largely from a goat pigment to another (Table 1). This local population large variability, regarding casein types, collaborates with the large morphologic and productive variability intra population for local goat concluded by several authors (Najari, 2005; Najari *et al*., 2006).

For the black local goat, which is the principal pigment type present in our population (Najari, 2005), the most present casein phenotype is the AB. However, the phenotype A is not observed for the pigment light belly.

AA homozygous genotype, which represents about 17% in our population (Table 2), is related with lower milk yield compared to AE, AF, EE and FF goat genotypes (Moioli *et al*., 1998). This can explain the low local population milk production which estimated to 97 kg per lactation in average (Najari, 2005). However, the homozygote AA were related to high protein percentage. Moioli *et al*., (1998), assigned for AA a genotype an average of 3.5 g protein/kg milk for Murciana-gradina breed. Milk fat percentage is also higher in AA and AE confirming, thus, the particular local goat milk quality. Table 3 presents the frequencies of the casein alpha s2 in the local population and its variation with respect to goat pigment type. Results show that the most represented milk phenotype is the AA and AC with, respectively 54 and 37%. The phenotypic variability seems to be more restricted than the variability of casein alpha s1. As well as for casein alpha s2, the genotypic polymorphism at casein alpha s2 affects significantly the milk production and composition
(Plan de développement de la R-D, 2002-2007)

With respect to goat pigment types, Table 2 shows the phenotypic frequencies of genotypes AA and AC for different goat morphological types. Note that some goat pigment types have more equilibrated genetic profile than others. The polymorphism variability at casein alpha s2 is shown between caprine breeds, populations and intra populations (Marie-Michèle Filion, 2006).

Allelic and Variants Frequency of the Casein Alpha S1 for the Local Goats' Population

Table 4-6 show the frequencies of alleles in the local population and by goat pigment type. Frequenciesy of allele B3 and B4 were 43 and 57%, respectively in local population. The allele A is considered as favourable with its contribution in the hypophysarian secretion which affects the dairy performances by the bias of the GH, PRL and TSH-B synthesis (Gengler *et al*., 2001). More ever, the same author indicated that the A allele presence can be related with the increase of the total milk

Table 4: Alleles frequencies of the casein αS1 for the local goats

Pigment type	A	B	O	E	F
Lada	0.10	0.10	0.03	0.00	0.00
Light belly	0.03	0.11	0.01	0.00	0.00
No pattern	0.08	0.13	0.04	0.01	0.00
Pinto	0.02	0.02	0.00	0.00	0.00
Head spotted	0.04	0.08	0.04	0.00	0.00
Gelé	0.05	0.07	0.00	0.00	0.00
Total	0.32	0.51	0.12	0.01	0.01

Table 5: Frequencies of allele A variant in local goat population

Aa	Ab	Ac	Ad
0.007	0.259	0.003	0.054

Table 6: Frequencies of all casein allele variants in local goat population

Alleles	Frequencies
Aa	0.007
Ab	0.259
Ac	0.003
Ad	0.054
B1	0.003
B2	0.003
B3	0.214
B4	0.286
O3	0.119
O4	0.003
E	0.010
F	0.007

production. Mean micellar size is lower in A milks (Pierre *et al*., 1998b; Remeuf, 1993). Lipolysis is lower in A milk and the relative proportions of FFA were different in A and O or F milks (Lamberet *et al*., 1996; Pierre *et al*., 1998a). Total solids, fat recovery and gross yield are significantly higher in cheese made from milks of goats with high rate of as1-casein synthesis (Remeuf, 1993; Vassal *et al*., 1994).

Cheeses made from FF milks exhibit a more intense goat flavour than that made from AA milks (Delacroix-Buchet *et al*., 1996; Lamberet *et al*., 1996; Vassal *et al*., 1994). Also, caprine milk, with very low as1-casein content, was found to be less allergenic than that with a high level of as1-casein (Bevilacqua *et al*., 2001). The B allele controls the cheese quantity and the coagulation time (Gengler *et al*., 2001). The high frequency of the allele B in the local population (Table 4) illustrates a relation with the adaptative behaviour of the indigenous goat and the quality of its milk.

The allele O presence in our population seems to be high with respect to other Mediterranean caprine breeds and populations (Moatsou *et al*., 2004). According to Chianese *et al*. (1993), allele O in goats is associated with very low casein content in milk. The presence of the allele O in the local goat population illustrates a consanguine mating traditionally adopted in the pastoral mode. In fact, in the pastoral breeding mode of the local goat, the bucks were mainly selected in the same herd progeny and they were kept during a long reproductive period (Najari, 2005; Najari *et al*., 2002). The consanguine mating, characterizing local goat husbandry, can explain the large genetic variability observed between genetic groups intra population.

The casein allele repartition according to the goat pigment shows the genetic variability of this population for the production of the milk in the arid zone is (Table 4). It is noticed that the alleles expressed differently for the various goats pigments especially for the allele B witch vary form 2% for the pigment pinto to 13% for the goat black.

For each casein allele, the variants frequencies were presented on Table 6. Regarding the allele O, the variant O3 is the more present in the local goat population. Four variants of the allele B were

present in our indigenous population; these four alleles were described by Ricordeau *et al*. (1999). The quantitative effects of goat αs1-casein variants on dairy traits were studied in both on-station (Barbieri *et al*., 1995) and on-farm experiments (Mahe *et al*., 1993).

CONCLUSIONS

The study of the casein polymorphism allowed to characterize the local goat population and to illustrate this genetic variability at the level of a dairy character. This study adds an important knowledge about the population structure and it can help to understand its genetic evolution. The polymorphism at the casein locus is now sufficiently documented as well as its relation with the population husbandry and consanguine mating.

Allelic frequencies illustrate the local population large genetic variability and its' dairy production characteristics genetically acquired through natural selection under arid harsh environment in the southern Tunisia. Also, some specificity towards Mediterranean breeds was detected. This can help to apprehend our local goat diversity studying and capacities valorisation. The large genetic population variability, at the casein polymorphism, contributes to identify some animal group able to select dairy local goat. A particular importance has to be allowed to the milk quality study in the aim to valorise local goat milk production. In fact, results underlined that, even though reduced, local goat milk production has genetically a determinism which supports protein high quality.

In spite of the results importance, they remain only a first step to characterize the local population genetic structure and capacities. In fact, scientific priorities should be directed firstly towards homogenous groups' identification and the study of the variants frequencies within the population sub groups. Further more, is necessary to determine if we can increase the local goat milk production and components without deteriorating qualities which differentiate indigenous goat's milk from other caprine selected breeds. Also, it will be necessary to found genetic markers associated proteins other than caseins and which would have an effect on quality of caprine milk.

Then, proteinic polymorphism describes only a part of DNA polymorphism reality and to evaluate genetic diversity, it necessary to identify some markers who describes better the whole genome polymorphism of the genome, that is to say microsatellites.

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