Short study of major milk protein polymorphism in local sheep breed

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There is an international interest for preservation and improvement of local animal breeds, due to their superior biological traits: rusticity, resistance and adaptability to very different local environment. The local Romanian **Teleorman Black Head Tsigai** sheep breed fits very well the current economic demands, such as milk production and prolificacy. The purpose of our study was to determine milk quality indices as well as milk protein polymorphism in local sheep breed, using 24 milk samples. The types of different milk proteins were identified by SDS-PAGE. The test day milk yield and chemical composition assays performed during the milking period of studied sheep showed that the yield of milk fat and protein ranged within the quality indices specific to breed (6.56% fat and 5.9% protein), described in literature. Milk samples were further analyzed for milk protein polymorphism. 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 74.16% of the total protein of sheep milk, followed by whey proteins. SDS-PAGE analysis showed that majority of milk samples is characterised by a medium expression level of both caseins and whey proteins (66.67-79.17%) followed by higher level of expression (12.6-29.17%). 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.

Aim of the study

The aim of this study was to determine the milk quality parameters and to study milk protein polymorphism in the **Teleorman Black Head Tsigai,** allowing thus a better knowledge of the breed, for sustainable genetic improvement and conservation. This study is part of a larger project concerning the use of milk protein genetic polymorphism as potential genetic marker for the local sheep breed **Teleorman Black Head Tsigai** milk production trait.

Methods

Animals and biological samples - milk samples were obtained from 24 local sheep breed, **Teleorman Black Head Tsigai**. For the determination of milk quality indices 6 test –day from two and two weeks were realized.

Determination of milk quality indices - the total percentage of milk solids non-fat (SNF) content, fat and protein content was determined using a Ekomilk M analyzer.

Milk protein separation - the Bradford method (Krauspe, 1986) was used to determine the concentration of total milk proteins before their separation. Milk proteins were further separated by SDS-PAGE electrophoresis in 15% polyacrylamide gel. 5µg of protein samples diluted in migration buffer 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 for 15 minutes. The apparent molecular weights of analyzed proteins were established in relation with a standard known protein. The relative expression of milk proteins were expressed as Arbitrary Units (AU).

Statistical analysis - Student's *t*- tests were used to analyze the differences between all samples. *p* values < 0.05 were considered significant. All data are expressed as mean ± standard error of the mean (SEM).

Conclusions

☐ the local sheep breed **Teleorman Black Head Tsigai** showed good performance for milk traits performance (quality parameters and protein polymorphism).

☐ percentage analysis of the protein fractions of interest revealed that **caseins** represented **74.16%** of the total protein of sheep milk, followed by whey proteins.

☐ most of the milk samples are characterised by a *medium intensity level of expression* of both caseins and whey proteins followed by the higher level of expression.

☐ an increase of the number of the animal along with further Real Time qPCR investigation are requisite for certitude of these results in order to identify the genetic variants from the locus of each protein.

Results

Table 1. *Indices of milk quality in the Teleorman Black Head Tsigai sheep during milking period*

Test Day	Milk parameters					
	Fat (g %)		Protein (g%)		SNF (g%)	
	Mean	SEM	Mean	SEM	Mean	SEM
1d	6.35	0.40	4.82	0.20	9.51	0.50
2d	8.93	0.20	5.57	0.10	10.87	0.10
3d	7.97	0.10	5.43	0.10	10.71	0.13
4d	10.19	0.20	5.37	0.10	10.30	0.27
5d	8.16	0.10	5.69	0.70	11.01	0.08
6d	9.07	0.20	5.63	0.10	10.92	0.13

Figure 2. *The expression level of milk proteins in Teleorman Black Head Tsigai sheep.*

Figure 3. *Distribution of milk samples related to the expression level (high, medium and low) of milk protein in Teleorman Black Head Tsigai sheep.*

Table 2.a. *Proportion of different protein fractions in milk of local sheep breed Teleorman Black Head Tsigai*

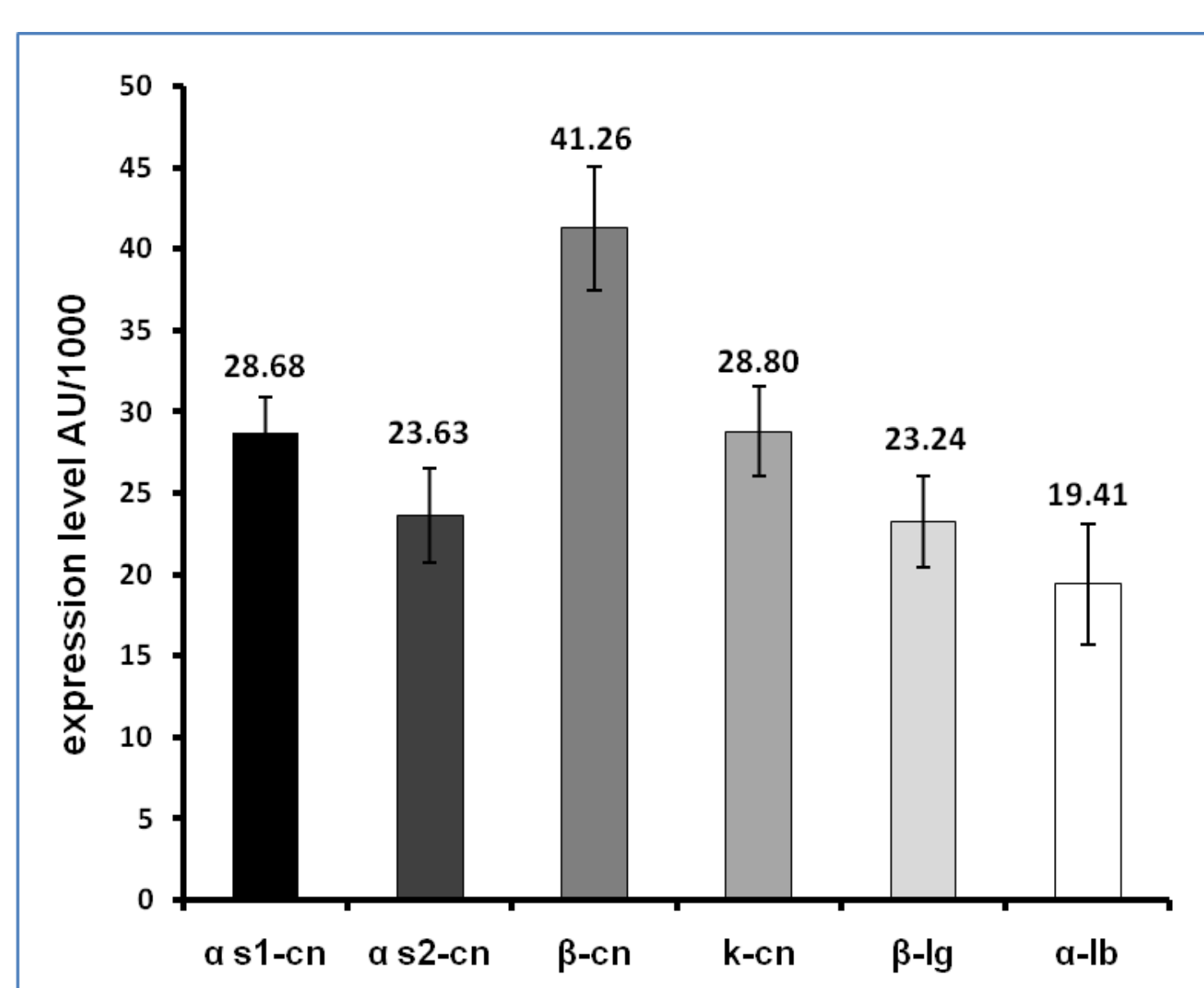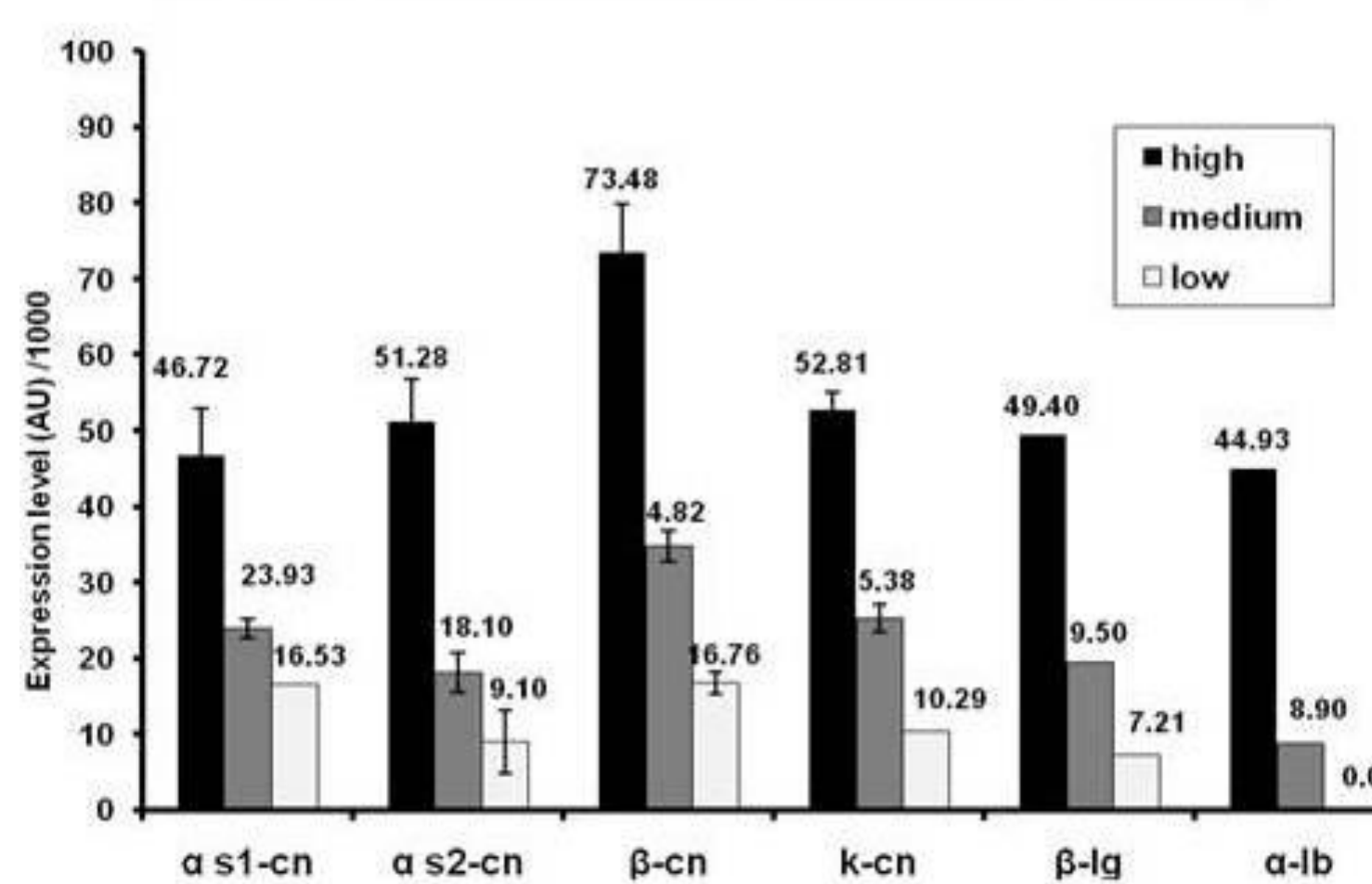| Protein fractions		% of total protein	
		Mean	SEM
Total caseins		**74.16**	**7.05**
Caseins	β-casein	25.00	2.29
	k-casein	17.45	1.66
	αs1- casein	17.38	1.32
	αs2- casein	14.32	1.78
Total whey proteins		**25.84**	**3.92**
Whey proteins	β-lactoglobulin	14.08	1.68
	α-lactalbumin	11.76	2.24

Table 2.b. *Proportion of casein fractions in milk of local sheep breed Teleorman Black Head Tsigai*

Casein fractions	% of total casein	
	Mean	SEM
β -casein	33.72	3.10
k -casein	23.54	2.24
αs1- casein	23.43	1.78
αs2- casein	19.31	2.40

Table 3. *Distribution of sheep groups depending on milk protein expression*

Group	Protein	% of total samples
High (n=4)	β-casein	16.67
Medium(n=18)		75.00
Low (n=2)		8.33
High (n=3)	k-casein	12.50
Medium(n=16)		66.67
Low (n=5)		20.83
High (n=5)	αs1-casein	20.83
Medium(n=18)		75.00
Low (n=1)		4.17
High (n=4)	αs2-casein	16.67
Medium(n=19)		79.17
Low (n=1)		4.17
High (n=3)	β-lactoglobulin	12.50
Medium(n=18)		75.00
Low (n=3)		12.50
High (n=7)	α-lactalbumin	29.17
Medium n=17)		70.83
Low (n=0)		0.00

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