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Milk protein polymorphism study in the Algerian sheep breed Hamra

A. AMEUR AMEUR[1,2*]., MT. BENYOUCEF[2], M. AMRANI[4]., SBS. GAOUAR[3]

[1] Department of agronomical sciences, University of Bouira (UAMO), Algeria
[2] National Superior School of Agronomy, (ENSA - ES1603), Algeria
[3] Department of Biology, University of Tlemcen, Algeria
[4] Laboratory of Biology of Microorganisms and Biotechnology, University of Oran, Algeria

* Auteur correspondent: ameurabdelkader@gmail.com

Abstract - In sheep, the polymorphism of major milk proteins is reduced as compared with other ruminant species, like cattle and goats. As in other ruminant species, in sheep these polymorphisms were studied relative to milk quantity, quality and its manufacturing properties or for authenticity identification of dairy products. In Algeria, data concerning the characterization of the sheep breeds in the six loci codifying for major milk proteins genes are almost absent. The purpose of our study was to determine milk protein polymorphism in local sheep breed, using 30 samples of milk from local sheep breeds Hamra. Our work consisted in the isolation and characterization of milk proteins. For this, we used the precipitation of caseins pHi (pH 4.6). After extraction, purification and assay, both casein and serum protein fractions were then assayed by the Bradford method and separated by SDS-PAGE. Milk protein variants were determined by the molecular weight in gel using a molecular weight proteins ranging from 10 to 250 kDa. The electrophoretic pattern of milk samples showed the presence of four major caseins variants (αs1-, αs2- β- and k-casein) and two whey proteins (β-lactoglobulin, α-lactalbumin). Our study on percentage analysis of protein fractions of interest revealed that caseins represented 79.16% of the total protein of sheep milk, followed by whey proteins with 20.43%. According to the standards, the total ovine milk proteins showed a molecular weights, which are respectively (33, 32, 28 and 27 kDa) for caseins fraction and The β-lg, α-la fractions were determined to be around 19 and 15 kDa respectively. Further studies (Real Time qPCR) are requisite for certitude of the results on the polymorphic genes of proteins from sheep milk, in order to identify the genetic variants from the locus of each protein.

Keywords: Caseins, Hamra, Protein Polymorphism, Sheep milk, SDS-PAGE

1. Introduction

There is a particular worldwide interest for the conservation and breeding of the local breeds of animals due to their superiod biological traits. The high efficiency of local sheep production for example is given by their superior traits – rusticity, resistance and the capacity to acclimatize to different environmental conditions, their better productive potential in relation to the level of improvement and technology of rearing and exploitation (Carta et al. 2009). Being increasingly aware of the benefits offered (meat and milk production with special nutritional qualities) the government, organizations and associations of state and private breeders and specialists in animal husbandry have created programs to study, to conserve and ameliorate these breeds. Hamra race, known as Beni Ighil is indigenous to North specifically the Moroccan High Atlas Africa where she was raised by the Beni-Ighil tribe from which it derives its name. This sheep characterized by its small size with a dark brown head and legs tending towards red. She has some ability in particular its strength, but now in sharp decline because of his non-preferred height for race Ouled Djellal (Chellig 1992). Despite the limited genetic knowledge on milk protein variability in sheep, interesting relationships between genetic milk

protein variants and traits of economical interest were already described (Rampilli et al. 1992 and Pirisi et al. 1999). Relationships of such variation with milk quality, composition, and technological characteristics have been found for α s1-casein (α s1-cn), where D allele is less favourable for cheese making than the most common C variant. Caseins are responsible for the enzymatic coagulation of milk and their hydrolysis pattern is an important characteristic of cheese ripening. Contradictory results have been obtained regarding β-lactoglobulin (β-lg) associated with the yield milk production. Some works found significant relationships between the main variants A and B and milk production (Bolla et al. 1989 ; Fraghi et al. 1996 and Nudda et al. 2000) fat and protein content (Garzon and Martinez. 1992 ; Giaccone et al. 1997 ; Rampilli et al. 1997 and Dario et al. 2003), while other studies did not reveal any influence on milk traits (Barillet et al. 1993 and Recio et al. 1997). Important relationships exist also between protein polymorphism and breed. For instance, This study is part of a larger project concerning the use of milk protein genetic polymorphism as potential genetic marker for the Algerian locals sheep breeds production trait. The purpose of this work was to determine the milk protein polymorphism in Algerian sheep breed Hamra, allowing thus a better knowledge of the breed, for sustainable genetic improvement and conservation. The two main protein groups found in sheep milk: caseins and whey proteins were analysed.

2. Materiels et methodes

2.1. Samples origin

The milk samples used in this study were obtained from sheep flocks of Hamra , They were raised in the same conditions at the National institute of breeding "ITELV" located in Ain Al Hajar region, ten (10) km far from the town center of the province of Saida. The size sample is 30 sheep. As the average age and weight of sheep it was 3.5 and 45.6 kg, respectively. The flock is led to the wrestling during the spring season (April-May) ending in autumnal births (September-October).

2.2. Preparation of milk samples for total protein determination

50 mL milk from each sample were centrifuged at 3500 rpm at 4°C for 20 minutes, The separation between the caseins and the whey proteins is obtained by precipitation at pH 4.6 in the presence of milk a hydrochloric acid solution 3N, followed by centrifugation at 3500xg / 15 min. The various fractions obtained (casein and serum protein) are dialyzed against distilled water for 48 h. They are then concentrated, frozen in cups and finally lyophilized and stored in that form.

2.3. Milk protein separation

For the purposes of the electrophoretic analysis, the content of the isolated protein fractions is estimated by spectrophotometric assay using the method of Bradford (1976). The reagent is prepared with 100 mg of Coomassie blue G 250 (Merck), 50 ml ethanol 96 ° and 100 ml of 85% acid octophosphorique. The mixture was adjusted to one liter with distilled water. The standard range is prepared with increasing amounts of bovine serum albumins (BSA) (1 mg / ml distilled water). From 10 to 50 mg; diluted with solvents containing the proteins to be assayed, the final volume is 100 .ml, 2 ml of reagent are added to each tube, then the tubes are incubated in the dark at room temperature (25 ° C) for 15 min, then reading the optical density is performed by a spectrophotometer at 595 nm (V530 JASCO UV / VIS Spectrophotometer).

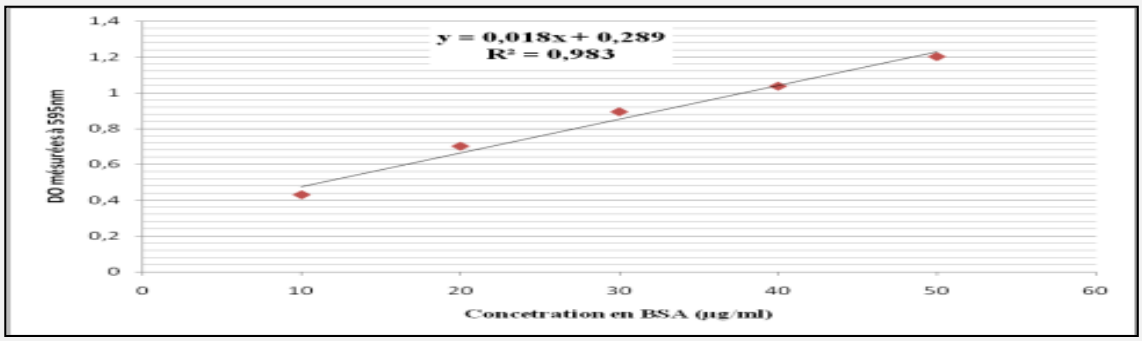

Figure 1. Standard curve for the protein assay by Bradford method (1976) as a protein standard with BSA.

Milk proteins were further separated by SDS-PAGE electrophoresis (maxi-tank (10x8 and 10x10cm. The Studier Model SE400). Following the protocol of Laemmli (1970), we performed a concentration gel 17 % polyacrylamide gel. 5µg of protein samples diluted in migration buffer (1.5M Tris-HCl pH 8.8, 17.4% glycerol, 8% sodium dodecylsulphate (SDS), 0.08% bromphenol blue and 3M beta-mercaptoethanol) were migrated for 1h and 30 minutes, to facilitate separation of proteins, based on their apparent molecular weight. After migration, gels were stained by immersion in staining dye (Coomassie Blue 250 R) for 15 minutes. The apparent molecular weights of analyzed proteins were established in relation with a standard known protein. The kit of protein standards, used for making the calibration curve Log MW = F (distance traveled), consisting of protein molecular weight ranging from 10 to 250 kDa (PageRuler™) (figure 2).

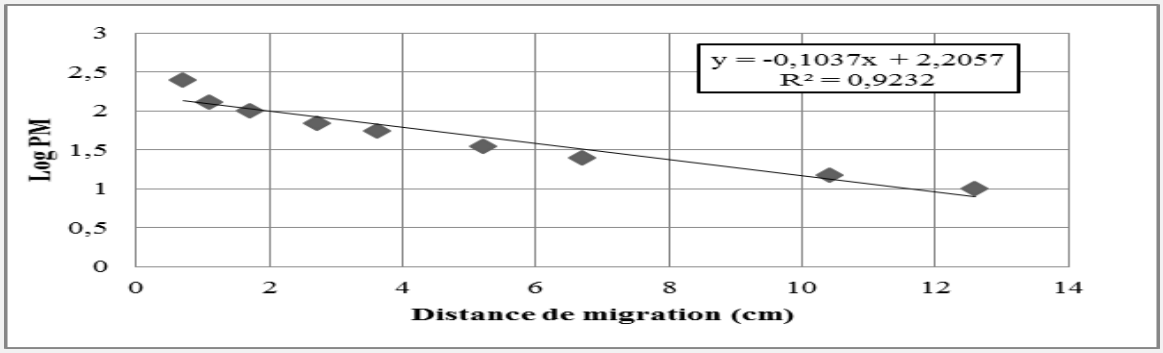

Figure 2. Calibration curve of the separation gel in SDS-PAGE using a protein standard (PageRuler ™) MW of from 10 to 250 kDa.

2.4. Gel visualization

The gels were subjected to densitometry quantification using a transluminator and the ImageJ software (http://rsb.info.nih.gov/ij/index.html). ImageJ converts pixel intensities into optical density using the function: Unc. OD = log 10(255/pixel value). The relative expression of milk proteins were expressed as percentage (%).

3. Results and discussion

Milk samples were further analyzed for protein polymorphism. SDS PAGE electrophoresis was used to determine the following proteins in the analysed milk samples: αs1-casein and αs2-casein, β-casein, κ-casein, α-lactalbumin and β-lactoglobulin. Migrated protein bands were obtained for each analysed milk sample, with different expressions within the same type of protein. The electrophoretic pattern of milk samples of Hamra sheep showed the presence of four major caseins variants αs1-casein, αs2-casein β-casein, k-casein and two whey proteins, β-lactoglobulin and α-lactalbumin. A representative selection of electrophoretic pattern of milk proteins (from sample 1 to 10 for each race) is shown in (Figure 3)

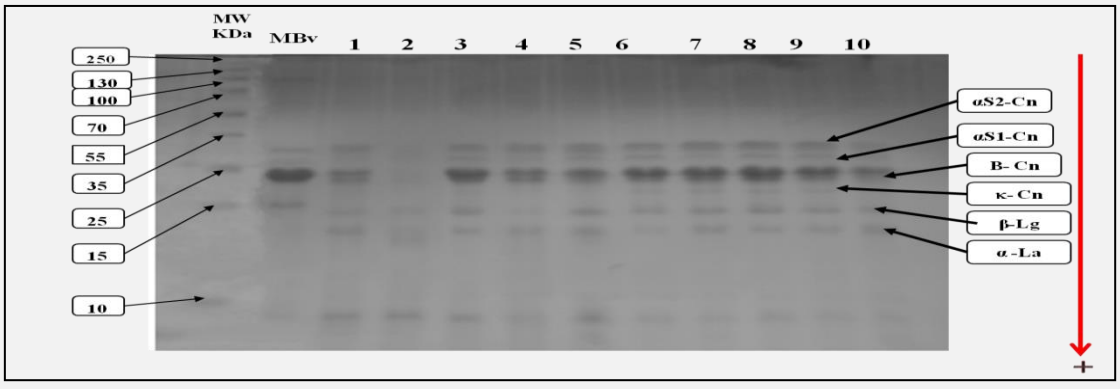

Figure 3. Representative selection of electrophoresis pattern of milk proteins (samples 1-10) in Hamra sheep. (T = 17%, C = 2.7%). **MW**: Molecular Weight, **MBv**: Milk bovin, **Cn**: Casein

Asia et al. (2011) and Ameur ameur et al (2014), describe the following ascending order of appearance of ovine casein SDS-PAGE: casein αS2 the αS1 casein, beta casein and kappa casein.
Such electrophoretic behavior is highlighted in table1. We can, through the work of authors cited, match 4 bands migration, αS2 casein, αS1 casein, β-casein and κ-casein respectively, which have respectively a molecular weight (30 674, 28 270, 26 398 and 27 214 Da).
Nevertheless, those we have obtained for our milk races do not correspond to the actual molecular weight of sheep proteins, and away from values reported by Trujillo et al. (2000), (Table1).
This difference between the actual and experimental values obtained can be explained by two factors, one being the strong association of caseins thus giving them a higher molecular weight than their actual molecular weight this results in low migration SDS-PAGE. The other is weak attachment of SDS with casein, this characteristic is well noted for other species casein (Boumahrou et al. 2009)

Table 1: Mlecular weight of sheep milk proteins

Proteins fraction	Molecular weight (Da)	
	Our study (SDS PAGE)	RP-HPLC/MS*
αS2 casein	30 674	25 616
αS1 casein	28 270	23 411
β casein	26 398	23 750
κ casein	23214	19 373
β-Lg	20.935	18 170
α-La	18.876	14 152

*Trujillo et al., (2000),

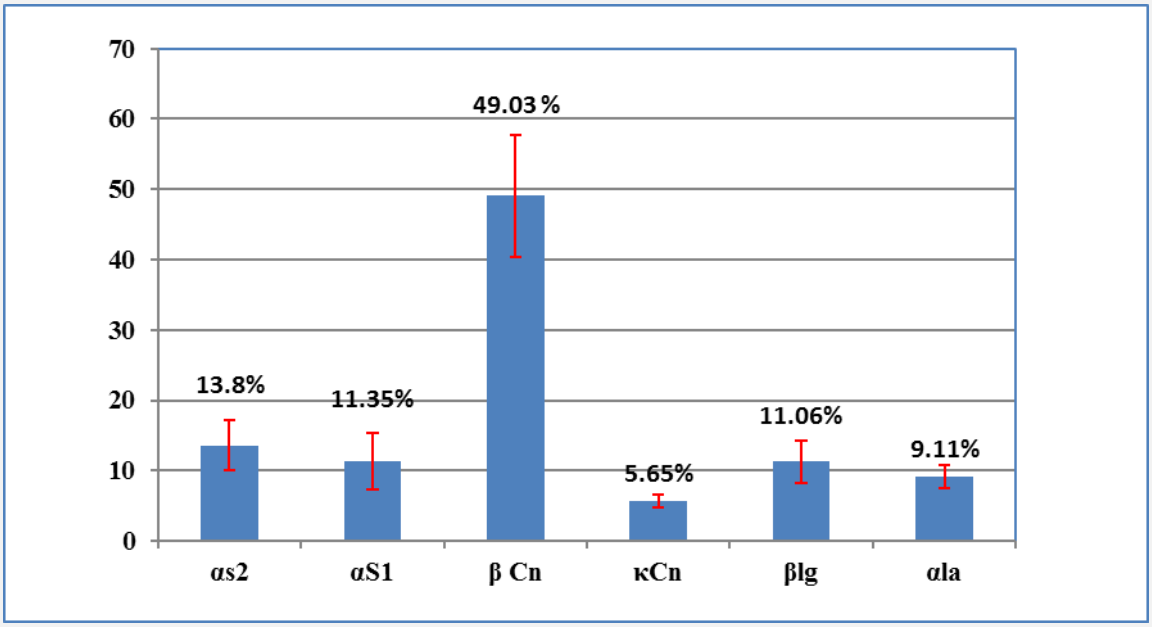

Figure 4: The expression level of milk proteins in Algeria sheep breed Hamra

Table 2: Proportion of different protein fractions in milk of local sheep breed

Protein fractions		% of total protein*	
		Mean	SEM
Total caseins		79.57%	17.25
Caseins	***β-casein***	49.03%	8.75
	k-casein	5.64%	09
	αs1- casein	11.3%	4
	αs2- casein	13.6%	3.6
Total whey proteins		20.43%	4.66
Whey proteins	***β-lactoglobulin***	11.25%	3
	α-lactalbumin	9.18%	1.66

*Protein fractions were separated by SDS-PAGE and intensity of bands was analyzed by software **ImageJ**. The mean values ± standard error of the mean (SEM) were calculated for each analyzed protein.

Caseins are the major proteins in sheep milk, accounting for 76-83% of the total proteins, being positively correlated with cheese production (Park et al., 2007). Furthermore, the milk of the local sheep breeds is more appropriate for cheese production because of the higher content of all types of casein (Revilla et al., 2009). These authors have found that the local breeds Churra and Castellana had a higher content of all the caseins in comparison with Assaf sheep. It was demonstrated that the proportion of the four milk caseins influences the physical-chemical, nutritional and technological properties of the milk (Ramuno et al., 2000). Indeed, the analysis of the milk proteins from Hamra sheep, separated by SDS-PAGE electrophoresis showed that the caseins account for 79.57% of the total milk proteins, while the major proteins from the whey represent 20.43% of the total protein (Table 2).

A higher expression for β-casein (49.03%) followed by αs2- casein (13.6%), αs1-casein (11.3%), κ–casein (5.64%), β-lactoglobulin (11.25) and α-lactalbumin (9.18 %) was observed (Table 2).

4. Conclusion

In summary, this study suggests that our locals sheep breeds Hamra showed good performance for milk traits performance (quality parameters and protein polymorphism).

In addition to our preliminary results, both increase the number of the animal studied and further studies (Real Time qPCR) are requisite for certitude of the results on the polymorphic genes of the milk proteins from sheep milk, in order to identify the genetic variants from the locus of each protein.

Also, correlations between diet and milk protein composition will be performed to estimate the potential effect of diet composition (different energy: protein ratio in the diet and dietary crude protein concentration) on the genetic potential of this breed.

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