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Milk Lipid and Protein Profiles of Abkhazian and Kackar Goats

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ABSTRACT

Fat and protein profiles of milk of Abkhazian and Kackar goats, Caucasian breeds, were compared in this study. The milk samples (n= 60) from 60 Abkhazian and Kackar goats were subjected to assessments of lipid profile using the high performance thin layer chromatography and protein profile using the sodium dodecyl sulphate polyacrylamide gel electrophoresis. The milk lipid and protein contents as well as their fractions were compared using student *t-test*. Total lipid content was 4.23±0.022 g/dl and 3.44±0.026 g/dl for Abkhazian and Kackar goat milk (P<0.0001). Milk triacylglycerol, free fatty acid and diacylglycerol fractions were different (P<0.05), but the cholesterol fraction was similar. Total protein content was 3.94 g/dl and 3.75 g/dl for Abkhazian and Kackar goat milk (P<0.007). The milk fat globule membrane protein mucine1 and xhantine oxidase, α-lactalbumin, α-casein, and κ-casein fractions were different (P<0.05). In conclusion, milk lipid and protein profile differs between Abkhazian and Kackar goats despite living in the same ecosystem. Differences in milk lipid and protein profile could be pertinent to human nutrition and health.

Keywords: goat milk, lipid profile, protein profile

INTRODUCTION

Milk is a complex biological fluid, abundant in nutrients (Drewnowski & Fulgoni, 2008). The consumption of goat milk increases as it has become a subject of a number of versatile research areas. Goat milk differs from cow or human milk, in terms of higher digestibility, distinct alkalinity, higher buffering capacity, and certain therapeutic values in human medicine and nutrition (Raynal-Ljutovaca *et al*., 2008). It exerts beneficial effects for paediatric and geriatric health and nutrition through aiding physiological functions and can be consumed without negative effects by people suffering from allergy to cow milk (Yangilar, 2013).

Compositions of human, sheep, goat, and cow milks are different, especially the structure, composition, and dimension of casein micelles, individual protein fractions and non-protein nitrogen amount as well as mineral concentration (Kucukcetin *et al*., 2011; Domagala, 2009). Moreover, in comparison with cow milk protein, goat milk proteins are more digestible (Ceballos *et al*., 2009) and the protein fraction has higher levels of six of the ten essential amino acids present (Costa *et al*., 2014). Another important property is that goat milk fat contains high amount of short and medium chain fatty acids and their fat globules are small (Silanikove *et al*., 2010). Goat milk fat is known to be rich in caproic ($C_{6:0}$), caprylic ($C_{8:0}$), and capric ($C_{10:0}$) acids as compared to sheep and cow milk (Markiewicz-Kęszycka *et al*., 2013).

Biochemical composition, technological properties, and bacteriological quality of goat milk vary depending on genetic factors, environmental conditions, and raising conditions (Yangilar, 2013). Different goat breeds are raised in many parts of the world for the purposes of food supply and economical gains. Traditionally, goat milk has been considered as a fundamental food in the diets of many cultures. Abkhazian & Kackar goats are local breeds and weigh about 40-135 kg and have wooly coat and short tail. They adapt to mountainous regions and rainy/foggy climate. Kacgar goat is raised in Northeastern Turkey by the Black Sea region. Abkhazian goat is originally Abkhazian region animal, but is also raised in the Black Sea Region of Turkey. This experiment was conducted to compare the fat and protein compositions of the milk of Abkhazian and Kackar goats traditionally raised in the Black Sea Region of Turkey.

MATERIALS AND METHODS

Sampling

The milk samples were collected from 30 Abkhazian and 30 Kackar goats that were in the age of 2-3 years and were reared in two different uplands in Artvin Province in the Black Sea Region of Turkey. Samples were shipped to the laboratory in a few hours under refrigerator conditions and stored at -80°C until analyses.

Determination of Triglyceride Concentration

To 100 µL milk sample, 1 mL triglyceride reagent [(4-Chlorophenol 3.5 mM, ATP >0.5 mM, magnesium salt 10 mM, 4-Aminophenazone 0.3 mM, microbial glycerol kinase >250 U/L, microbial glycerol phosphate oxidase >4500U/L, horseradish peroxidase >2000 U/L, microbial lipase >200.000 U/L, buffer (pH 7.3), sodium azide (0.01%)] was added. After 30 min of incubation, absorbances of samples were measured at 505 nm (Fossati & Prencipe, 1982). Results were calculated by using a standard triglyceride (TG) solution (50 mg/dl) and expressed as g TG/dl.

Lipid Profile Analysis

Milk lipid profile was assessed using the high performance thin layer chromatography (HPTLC). Five hundred µl of n-hexane:iso-propanol 3:2 (v/v) mixture were added into 1 mL of milk. After vortexing vigorously, the tubes were centrifuged at 5.000 *g* for 5 min at +4°C, and the upper phase were used for HPTLC analysis (Hara & Radin, 1978). A standard lipid mixture comprising L-*α*-phosphatidylcholine, cholesterol, palmitic acid, triolein, glycerol di-oleate, and cholesterol 3-oleate was used to identify milk lipid classes. One µL portion of the control standard and extracted lipid was spotted with a micropipette 2 cm away from the bottom of the HPTLC plates.

The lipid spots were developed using developing solvent. Then, the entire plate was sprayed with a 10% $CuSO_4$ (w/v) in 8% H_3PO_4 (v/v) and lipid classes were visualized by charring at 180°C for about 10 min. Milk lipids were separated into the following classes: cholesteryl ester (CE), triacylglycerol (TAG), free fatty acids (FFA), cholesterol (CHOL), diacylglycerol (DAG), and phospholipids (PL) (Table 1, Figure 1).

HPTL chromatograms were scanned with photoscanner and analyzed with TL 120 software. Results were obtained as percentage of individual lipid class in total lipid of milk samples (Kaynar *et al*., 2013).

Determination of Total Protein Concentration

In order to determine total protein concentration, 1 mL of milk sample was mixed with 0.5 mL sodiumdeoxycholate (10%) and 0.5 mL trichloroacetic acid (10%). The mixture was then incubated in 37°C for 30 min and centrifuged at 5,000xg at +4°C for 5 min. The precipitate was dissolved in 5.0 mL 0.1 N NaOH. Further, 5.0 mL alkaline copper reagent was added into the same tube. After 10 min, 0.5 mL folin reagent was added and incubated at room temperature for 30 min. Finally absorbance values of the samples and protein standards were recorded at 660 nm at spectrophotometer (µ-Quant, BioTek) against the blank solution (Lowry *et al*., 1951). Results were calculated by using a standard albumin solution (5 g/dL) and expressed as g TP/dl.

Protein Profile Analysis

Individual milk proteins were attained using the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), consisting of 4% stacking and 10% resolving gels (Laemmli, 1970). Briefly, 10 µL of milk sample and 90 µL electrophoresis denaturing sample buffer (0.8 mL glycerol, 1.6 mL 10% SDS, 0.2 mL 0.05% bromophenol blue in ethanol, 1 mL 0.5 M tris-HCl pH 6.8, 0.4 mL β-mercaptoethanol, and 4 mL distilled water) were mixed and 15 µL of mixtures were loaded into each well. The electrophoresis was carried out in tris-glycine running buffer pH 8.3 (Trizma base 1.515 g, glycine 7.2 g, SDS 0.25 g, 500 mL) at 20 mA/gel constant current for 90 min and proteins were visualized by Oriole fluorescent staining.

SDS-PAGE electrophoretograms (Figure 2) were visualized with GelDoc XR (BioRad) and analyzed with Image Lab software (Figure 3, Table 2). Milk proteins [MFGM (milk fat globule membrane protein/mucine1 (MUC1), xhantine oxidase (XO), cluster of differentiation (CD), butyrophilin (BTN) periodic acid schift (PAS), and WAP (whey acidic protein)] were reported as the percentages within the total protein.

Statistical Analysis

The difference between lipid and protein parameters of goat milk were determined using student *t*-test (SAS, 2002). Due to missing data and possible unequal variance within groups for some parameters, the Satterthwaite approximation was employed to attain true probability of significance. The data were presented as mean±SE as well as group mean difference±SE. Group differences were considered at $P<0.05$.

RESULTS

Lipid Profile

The lipid profiles of Abkhazian and Kackar goats are shown in Table 1. Milk fat content for Abkhazian goat was higher than that for Kackar goat (4.23 vs. 3.44 g/dl; $P<0.0001$). The TAG percentage was higher in Abkhazian goat (68.65 vs. 67.37%; $P<0.0001$), while the FFA (2.87 vs. 2.08%; $P<0.0002$) and DAG (2.87 vs. 2.37%; $P<0.0001$) percentages were higher in Kackar goat. They had similar percentages of CHOL and CE.

Protein Profile

Total protein amounts were 3.94 and 3.75 g/dl for Abkhazian and Kackar goat milk ($P<0.007$; Table 2). As revealed by the SDS-PAGE method, the molecule weights of MFGM proteins, caseins, and whey proteins

were determined to be in the range of 10 kDa to 162 kDa (Figure 1). The molecular weights of XO, CD36, BTN, and PAS 6/7, which are the other MFGM proteins in the milk, were calculated to be 80.7 kDa, 66.0 kDa, 59.9 kDa, and 54.49 kDa, respectively (Figure 1). The percentages of MUC1, XO, CD36, BTN, and PAS 6/7 in total protein were 1.43%, 1.45%, 2.53%, 1.25%, and 2.70% for Abkhazian goat and 0.78%, 2.18%, 2.49%,

Table 1. Comparison of milk lipid profile

Variables	Caucasian goat breed		Difference	*t*	*P*>|*t*|
	Abkhazian	Kackar			
Total lipid, g/dl	4.23±0.02 (4.19-4.28) [a]	3.44±0.03 (3.39-3.50) [b]	0.79±0.03	22.93	0.000
Lipid classes, %[1]					
CE	19.59±0.23 (19.11-20.07)	19.37±0.17 (19.01-19.72)	0.23±0.29	0.79	0.43
TAG	68.65±0.24 (68.16-69.14) [a]	67.37±0.18 (67.01-67.73) [b]	1.28±3.30	4.31	0.000
FFA	2.08±0.11 (1.86-2.30) [b]	2.87±0.16 (2.53-3.20) a	-0.79±0.20	-4.04	0.000
CHOL	3.49±0.12 (3.25-3.73)	3.49±0.14 (3.22-3.77)	-0.01±0.18	-0.04	0.97
DAG	2.37±0.07 (2.23-2.51) [b]	2.87±0.08 (2.70-3.04) [a]	-0.50±0.11	-4.6	0.000
PL	3.82±0.08 (3.66-3.99)	4.00±0.09 (3.82-4.18)	-0.18±0.12	-1.48	0.14

Note: *Data are mean±SE (lower and upper CI, 95%). Fraction was calculated based on relative volume in HPTLC. Means in the same row with different superscripts differ significantly (P<0.05). [1]HC+CE= hydrocarbon + cholesterol ester; TAG= triacylglycerol; FFA= free fatty acids; CHOL= cholesterol; DAG= diacylglycerol; PL= phospholipids.

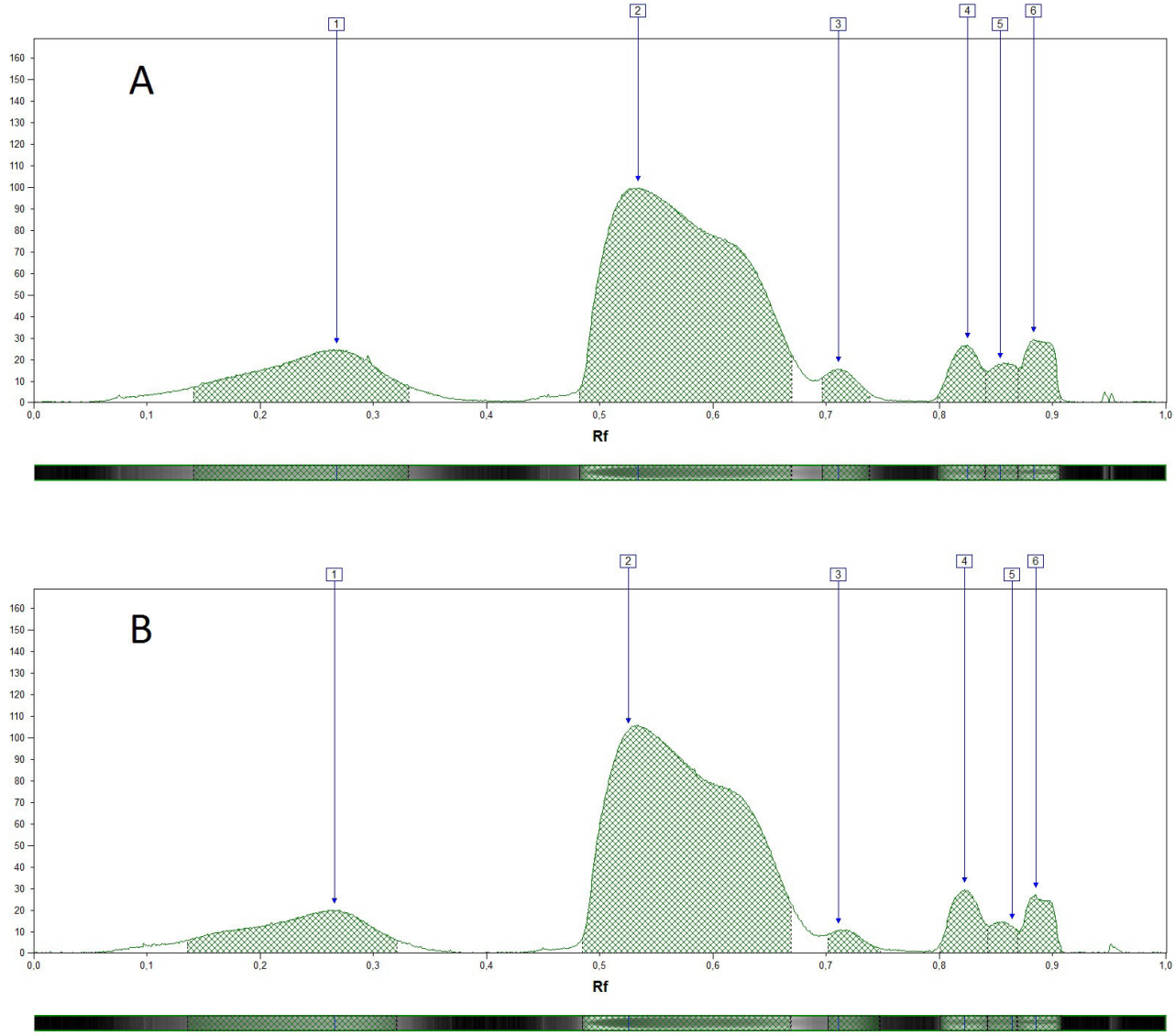

Figure 1. Abkhazian (upper panel) and Kackar (lower panel) goat milk lipid densitograms

1.36%, and 2.50% for Kackar goat, respectively (Table 2). The percentages of MUC1 and XO proteins among MFGM proteins were significantly different between goat breeds. It was determined that the percentage of MUC1 protein was higher in Abkhazian goats (1.43% vs. 0.78%; P<0.0001), while the percentage of XO protein was higher in Kackar goats (2.18% vs. 1.45%; P<0.0002).

The percentages of α-casein, β-casein, and κ-casein were 7.13%, 40.01%, and 7.6%, respectively, for Abkhazian goat and 10.24%, 39.46%, and 9.20%, respectively, for Kackar goat. The percentages of α-casein and κ-casein for Kackar goat were higher than those for Abkhazian goat (P<0.0001).

The percentages of β-lactoglobin in Abkhazian and Kackar goats were 6.56% and 6.78%, respectively, while percentages of α-lactalbumin were 7.94% and 6.68%,

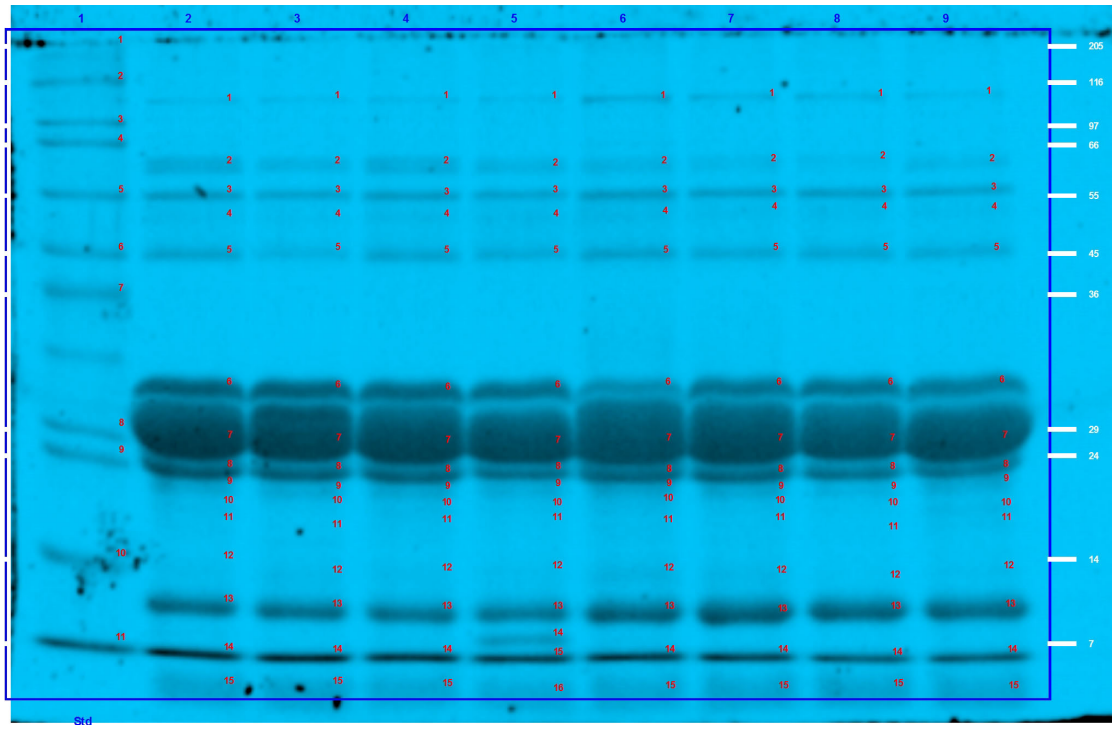

Figure 2. SDS-PAG electrophoretogram of Abkhazian and Kackar goat milk proteins (x-axis: Lanes, y-axis: kD).

Table 2. Comparison of milk protein profile

Variables		Caucasian goat breed		Difference	*t*	*P*>|*t*|
		Abkhazian	Kackar			
Total protein, g/dl		3.94±0.06 (3.83-4.06) [a]	3.75±0.04 (3.67-3.83) [b]	0.58±0.21	2.8	0.007
Protein, %[1]	MW, Da					
MFGM proteins MUC1	161921	1.43±0.04 (1.34-1.52) [a]	0.78±0.03 (0.72-0.85) [b]	0.64±0.05	12.47	0.000
MFGM proteins XO	80709	1.45±0.14 (1.16-1.75) [b]	2.18±0.10 (1.98-2.39) [a]	-0.73±0.17	-4.31	0.000
MFGM proteins CD36	66000	2.53±0.06 (2.40-2.66)	2.49±0.07(2.34-2.63)	0.04±0.09	0.43	0.67
MFGM proteins BTN	59988	1.25±0.04 (1.16-1.34)	1.36±0.05 (1.25-1.48)	-0.11±0.07	-1.66	0.11
MFGM proteins PAS6/7	54498	2.70±0.10 (2.48-2.92)	2.50±0.08 (2.33-2.67)	0.20±0.13	1.53	0.14
α-casein	33754	7.13±0.47 (6.44-8.14) [b]	10.24±0.26 (9.68-10.80) [a]	-3.11±0.54	-5.74	0.000
β-casein	26589	40.01±0.63 (38.68-41.34)	39.46±0.84 (37.68-41.25)	0.55±1.05	0.53	0.6
κ-casein	21670	7.68±0.15 (7.35-8.01) [b]	9.20±0.23 (8.71-9.69) [a]	-1.52±0.28	-5.5	0.000
WAP	20505	2.22±0.08 (2.04-2.40)	2.31±0.14 (2.01-2.61)	-0.09±0.16	-0.56	0.58
WAP	20007	1.59±0.07 (1.44-1.72)	1.52±0.13 (1.25-1.80)	0.06±0.14	0.42	0.68
WAP	19968	1.09±0.06 (0.97-1.20)	1.04±0.08 (0.88-1.20)	0.05±0.09	0.5	0.62
WAP	19665	2.36±0.12 (2.10-2.61) [a]	1.86±0.12 (1.60-2.13) [b]	0.49±0.17	2.87	0.008
WAP	17723	14.08±0.42 (13.19-14.98) [a]	11.60±0.26 (11.05-12.14) [b]	2.49±0.49	5.06	0.000
β-lactoglobulin	13547	6.56±0.24 (6.04-7.08)	6.78±0.26 (6.22-7.35)	-0.22±0.36	-0.6	0.55
α-lactalbumin	10528	7.94±0.37 (7.15-8.74) [a]	6.68±0.18 (6.30-7.05) [b]	1.27±0.41	3.07	0.006

Note: *Data are mean±SE (lower and upper CI, 95%). Fraction was calculated based on relative volume in SDS-PAGE. Means in the same row with different superscripts differ significantly (P<0.05). [1]MFGM = milk fat globule membrane protein/mucine1 (MUC1), xhantine oxidase (XO), cluster of differentiation (CD), butyrophilin (BTN) periodic acid schift; WAP = whey acidic protein.

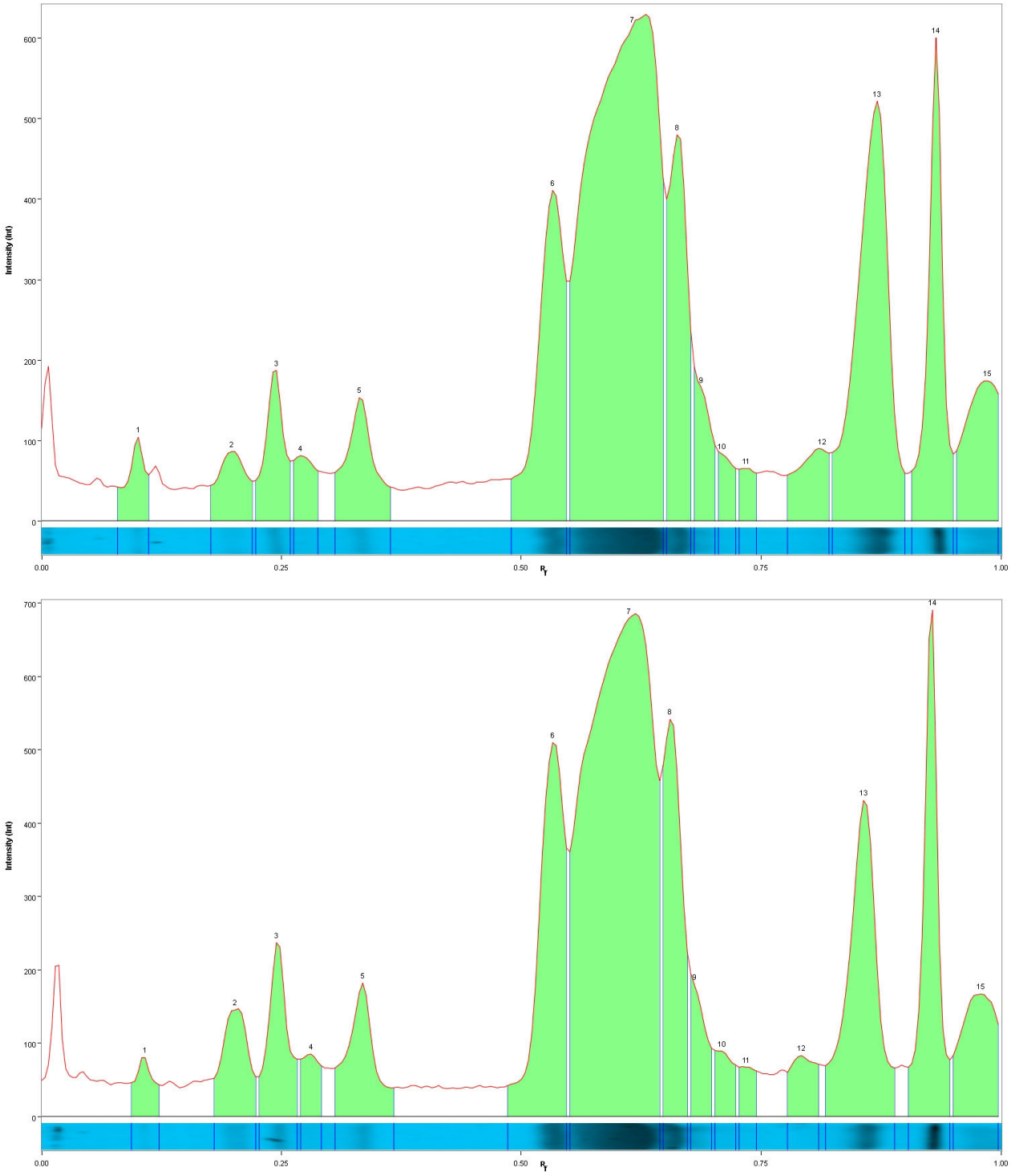

Figure 3. Abkhazian (upper panel) and Kackar (lower panel) goat milk protein densitograms

respectively. α-Lactalbumin fraction between breeds was different (P<0.006), but β-lactoglobin fraction was similar.

DISCUSSION

It is known that significant variations occur in milk composition and yield during different seasons, breeds, environmental conditions, feedings, and stages of lactation within a milking goat. Nutrition (forage-to-concentrate ratio, type of forages, etc.) is the main environmental factor regulating milk fat synthesis and fatty acid composition in ruminants (Bernard *et al*., 2009). Abkhazian and Kackar goat are local breeds, and their milk nutrient contents/profiles have not been studied.

Milk lipids commonly consist of 98%-99% TAG, which are located in the fat globule. The remaining 1%-2% are minor lipid components, including DAG (0.3%-

1.6%), monoacylglycerols (0.002%-0.1%), PL (0.2%-1.0%), cerebrosides (0.01%-0.07%), sterols (0.2%-0.4%), and FFA (0.1%-0.4%) (Renner *et al*., 1989). In this study, Abkhazian and Kackar goat milk fats had lower TAG, while higher DAG, FFA, CHOL, and PL percentages in total lipid (Table 1) than those reported in the literature.

Breed appears to be a major factor regulating protein synthesis in mammary gland. Costa *et al*. (2014) reported that the total amount of protein was 3.15 g/dl and 3.60 g/dl, respectively, in Saanen and Alpine goat milk in the north-east of Brazil. The total amount of protein in human, cow, and goat milks were reported to be 10 g/L, 34 g/L, and 33 g/L, respectively (Greppi *et al*., 2008). The protein concentration in these local goat species (Table 2) was greater than dairy goat species previously reported (Silanikove *et al*., 2010).

Milk proteins can be classified in 3 major classes: milk fat globule proteins (MFGM), caseins, and whey proteins. Casein is insoluble, while whey proteins are soluble proteins. Milk fat globules are surrounded by a membrane that mainly consists of proteins, phospholipids, glycoprotein, triglycerides, cholesterol, and enzymes. This membrane is known as milk fat globule membrane (MFGM) and consists of a few layers of different origins (Zamora *et al*., 2009). Milk fat globules are produced in mammary glands during breast feeding and their structures consists of double phospholipid membrane. 25%-70% of MFGM consists of proteins depending on the milk source and process. As the globule typology and protein content of milk fat vary among farm animal species, the structure and function of MFGM proteins attract great attention (Roncada *et al*., 2012).

The MGFM proteins have certain physiological benefits, which include to act as natural emulsifying agents, prevent flocculation and the unification of fat globules and protect fat against enzyme activity. The biggest MFGM protein is MUC1 (Zamora *et al*., 2009). The molecular weight was approximately 162 kDa and made up 0.78% of the total protein. Mather (2000) found the molecule weight of CD36 in cow milk was in the range of 75 and 88 kDa, while Zamora *et al*. (2009) found that the molecule weight of CD36 in goats was about 83 kDa. The percentage of BTN protein in cows depending on delivery varies within MFGM proteins. In many studies, the BTN proteins gave 2 bands at 67 and 64 kDa when they were fragmented as a result of proteolysis from SDS-PAGE (Heid *et al*., 1983). Zamora *et al*. (2009) indicated that the molecular weight of BTN protein was 68 kDa in goat milk, being the second biggest MFGM protein. The PAS 6/7, another MFGM protein, is the most abundant MFGM glycoprotein after BTN protein, and it was reported that range of their molecular weights was from 43 kDa to 59 kDa (Mather, 2000). Hvarregaard *et al*. (1996) determined that the PAS 6/7 presented two bands in 50 and 47 kDa, respectively. Atmani *et al*. (2004) found that the molecular weight of goat XO as a single band was 150 kDa and it constituted 0.69%-1.81% of total protein. The molecular weights and fractional percentages of MFGM proteins in local goat milk (Figure 1; Table 2) were in agreement with those reported in literature.

Caseins consist of micelles in suspension, which are approximately 190 nm in diameter. Caseins are connected with calcium phosphate, small amount of magnesium, sodium, and citrate. These disperse the light and give a white opaque look to milk (Park *et al*., 2007). The main caseins in goat milk are the same as those in sheep and cow, and named as α_{S1}-casein (α_{S1}-CN), α_{S2}-casein (α_{S2}-CN), β-casein (β-CN), and κ-casein (κ-CN) (Park *et al*., 2007; Hama *et al*., 2010). Furthermore, the genetic polymorphism and fractions of casein content in goat breeds are different (Raynal-Ljutovaca *et al*., 2008). Potocnik *et al*. (2011) reported that the percentages of α-casein were 40.2%-59.0% in mares, 48.3%-48.5% in cows, 21.2%-32% in goats, and 11.1%-12.5% in humans; the percentage of β-casein as 40.1%-51.4% in mares, 35.8%-37.9% in cows, 39.95% in sheep, 48%-60% in goats, and 62.5%-66.7% in humans; the percentages of κ-casein were 7.71% in mares, 9.32% in sheep, between 12.7%-13.8% in cows, 12%-20% in goats, and 22.2%-25.0% in humans. The amount of casein is low in goat breeds and the amount of α-casein among casein fractions is lower than that of β-casein. Salem *et al*. (2009) reported that the proportion of β-casein (70.2%) was predominant fraction and the proportion of α-casein (29.85%) was minimal fraction. All casein fractions in Abkhazian and Kackar goat milk were lower than those reported in dairy goats (Table 2), but their fractional rank orders were similar.

Whey proteins (more than 80%) are immunoglobines, α-lactalbumin, lactoferrin, β-lactoglobin, serum albumin, and lactoperoxidase (Gupta *et al*., 2012; Casado *et al*., 2009). Certain whey proteins support health and reduce disease risks. When whey proteins are used as dietary substances or supplements, they ensure the increase of antimicrobial activity, immune modulator, and muscle power. They can also delay the onset and/or help the heal of diseases such as osteoporosis, cardiovascular diseases, obesity, and cancer (Casado *et al*., 2009). The proportion of α-lactalbumin is high in cows milk, which leads to allergic reactions in many individuals. β-lactoglobin is the main whey protein in sheep, mares, and goats although it is not present in human milk (Potocnik *et al*., 2011). This protein type is allergic form in milk proteins and responsible for the onset of the allergy that affect numerous babies that are fed on other milk than breast milk (Hochwallner *et al*., 2014). Potocnik *et al*. (2011) reported that the percentage of β-lactoglobin was 25.3%-36.3% in mares, 18.4%-20.1% in cows, 59.24%-77.70% in sheep, and 43.54%-63.80% in goats. The percentage of α-lactalbumin was 27.5%-29.7% in mares, 52.9%-53.6% in cows, 8.97%-17.00% in sheep, 13.31%-34.70% in goats, and 30.3%-45.4% in humans. Both local goat species had much lower β-lactoglobin and α-lactalbumin percetages than dairy goat species (Table 2), which could replace milk causing allergy in infant nutrition.

CONCLUSION

Despite reared under the same ecosystem and subjected to the same management protocols, Abkhazian and Kackar goat milk differed in total fat concentration

and percentages of TAG, FFA, and DAG. The concentration of total protein and the fractions of whey proteins, MFGM proteins, and caseins were also different between Kackar and Abkhazian goats. The differences in the fat and protein profiles in milk between goat breeds may depend on genetic factors. Milk from both local goats can be an option to eliminate problems arising due to allergic reactions in infant nutrition.

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