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CONTRIBUTION TO THE GENETIC DETERMINISM OF THE SHEEP'S MILK QUALITY. RACE INFLUENCE (HAMRA AND OULED DJELL0041L) ON PHYSICO-CHEMICAL PARAMETERS AND PROTEIN PROFILE

[1]Ameur Ameur, A., [2]F. Chougrani, [2]A. Cheriguene, [1]M. Halbouche and [3]N. Karam

[1]Laboratory of Animal Applied Physiology, University of Mostaganem (UMAB), Algeria
[2]Laboratoryof Microbiology and Plant Biology, University of Mostaganem (UMAB), Algeria
[3]Laboratory of Biology of Microorganisms and Biotechnology, University of Oran Es-Senia, Algeria

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ABSTRACT

In order to study the race effect on the physico-chemical characteristics and protein profile, two sheep or ovine breeds Hamra and Ouled Djellal were raised in the same condition sat the ITELV (National institute of breeding) (Ain Hajar-Saida, Algeria). The analyses were focused on the pH, density, freezing, fat, total protein, dry defatted extract and total dry extract. The results obtained or these parameters showed no significant differences between the two races. Then the protein fractions were isolated and analyzed by Poly Acryl Amide Gel Electrophoresis (PAGE) under different conditions (native and in presence of urea). The resulting electrophoretic profiles have identified similarities between the two breeds milk.

Keywords: Sheep Milk, Casein, Hamra, Ouled Djellal, Race and Proteins

1. INTRODUCTION

Dairy sheep farming is a vital part of the national economy in many countries, especially in the Mediterranean and the Middle East (Park *et al*., 2007). In Algeria, the production of sheep milk for human consumption depends mainly on rain fall during the year and therefore the condition of natural pastures (Benyoucef and Ayachi, 1991).

The sheep's population is estimated at 20 million head. It is consisted of main dominant races (Ouled Djellal, Hamra, Rembi) and secondary breeds such as Berber, Barbarine, D'men and Sidahou (Chellig, 1992).

Ouled Djellal race is the true sheep of the steppe. It's an entirely white race with fine wool and fine tail, tall and long, powerful legs. These features provide a spreading in all regions where it tends to replace some races in their birth place the case of the race Hamra (Chellig, 1992).

Hamra race, called Beni Ighil is native to North Africa specifically the Moroccan High Atlas, where it was raised by the Beni Ighil tribe from which it derives its name. This sheep is characterized by its small size with a dark brown head and legs tending towards red. It has some ability including strength, but now in sharp decline due to its non-preferred size comparing to the race Ouled Djellal (Chellig, 1992).

In this perspective, this study aims to analyze and compare the results of the milk quality of two Algerian sheep breeds, Hamra and Ouled Djellal. In the other hand, the isolation and the identification of the major proteins of these two types of milk were carried out. The aim is extended to characterize them in terms of their electrophoresis behavior.

2. MATERIALS AND METHODS

2.1. Samples Origin

The milk samples used in this study were obtained from sheep flocks of Hamra and Ouled Djellal. They were raised in the same conditions at the National institute of breeding "ITELV" located in Ain Al Hajar

Corresponding Author: Ameur Ameur, A., Laboratory of Animal Applied Physiology, University of Mostaganem (UMAB), Algeria

region, ten (10) km far from the town center of the province of Saida. The size sample of both races is 30 sheep for each. As the average age, it was 3.5 and 3.7 years for Ouled Djellal race and Hamra race, respectively. The average weight of sheep was 45.6 kg for Hamra race and 49.5 kg for the race of Ouled Djellal. The flock is led to the wrestling during the spring season (April-May) ending in autumnal births (September-October).

Sheep population of the farm is conducted in semi-intensive. Ewes received basal diet of hay and vetch-oats with a concentrated complement in the dose of 0.79 UF and 130 g. DCP/kg and distributed at a rate of 0.6 kg/day per mother in late pregnancy and during lactation. The water is distributed at will. Following the experiment al objective, milk is fractionated, a portion (10 mL for each sample) is for physico-chemical analysis and the other part of the milk is processed according to a protocol of separation to isolate act proteins which will be then analyzed by electrophoresis in the laboratory LBMB of the University of Oran Es-Sénia.

2.2. Physico-Chemical Analysis

Milk is processed manually from healthy sheep in early lactation stage, then it is analyzed by Ekomilk®machine, an ULTRA-analyzer using the new technology, such as the principle of ultrasound in laboratory of milk analysis of ITELV Sidi Belabbes.

2.3. Electrophoretic Characterization of Milk Proteins

Samples preparation for electrophoretic analysis is performed according to the following steps. Skimming is done by centrifugation of the milk at 3500 rpm for 20 min at 4°C. The separation of caseins from whey proteins is obtained by precipitation of milk at pH 4.6 in the presence of a hydrochloric acid solution 3N, followed by centrifugation at 3500 rpm for 15 min. The obtained different fractions (serum and casein proteins) were dialyzed against distilled water for 48 h. They are then concentrated, frozen in cups and finally lyophilized and stored in this form. Then the spectrophotometric determination of these proteinous fractions is carried out by Bradford method (Bradford, 1976). Protein separation was carried out on maxi-tanks (The studier model SE400). Several separation conditions were used: In non-denaturing medium (native-PAGE) and in denaturing conditions in the presence of urea (UREA-PAGE).

We performed electrophoretic separation on Poly Acrylamide Gels (PAGE), which is the product of polymerization of acrylamide monomer (CH_2 = CH-CO-NH_2) and the comonomer N, N'-methylene-bis acrylamide (CH_2 = CH-CO-NH-CH_2 = NH-CO-CH = CH) and in the presence of ammonium per Sulphate $(NH_4)_2S_2O_8$ and N, N, N, N'-Tetramethyl-Ethylenedia Mine (TEMED) as a catalyst for the reaction.

Separations were conducted on tanks maximum 10×10 and 10×8 cm (Studier Model SE400), which have the advantage of very little use of products and do not require cooling of the gel. Several conditions have been employed for separations in non-dissociating and non-denaturing medium (native PAGE) and in dissociating and denaturing conditions in the presence of urea (urea-PAGE) and Sodium Dodecyl Sulfate (SDS-PAGE). Protocols of electrophoresis separations required several optimization tests to obtain reproducible results.

Electrophoresis in the presence of urea and 2-mercaptoethanol was performed using the protocol described by (Kwai-Hang and Kroeker, 1984) by which we performed stacking gel (T = 4% and C = 2.7%) in the buffer (urea, 0.08 M Tris, 0.49M pH 6.8) and a separating gel (T = 13% and C = 2.7%) in the buffer (urea, 4 M, Tris, 1.5 M, pH 8.8), the samples were dissolved in buffer of the same composition as that of the stacking gel (8% v/v urea to 3.3 M 2-mercaptoethanol, 0.3% of 10% glycerol). The running buffer is composed of the same components as those used in native-PAGE.

3. RESULTS

3.1. Physico-Chemical Quality

3.1.1. Physical Parameters

The values obtained during this measurement indicated a mean pH for milk samples of sheep breed Hamra and Ouled Djellal which are respectively 6.50 and 6.61. It appears that the Hamra race has the lowest pH compared to Ouled Djellal race. The analysis of variance showed that the rewash a significant difference ($p<0.05\%$) between the pH of the two races, which may be due to the technique of milking. However, the average density of the milk samples (**Table 1**) is equal to 1.038 g/cm^3 for both races and a value of freezing point of 0,649°C, 0,651°C for samples of Hamra race and Ouled Djellal, respectively. No significant difference ($p<0.05\%$) was recorded for these two parameters.

Table 1. Physico-chemical analysis of collected samples of sheep milk (Hamra and Ouled Djellal race)

		Race HAMRA (n = 30)			Race Ouled Djellal (n = 30)			
		Av (±) SD	Max	Min	Av (±) SD	Max	Min	Fisher test p<0,05
Physical parameters	pH at 20°C	6,50±0,09	6,90	6,37	6,61±0,10	6,88	6,44	P*
	Density (g/cm^3)	1,038±0,0023	1,043	1,033	1,038±0,0047	1,048	1,032	NS
	Freezing point (°C)	-0,649±0,028	-0,570	-0,709	-0,651±0,04	-0,586	-0,781	NS
Chimical parameters	Fat %	5,69±0,93	8,08	4,15	5,945±1,06	8,85	4,16	NS
	Protein %	6,41±0,46	7,29	5,37	6,23±0,61	7,95	5,25	NS
	DDE%	11,93±0,56	13	10,7	11,75±0,81	13,8	10,3	NS
	MST %	17,42±1,75	21,08	12,24	17,36±1,78	18,01	14,65	NS
	H_2O %	82,57±1,75	86,76	78,92	83,63±1,78	86,51	79,56	NS

DDE: Deffated Dry Extract, TDM: Total Dry Matter, Av: Average, DCP: Digestible Crude Protein; *p<0, 05: Significant difference, NS: No Significant difference

Results showed that the mean fat content of the samples was 5.69 and 5.94% in the Hamra and Ouled Djellal race, respectively. These results are close to one another, It may be mean that the race fact or does not influence the milk composition. In addition, the protein content of milk for Hamra race is relatively close to that recorded for the milk of Ouled Djellal race. The dry defatted extract is very important particularly in cheese industry, because the extraction of the lipid fraction of milk provides better calculation of the protein fraction. For this parameter we have recorded mean of 11.93% for milk of Hamra race and 11.75% for milk of Ouled Djellal race. DDE sheep's milk is, however, as in cow's milk, a very stable value. This may explain the similarity of our samples for this parameter. Analysis of variance showed that there was no significant difference (p<0.05%) for chemical parameters studied between the milk of both breeds.

3.2. Electrophoretic Analysis of the Protein Fraction

3.2.1. Electrophoretic Analysis of Total Serum Proteins

Results indicate that serum proteins of bovine milk in native conditions showed 5 bands in ascending order of their electrophoretic mobility corresponding to Ig, PP3, BSA, the α-La and finally to β-Lg (A and B). Serum proteins of ewe's milk of race of Hamra and Ouled Djellal also were separated into five bands (I to V), compared to their bovine counterparts. Bands (I) (II) and (III) have respectively the same level of migration as Ig, PP3 and BSA, suggesting that they are of the same nature. However, the bands (IV) and (V) have a different level of migration. The bovine bands α-La and β-Lg are more forward than their ovine counterparts (**Fig. 1**).

Deffated Dry Extract (DDE), Total Dry Matter (TDM), Average (Av), Digestible Crude Protein (DCP) *p<0, 05: Significant difference, No Significant difference (NS).

3.3. Electrophoretic Analysis of Total Casein

The electrophoretic behavior of ovine caseins in Urea-PAGE, **Fig. 2** shows the following pattern of migration: First, the β casein with the lowest mobility, followed by αS-Cn complex (αS2 then αS1), these two groups are separated by a diffuse band just focused, κ-casein. Compared to the electrophoretic profile of bovine casein, a pre-identification of casein bands of our two races is possible. Thus, the first level of migration of bovine milk, β-casein, well focused, finds a match in the tracks of casein migration of our samples; it may be similar according to the criteria described by (Mayer, 2005).

The second level of milk migration of two races (Hamra and Ouled Djellal) corresponds to the diffuse band representing the k-casein. This is similar to observations reported by (Alais and Jollès, 1967; Chianese *et al*., 1992; Moatsou *et al*., 2004).

The complex αS casein, in term, also has an identical arrangement (αS2 then αS1) in both races, except those affected by the proteolysis phenomenon. Compared to bovine αS-caseins, the ovine αS caseins complex has a retarded electrophoretic mobility. This can be used as a marker for the presence of bovine milk in a mixture of sheep's milk (Mayer, 2005).

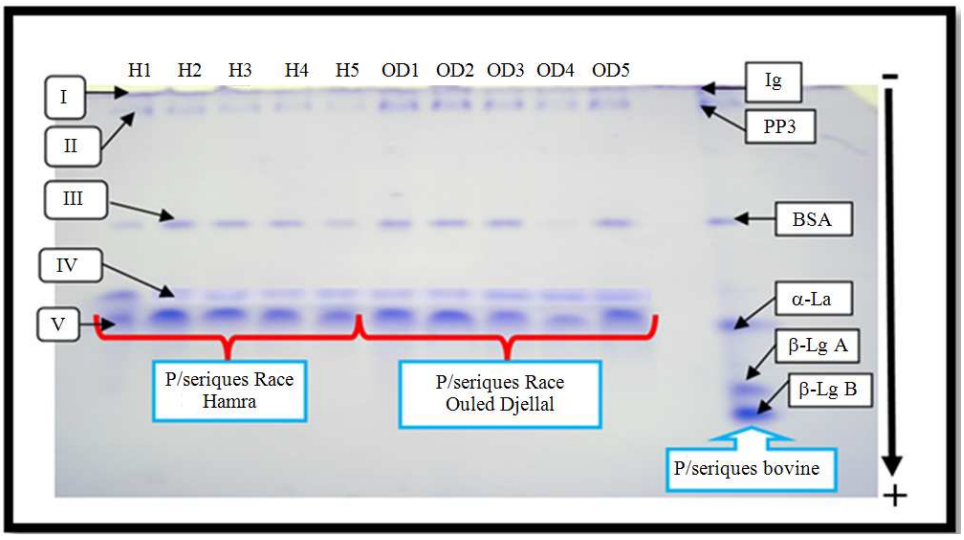


Fig. 1. Electrophoretic pattern of serum proteins in ewe's milk race Hamra and Ouled Djellal compared to bovine milk (T = 12%, C = 2.7%)

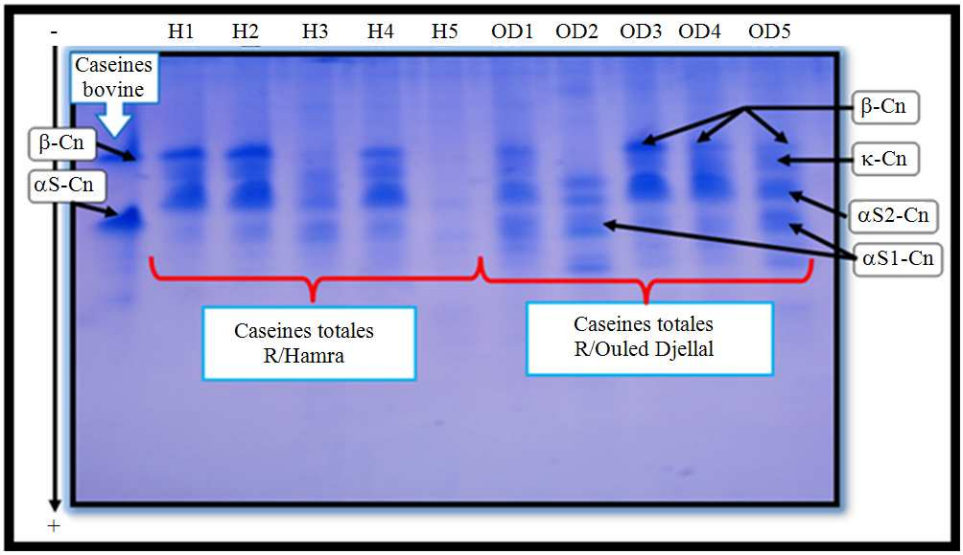


Fig. 2. Electrophoretic casein ewe's milk race Hamra and Ouled Djellal compared to bovine casein- PAGE Urea (5.7 M) (T = 15%, C = 2.7)

4. DISCUSSION

4.1. Physico-Chemical Quality

Compared to cow's milk (6.71) (Jenness *et al*., 1974), sheep's milk of our races is slightly acide (low pH), probably because of its high protein content (Alichanidis and Polychroniadou 1996; Juarez and Ramos, 1986). Indeed, the pH of sheep milk was always less therefore more acidic due to its alkalinity. Aganga *et al*. (2002; Manfredini *et al*., 1993) reported lower pH values for sheep milk in early lactation compared to the terminal phase (6.57-7.01).

4.2. The Chemical Parameters

These pH values correspond with those reported by many authors such as (Kurkdjian and Gabrielian, 1962; Haenlein and Wendorff, 2006) they reported values 6.51 and 6.68 respectively in sheep's milk.

Results of density shows that they are characterized by similar average values of those reported for sheep milk (1.0347-1.0384) as mentioned by (Kurkdjian and Gabrielian, 1962; Anifantakis *et al*., 1980; Haenlein and Wendorff, 2006; Park *et al*., 2007; Juarez and Ramos 1986).

The density of goat milk (1029-1039) is comparable to that of cow's milk (1.0231-1.0398), but is lower than sheep's milk (Parkash and Jenness, 1968; Haenlein and Wendorff, 2006). This can be explained by the high content of solid non-fat in sheep milk (Mahmoud and Usman, 2010).

Compared to cow's milk, the freezing point of sheep's milk is lower due to higher solids-non-fat (Alichanidis and Polychroniadou, 1996).

The values recorded in our work are less than those reported by (Kurkdjian and Gabrielian, 1962; Haenlein and Wendorff, 2006; Park *et al*., 2007; Ramos and Juarez, 2011), which give the average freezing point for sheep's milk being -0.570°C, which can beheld to the freezing point which characterizes sheep's milk. This may be due to the stage of lactation that is significantly correlated with the value of freezing point (Pavić *et al*., 2002).

However, a study conducted by Timar (2011) records a value relatively very close freezing point which is of the order of -0.710°C.

The results shows that mean contents of fats are close to each other, which means that the race factor do not affect the composition of the milk. This has been reported by several authors such as (Moore, 1966; Bencini and Purvis, 1990; Bencini *et al*., 1992) who observed no difference in the composition of milk from both races.

Usually, sheep's milk contains 6-9% fat (Simos *et al*., 1996). This content is higher than in cow or goat milk (Haenlein and Wendoff, 2006).

Substantially similar values to those obtained are recorded by (Miocinovic *et al*., 1981) with 6.77% (Baltadjieva *et al*., 1982) with 6.45%, (Konar *et al*., 1991) with 6.3% (Ploumi *et al*., 1998) with 6.77% (Hilali, 2001) with 6.76% and (Paccard and Lagriffoul, 2006) with 6.82%, (Hilali *et al*., 2010) with 6.50% and (Mayer and Fiechter, 2011) with 5.58%.

Nevertheless we find very distant values of our samples with lower values of 5.40 and 5.30% respectively recorded by (Malau-Aduli and Anlade, 1997; Pugliese *et al*., 2000) and others more important with 9.05, 7.11 and 7.52% recorde drespectively by (Anifantakis *et al*., 1976; Sevi *et al*., 2004; Kondyli *et al*., 2011). These reflect the high variability in the rate of fat of sheep milk.

4.3. Electrophoretic Analysis of the Protein Fraction

Serum proteins of bovine milk in native conditions migration to five bands corresponding in ascending order of their electrophoretic mobility to immunoglobulins, the component 3 of proteose-peptone fractions, bovine serumalbumin, alpha-lactalbumin and beta-lactoglobulin (Egito *et al*., 2001; Groeneboom *et al*., 2010).

Serum proteins of milk samples of Hamra and Ouled Djellal sheep breed studied also migratein to five bands, from I to V. the semay correspond to the samep rote in species found in these rumfraction of bovine milk.

According to Mati *et al*. (1991), it was concluded that there is a great similarity between the proteose-peptone fractions (band II) of cow's and sheep's milk. It is a particularly distinguished from their bovine counter part with a lower electrophoretic mobility in native PAGE.

The migration bands (IV) and (V), according to the order of migration of bovine whey proteins, could correspond respectively to alpha-lactalbumin and beta-lactoglobulin, find no similarity in the migration track of bovine serum proteins. Such observations are also reported by (Amigo *et al*., 1992), which locate characteristic bands corresponding to bovine alpha-lactalbumin and beta-lactoglobulin further forward than its ovine counterpart.

Based on the work of (Kiddy *et al*., 1972), we can confirm the position of the two genetic variants of bovine β-Lg (β-LGA and β-LGB). The fraction of β-Lg in our samples appear as a single intense band with the similar electrophoretic mobility to the bovine α-La. Similar observations were made by (Amigo *et al*., 1992; Blitzsteina and Diaconisb, 2011) which located and indicated as in the bovine counter part the existence of two genetic variants of ovine β-Lg but could not be distinguished.

The electrophoretic behavior of the ovine casein Urea-PAGE and the order of appearance of these was described by (Arave *et al*., 1973; Chianese *et al*., 1992; Moatsou *et al*., 2004).

The general pattern of migration presents first the β-casein with the lowest mobility, followed by αS-Cncomplex (αS2 then αS1), these two groups are separated by diffuse band focused casein-κ.

However and in contrast to bovine profile (Egito *et al*., 2001) the profile of ewe's casein in urea-PAGE is similar to that of goat's profile (Moatsou *et al*., 2004).

As described by Pardo and Natalucci (2002; Le Bars and Gripon, 1993) the electrophoretic diagram

shows for the bovine milk an identical arrangement as described by the authors (β-Cn and Cn-αS2 and αS1-Cn). Caseins αS2-Cn and Cn-αS1 appear as a single well-focused band and κ-casein is not detectable.

5. CONCLUSION

The study of the physico-chemical quality of milk produced by two sheep breeds Hamra and Ouled Djellal showed that there is no significant difference or most studied parameters. The electrophoretic behavior (native and using urea) and electrophoretic profiles obtained are very similar despite the variety of sample origin (Hamra and Ouled Djellal race). However, we found out a different migration no fovine α-lactalbumin and β-lactoglobulin compared to their bovine counter parts. UREA-PAGE highlighted the particularity of the migration of ovine caseins, especially for αS-Cn complex that has a delayed electrophoretic mobility compared to bovine ones.

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