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Physicochemical and microbiological characteristics of goat milk from animals grown in a mountainous area in Bulgaria

D. Pamukova[1], N. Rusenova[2], T. Kolev[3], S. Chobanova[4], N. Naydenova[1]*

[1]Department of Animal husbandry – Ruminants and Dairy farming, Faculty of Agriculture, Trakia University, 6015 Stara Zagora, Bulgaria
[2]Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, 6015 Stara Zagora, Bulgaria
[3]Agricultural Academy, Agricultural Institute, 6000 Stara Zagora, Bulgaria
[4]Department of Morphology, Physiology and Animal Nutrition, Faculty of Agriculture, Trakia University, 6015 Stara Zagora, Bulgaria

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Abstract. *The aim of the study was to determine the goat milk quality from animals grown in a mountainous area in Bulgaria based on physicochemical and microbiological parameters. The study was carried out in a farm that breeds local goats and goats of the Bulgarian White Dairy Goat (BWD). Individual milk samples were taken on a monthly basis from morning milking. A total of 100 individual and 10 bulk milk samples were examined for fat, solids non fat, protein and density. A total of 62 samples were collected at a time to determine the microbiological characteristics of milk. For the period May-September 2017, the percentage of fat in the milk of local goats averaged 3.61% and of goats from BWD goat - 3.54%. The solids non fat were 8.27% and 8.19%, total protein - 3.13% and 3.10%, and the dry matter - 11.89% and 11.74%, respectively. For the period May-August 2017 the individual constituents of milk changed to varying degrees with the most variable being milk fat (decrease of 0.97% in local goats' milk and 1.09% in milk from BWD goat) followed by solids non fat (0.56% and 0.7%, respectively). The slightest change was in protein - 0.21% and 0.26%, respectively. Coagulase-negative staphylococci were the predominant bacterial species in the goats' milk samples.*

Keywords: Bulgarian white dairy goat, coagulase, local goat, quality of milk

Introduction

Goat milk is defined as functional food because of the content of easily digestible and essential nutrients for the human body (Mihailova et al., 2005; Bernacka, 2005). The physical and chemical properties of milk are influenced by the breed, health, nutrition and feeding practices, production systems, season (Goetsch et al., 2011), stage of lactation (Bhosale et al., 2009).

In a number of studies the authors established that the lactation phase in all goat breeds has the most significant effect on fluctuations in milk productivity and the main components of milk. The daily milkiness varies significantly throughout lactation peaking by 3-5 weeks after birth, then it decreases (Gipson and Grossman, 1990; Fernández et al., 2002). The percentage of dry matter is relatively constant during lactation. It follows that the concentration of the ingredients is the lowest during the period of the highest daily milkiness (Zeng and Escobar, 1996; Clark and Sherbon, 2000). According to Oprean et al. (2011) goats fed high concentrate level and pasture grazing produced milk with significantly higher contents of fat, protein, and total solids than goats kept under a confined feeding system with concentrate and hays. Higher concentrations of milk components (fat, protein, and dry matter) at the beginning and end of lactation and the lowest in the middle of lactation are reported by Mestawet et al. (2012) and Mahmoud (2014). The variation in the percentage of milk fat is the greatest compared to that of protein and dry matter. Total protein content varies according to the breed and lactation phase (Singh and Sengar, 1990).

According to Prasad et al. (2005) and Coulon et al. (2004) the effect of season and climatic conditions on milk composition may be due to the botanical composition of the ration. Fedele et al. (2000) found that at the beginning of May goat pasture grazing produced milk with high content of aliphatic hydro carbonates, while at the end of May alcohol and phenolics dominated. These changes are due to changes in the botanical composition of the herbs that determine the type and amount of volatile components in milk (Mariaca et al., 1997). Climatic parameters can also affect the content of volatile components (Rajeswara Rao et al., 1996).These factors are of particular importance for milk composition in year-round grazing of goats when the botanical composition and the grazing portion of plants change dramatically (Fedele et al., 1993).

The objective of this study was to determine goat milk quality from animals grown in a mountainous area in Bulgaria based on physicochemical and microbiological parameters.

*e-mail: n_naidenowa@abv.bg

Material and methods

The study was performed in a goat farm rearing local goats (LG) and Bulgarian White Dairy goats (BWD) during spring – summer 2017. The farm was located in the Sashtinska Sredna Gora Mountain and housed 150 goat does (83 from local goat and 67 from BWD). The animals were grazed during the period May-September and milking was manual.

Two groups of 10 goats each were formed (a total of 20 goats). The study was conducted from May to September. Individual milk samples were collected from the morning milking, proportionally to the milk yield, according to rules for milk sampling. Five controls (from May to September) were carried out and a total of 100 individual and 10 bulk milk samples were collected. In individual and bulk milk samples, the following parameters were assayed: fat content, solids-non-fat, total protein, density and titratable acidity (only bulk milk).

In order to establish the microbiological quality of milk during manual milking, 62 individual milk samples were obtained from the left (L) and right (R) udder halves of 31 goat does. Samples for microbiological analysis were collected observing general rules (Markey et al., 2013). Ten microliters of each sample were inoculated on tryptic soy agar (TSA, Fluka, India) with 5% defibrinated sheep blood and on McConkey agar (BulBio-NCIPD Ltd., Sofia) for detection of Gram-negative microflora. Plates were aerobically incubated at 37°C for 24-48 h.

The following milk parameters were monitored: dry matter content, % - by oven drying to constant weight at 102°C ± 2°C (Bulgarian State Standard /BSS/ 1109:1989); fat content, % – ISO 2446:2008 (International Dairy Federation standards /IDF/ 226:2008); solids non-fat content, % – by calculation based on the dry matter and milk fat; total protein content, % - by Kjeldahl method, according to BSS EN ISO 8968-1:2014; density, g/cm^3 – according to BSS 1110:1973; titratable acidity - by Thörner method (BSS 1111:1980).

Results from laboratory analyses were statistically processed with Statistica software.

Results and discussion

Physico-chemical analysis of milk

Physico-chemical analysis of bulk milk samples

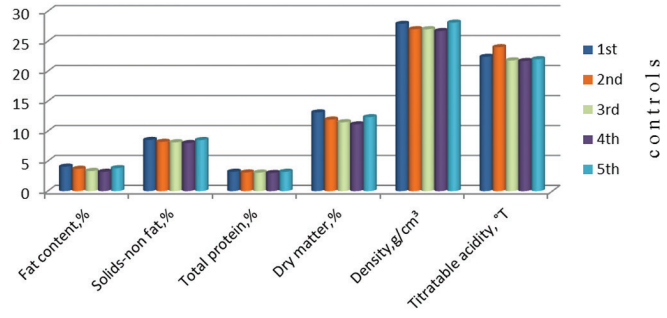
The study results showed insignificant differences in milk physico-chemical indices in local goats and Bulgarian White Dairy (BWD) goats (Table 1). For May-September, average fat content in the milk of local goats was 3.61% and that of BWD goats – 3.54%. Milk solids-non-fat content was 8.27% and 8.19%, total protein – 3.13% and 3.10%, and dry matter – 11.89% and 11.74%. The values of the studied parameters were slightly higher in the milk of local goats, yet statistically insignificantly.

Test day milk parameters of both groups were also comparable. In local goats, milk fat percentage, solids-non-fat, total protein, dry matter and density decreased from the 1st to 4th test day (from May to August), whereas levels by the 5th test day were similar to those of the 1st test day (Figure 1).

Similar changes were found in the milk of BDW goats (Figure 2). By test days, differences in fat percentage, solids-non-fat, total protein, dry matter and density between the two groups were small, higher in local goats' milk.

Table 1. Physico-chemical analysis of bulk milk from local goats and Bulgarian White Dairy (BWD) goats (n=5)

Parameters	Local goats		BWD goats	
	x ± Sx	C	x ± Sx	C
Fat content, %	3.61±0.17	10.40	3.54±0.16	9.94
Solids-non-fat, %	8.27±0.10	2.75	8.19±0.09	2.67
Total protein, %	3.13±0.04	2.77	3.10±0.04	2.68
Dry matter, %	11.89±0.27	5.01	11.74±0.23	4.31
Density, g/cm^3	1.027±0.27	2.22	1.027±0.32	2.68
Titratable acidity	22.38±0.42	4.22	19.38±1.50	17.34



Figue 1. Physico-chemical analysis of bulk milk from local goats by test days

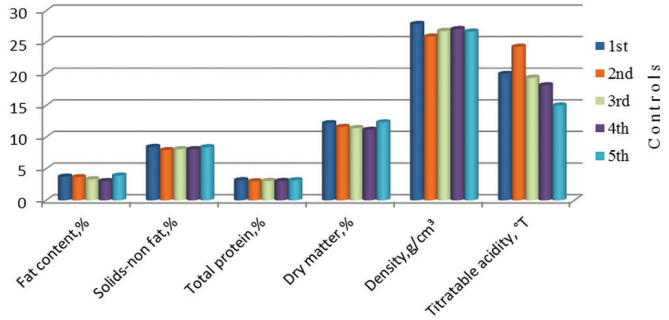


Figure 2. Physico-chemical analysis of bulk milk from BDW goats by test days

Physico-chemical analysis of individual milk samples

Results from analysis of individual milk samples showed that fat content, solids-non-fat, total protein and dry matter in the milk of local goats decreased from May to August, and in September increased statistically significantly compared to the preceding test day. The proportion of milk fat decreased from 4.03% (in the beginning of the study) to 3.06% (in August), for solids-non-fat: from 8.54% to 7.98%, for milk protein – from 3.22% to 3.01% and for dry matter – from 12.56% to 11.04% (Table 2).

Table 2. Physico-chemical analysis of individual milk from local goats by test days (n=10)

Control	Fat content, %		Solids-non-fat, %		Total protein, %		Dry matter, %		Density, g/cm^3	
	x ± Sx	C	x ± Sx	C	x ± Sx	C	x ± Sx	C	x ± Sx	C
1st	4.03±0.29^{a,b}	22.59	8.54±0.11^{a,b}	4.22	3.22±0.04^{a,b}	4.12	12.56±0.33^{a,b}	8.29	1.028±0.47^{a}	5.35
2nd	3.71±0.16^{c}	13.55	8.33±0.07^{c}	2.72	3.15±0.02^{c}	2.66	12.03±0.18^{c}	4.69	1.027±0.31	4.58
3rd	3.30±0.19^{a}	18.05	8.20±0.07^{a,d}	2.90	3.10±0.03^{a,d}	2.88	11.50±0.23^{a}	6.24	1.027±0.28	3.29
4th	3.06±0.19^{b,c,d}	19.34	7.98±0.09^{b,c,e}	3.43	3.01±0.03^{b,c,e}	3.39	11.04±0.23^{b,c,d}	6.50	1.026±0.34^{a,b}	4.01
5th	3.72±0.24^{d}	20.44	8.44±0.08^{d,e}	2.99	3.19±0.03^{d,e}	2.88	12.15±0.22^{d}	5.88	1.028±0.44^{b}	4.99
	a,b,c,d : p<0.05 c,e : p<0.01		a,d : p<0.05 c : p<0.01		a,d : p<0.05 b,c,d : p<0.01		a : p<0,05 b : p<0.001		a,b : p<0.05 b,e : p<0.001	

Table 3. Physico-chemical analysis of individual milk from BDW goats by test days (n=10)

Control	Fat content, %		Solids-non-fat, %		Total protein, %		Dry matter, %		Density, g/cm^3	
	x ± Sx	C	x ± Sx	C	x ± Sx	C	x ± Sx	C	x ± Sx	C
1st	4.12±0.18^{a,b}	14.19	8.68±0.16^{a,b,c}	6.01	3.27±0.06^{a,b}	5.80	12.80±0.31^{a,b,c}	7.65	1.029±0.59^{a,b}	5.56
2nd	3.75±0.14^{c,d}	11.77	8.19±0.16^{a}	6.08	3.09±0.06	5.97	11.94±0.27^{a,d}	7.06	1.027±0.56	6.60
3rd	3.32±0.14^{a,c}	13.46	8.10±0.11^{b}	4.29	3.06±0.04^{a}	4.26	11.42±0.21^{b,e}	5.92	1.027±0.40^{a}	4.70
4th	3.03±0.14^{b,d,e}	14.41	7.98±0.12^{c,d}	4.64	3.01±0.04^{b,c}	4.61	11.00±0.23^{c,d,f}	6.66	1.027±0.39^{b}	4.68
5th	3.98±0.28^{e}	22.37	8.47±0.17^{d}	6.23	3.21±0.06^{c}	6.24	12.44±0.41^{e,f}	10.46	1.028±0.53	6.07
	c : p<0.05 a,d,e : p< 0.01		a,d : p<0.05 b,c : p<0.01		a,b,c : p<0.05 b,f : p<0.01		a,d,e : p< 0.05 b : p <0.001		a,b : p< 0.05 c: p <0.001	

The differences between the parameters determined at individual test days exhibited various levels of statistical significance (p<0.01; p<0.05; p<0.001). As lactation advanced, the milk constituents changed at a various extent. Milk fat was the most dynamic, decreasing by 0.97% for May-August followed by solids-non-fat – by 0.56%. The total protein change was the least – by 0.21%. Milk dry matter as a function of solids-non-fat and milk fat was by 1.52% lower on the 4th test day (in August) compared to the 1st test day (in May). The changes in milk density followed the same trend as the solids-non-fat. The density of milk also decreased along with solids-non-fat but the differences were statistically significant only between the 1st and 4th test days (1.38 °L, p<0.05) and 4th and 5th test days (1.27 °L, p<0.05). The association of milk density with fat content and solids-non-fat is presented in Regulation (EC) 854/2004 of the European Parliament. With solids-non-fat reduction from 8.95% to 7.75%, the density at 20°C decreased from 1.030 to 1.020 g/cm^3.

In BDW goats and local goats, physico-chemical parameters of individual samples decreased substantially from the 1st to the 4th test day (Table 3). The greatest decrease was noted for milk fat – by 1.09% (from 4.12% in May to 3.03% in August), and the lowest – for total protein– by 0.26% (from 3.27% to 3.01%). For solids-non-fat, the difference between maximum (8.68% in May) and minimum (7.98% in August) value was 0.7%, and for dry matter – 1.8%. Milk density was statistically significantly lower by the 3rd test day (by 1.58 °L, p<0.05) and 4th test day (by 1.82 °L, p<0.05), vs the values by the 1st test-day (28.5 °L).

Mayer and Fiechter (2012) reported seasonal changes in dry matter, protein and fat content in goat milk from March to October – decreasing concentrations from the beginning of lactation (March), lowest values in June-August and highest levels in October, at the end of lactation.

The obtained values of physico-chemical parameters of local and BWD goats were similar to those of Terziyska et al. (1994) for BWD goat milk (fat content – 3.49%, total protein – 3.11%, dry matter – 11.95%) and Odzhakova (2002) for local goats and their crosses with Saanen bucks (3.45-3.56% for milk fat; 8.29-8.35% for solids-non-fat; 3.08-3.22% for total protein and 11.74-11.91% for dry matter).

The present study demonstrated that milk composition was largely influenced by feeding. In mountainous regions goats were reared on pastures in the summer (without supplementation). Advancement of vegetation changes the sward chemical composition, which has an effect on physico-chemical composition of milk.

Evaluation of microbiological characteristics of milk

A total of 62 goat milk samples were examined (Local and BWD goats) (Table 4). Sixteen milk samples from healthy animals showed microbiological growth on blood agar (pure cultures). On Gram-stained microscopic preparations, Gram-positive cocci were observed in 15 samples, arranged specifically in grape-like formations as well as in short chains or single ones. Yeast cells were visualised in one sample.

Table 4. Bacterial isolates from milk from local and BDW goats

No.	Positive samples	Isolates
1	BWD14 L	CoNS
2	BWD 7 L	CoNS
3	BWD 4R	CoNS
4	BWD 7 R	CoNS
5	BWD13 L	CoNS
6	BWD10 R	CoNS
7	BWD11 L	CoNS
8	LG 31 R	CoNS
9	LG 29 L	CoNS
10	LG 28 R	CoNS
11	LG 30 L	CoNS
12	LG 15 R	CoNS
13	LG 30 R	CoNS
14	LG 23 L	CoNS
16	LG 26 L	*Streptococcus uberis*
	LG 23 R	Yeasts

*BWD - Bulgarian White Dairy goats; LG - local goats;
**The number shows the sample number:
L - left udder halves
R - right udder halves

For genus identification of Gram-positive cocci, catalase and oxidase production tests and O-F test were carried out. Fourteen isolates were catalase-positive and oxidase-negative, and demonstrated fermentation of substrates specific for *Staphylococcus* spp. Additionally, these isolates were gamma haemolytic and coagulase negative, therefore they were assigned to the group of coagulase-negative staphylococci (CoNS) – 22.6%. One of the isolates was catalase-negative, hence belonging to genus *Streptococcus*. The CAMP test with esculin gave the specific black coloration, which along with lack of growth on MacConkey agar allowed identification of *Streptococcus uberis*. Isolates were identified using conventional microbiological examination methods (Markey al., 2013). The predominance of CoNS in the samples of our study is in line with previous publications (Callon et al., 2007; Tormo et al., 2011) focusing on microbial profile of the raw goat's milk. Moreover, other taxonomic groups of bacteria were not isolated on the used culture media which supports the fact for the good quality of milk from animals raised in the mountainous areas (Kawęcka et al., 2020).

Conclusion

The study results showed insignificant differences in the physico-chemical parameters of milk from local goats and Bulgarian White Dairy (BWD) goats. In the milk of local and BWD goats, fat content, solids-non-fat, total protein and dry matter decreased statistically significantly from May to August, while in September their values increased considerably vs the preceding test day. By test days, the differences between both groups were small and statistically insignificant. Within the groups, parameters' variation was at different levels of significance ($p<0.01$; $p<0.05$; $p<0.001$). Between May and August (from 1st to 4th test days), the different milk constituents changed at a various extent; milk fat being the most dynamic parameter followed by solids-non-fat. Total milk protein changed at the least extent. Microbiological characteristics of the goat's milk samples showed predominance of coagulase-negative staphylococci, followed by *Streptococcus uberis* and yeasts.

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