

# Physiological Characterization of Dubska Pramenka

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## Abstract

Scientific knowledge about the predispositions of Bosnian native breeds of sheep (Dubska) for the production of milk and milk products until recently were quite sparse and very scarce. Therefore, the aim of this study was to investigate the biochemical indicators and serum minerals, for monitoring the nutritional status. Until now subject research did not include testing of the effect of biochemical parameters and hormonal status of the blood of sheep on the quality of milk and milk products. Persistence of this connection is justified by the fact that most of the ingredients of milk just derived from the blood. The main objective of this research was to explore metabolic parameters of sheep's blood, and their relative importance on the quality and quantity of milk components. The study included a total of 127 sheep blood samples from two different areas (Livno and Travnik) in summer feeding period (July, August and September). The animals were marked with the appropriate number of ear tags on the basis of which we always took samples from the same animals through different periods. In sheep blood serum, the concentration of biochemical parameters (glucose, cholesterol, triglycerides, total protein, albumin, urea, AST, ALT, AP, GGT, LDH, calcium, phosphorus and magnesium) and metabolic hormones (triiodothyronine, thyroxine, and insulin cortisol), and samples of milk: milk parameters (milk fat, lactose and protein) as well as fatty acid composition of the milk. The concentrations of blood biochemical parameters were determined by spectrophotometry with

automated analysis with ready-made package of slides. Concentrations of metabolic hormones (T3, T4, cortisol and insulin) in the serum were determined by ELISA method. The percentages of fat, protein and lactose in milk were determined by infrared spectrophotometry. Fatty acids in milk were determined by gas chromatography (GC). Our research showed hypoglycemia, mild hypoproteinemia and hypoalbuminemia probably due to nutritional deficiencies during lactation period. Negative energy balance could be responsible for changes found in the cholesterol concentration as well as in the activity of ALT, AST, GGT, LDH. Significant differences in milk fat and milk protein, as well as fatty acids composition were determined between localities and season sampling. In general, our results showed variation of blood biochemistry and milk quality. Due to differences in climate, botanical differences of soil and pasture these variations are considered to be acceptable. Statistical analysis was performed using the software package SPSS 15.00 (for biochemical parameters and parameters of milk) and 21.00 (for metabolic hormones and fatty acid composition of the milk).

## Keywords

Sheep, Serum, Biochemical Parameters, Hormones, Milk

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## 1. Introduction

Natural conditions of Bosnia and Herzegovina (BiH) allow the raising and keeping of economically sustainable and profitable production of sheep milk, meat and wool. Being resilient and able to adapt to unpredictable climatic conditions, the sheep breed *Pramenka* was and will be an important economic resource for the occupancy and existence of the farms in the most remote rural areas of BiH. Milk should be seen as a complete and complex raw material containing active substances potentially effective in promoting a good health. Therefore, the main objective of this work was to explore metabolic parameters of sheep's blood, and their relative importance on the quality and quantity of milk components. Particular emphasis was paid to the content of bioactive substances with a possible positive effect on human health. Biochemical parameters responsible for various body functions and its deficiency result in impairment of functions induce structural and physiological abnormalities. The physiological responses of animals to environmental stress during the winter and summer and their energy balance, showed that seasonal heat and cold stress have profound effects on some serum biochemical parameters [1] [2]. Research conducted by Rasooli *et al.* [3] showed a significant difference in the concentration of serum total protein, albumin, glucose, cholesterol, calcium, T3 and T4 and activity of AST between hot and cold seasons, whereas the concentration of serum inorganic phosphorus was not significant difference between these two seasons.

## 2. Materials and Methods

The research was conducted during July, August and September at Livno area

(village Guber) and Vlasic Mountain (village Mudrike) in BiH. A total of 127 sheep of the breed Pramenka was investigated. The animals were marked with numbered ear tags and the sampling was always of the same animal through the different sampling periods (July-I, August-II and September-III).

### 2.1. Biochemical Parameters

Physiological characterization included the examination of the following values of the biochemical parameters (glucose, cholesterol, triglycerides, total protein, albumin, urea, aspartate aminotransferase (AST), alanin aminotransferase (ALT), alkaline phosphatase (AP), gamma glutamyltransferase (GGT), lactate dehydrogenase (LDH), calcium, phosphorus and magnesium). The sampling included parallel collection of blood samples as well as fresh milk from each animal. The concentrations of blood biochemical parameters were determined by spectrophotometry with automated analysis carried out by the company Johnson & Johnson Clinical Diagnostics Kodak (100 Indigo Creek Drive Rochester, New York 14650) with ready-made package of slides.

### 2.2. Metabolic Hormones

At the “Institute of Physiology, Pharmacology and Toxicology” of the Veterinary Faculty University of Ljubljana, Slovenia the value of metabolic hormones (triiodothyronine— $T_3$ , thyroxine— $T_4$ , cortisol and insulin) in the blood serum of sheep were determined by ELISA (ACTIVE Cortisol ELISA—Diagnostic Systems Laboratories, Webster, USA and Demeditec Diagnostics Germany).

### 2.3. Parameters of Milk

The percentages of fat, protein and lactose in milk were determined by infrared spectrophotometry. Fatty acids in milk were determined by gas chromatography (GC) in the laboratory “Vitas As” Oslo Innovation Centre, Norway. The following parameters of milk were determined: milk fat, lactose and protein and fatty acid composition (butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), stearic acid (C18:0), oleic acid (C18:1 cis-9), linoleic acid (C18:2 n-6), vaccenic acid (C18:1 trans-11), alpha linolenic acid (C18:3 n-3), arachidonic acid (C20:4 n-6, ARA), eicosapentaenoic acid (C20:5 n-3, (EPA), docosahexaenoic acid (C22:6 n-3, DHA), rumenic acid (C18:2 cis-9, trans-11, CLA)). Statistical analysis was performed using the software package/SPSS 15.00 and SPSS 21.00.

## 3. Results and Discussion

In this study we investigated metabolites concentrations in the blood serum of sheep, and their connection with the basic parameters of the quality of milk. Beside the metabolic profile of sheep, including biochemical indicators and serum minerals, it is important to monitor the nutritional status. The necessity for the body of mammals is maintaining the constancy of the internal environment. However, the process of maintaining the constancy of the internal en-

vironment, depends on the variety of mechanisms that tend to force intensity of metabolic processes subordinated to the needs of the body that depends on conditions such as pregnancy and lactation.

### 3.1. Biochemical Parameters

Results from the 127 blood samples indicated the presence of hypoglycemia, mild hypoproteinemia and hypoalbuminemia in the test animals. These are possible consequence of energy deficit in lactating animal as lactation is a metabolic demanding process. A tendency of the various blood values to be more normal towards the end of the lactation period is a sign of a normalization of the metabolic status at the end of the production cycle. The values of analyzed enzymes in the blood serum were near or above the upper physiological range, an indication of the compensatory intensification of metabolic processes as a response to a negative energy balance. In the area of Mount Vlasic hypocalcemia was determined at the beginning of the sampling. This is probably the result of poor quality and quantity of food. Lower blood glucose concentration may occur as a result of the increase in glucose utilization in the synthesis of lactose [4]. The concentration of cholesterol in both areas of sampling is varied after the sample period which is attributed to the diet as a key influence on the value of total cholesterol (Table 1 and Table 2). The content of cholesterol in plasma is variable and represent the result of its entry into the bloodstream in a complex with lipoproteins. The results of our analysis indicate that the concentration of cholesterol ranged within the reference value specified by the Kaneko *et al.* [5], while a slightly higher concentration of cholesterol in the second period of sampling in Livno area can be associated with increased metabolic activity of the liver and at the same time increased activity of the mammary gland [6]. Triglycerides as a parameter status of metabolism in the Travnik area showed a tendency of lowering towards the end of lactation (Table 2). Maximum concentrations of triglycerides, we determined the sampling period II for casting, with statistical significance compared to other periods of sampling both areas. The concentration of urea in the tested sheep in both areas has downward trend, but still within the physiological reference values listed by Kaneko *et al.*, [5]. In the area of Travnik in all three periods of sampling urea concentrations were significantly ( $p < 0.05$ ) (Table 2) higher compared to the same periods in the area of Livno (Table 1). Research and associates is Kohn [7] show a significant correlation between the concentration of urea in blood and protein levels in sheep food. Katunguka-Rwakishaya *et al.* [8] found that sheep's diet has a significant effect on the concentration of urea; also confirmed by the research Ašimović [9]. The specificity of the metabolism of sheep in the fact that degradation of feed proteins results in increased ammonia, and thus the higher intensity of the urea cycle. Excess ammonia comes from the amino acid foods that are not used immediately for protein synthesis. In terms of the lack of protein in a diet rich in fats and carbohydrates will not stop making clocks [9]. The value of serum enzyme ALT, AST, GGT and LDH, which were near or

**Table 1.** Concentrations of serum biochemical parameters in sheep in the area of Livno.

Biochemical parameters	Area "Livno"		
	I sampling	II sampling	III sampling
Glucose (mmol/l)	1.45 ± 0.07 <sup>a</sup>	1.58 ± 0.03 <sup>a</sup>	1.69 ± 0.04 <sup>b</sup>
Cholesterol (mmol/l)	1.97 ± 0.11 <sup>a*</sup>	2.47 ± 0.08 <sup>b*</sup>	1.77 ± 0.06 <sup>a</sup>
Triglycerides (mmol/l)	0.302 ± 0.015 <sup>a</sup>	0.351 ± 0.013 <sup>b*</sup>	0.276 ± 0.015 <sup>a</sup>
Total protein (g/l)	62.89 ± 2.76 <sup>a*</sup>	63.11 ± 1.04 <sup>a*</sup>	58.67 ± 0.97 <sup>a</sup>
Albumin (g/l)	27.68 ± 1.40 <sup>a*</sup>	29.68 ± 0.45 <sup>b*</sup>	27.11 ± 0.55 <sup>a*</sup>
Urea (mmol/l)	4.87 ± 0.22 <sup>c</sup>	2.85 ± 0.19 <sup>b</sup>	2.24 ± 0.17 <sup>a</sup>
Aspartate aminotransferase (U/L)	140.84 ± 8.33 <sup>b*</sup>	155.53 ± 6.29 <sup>b*</sup>	110.47 ± 3.54 <sup>a</sup>
Alanin aminotransferase (U/L)	44.29 ± 1.90 <sup>b*</sup>	40.24 ± 1.65 <sup>a</sup>	36.71 ± 1.58 <sup>a</sup>
Gamma glutamyltransferase (U/L)	88.37 ± 6.72 <sup>a</sup>	91.26 ± 5.31 <sup>a</sup>	82.63 ± 6.76 <sup>a</sup>
Alkaline phosphatase (U/L)	134.32 ± 11.48 <sup>a*</sup>	134.37 ± 15.72 <sup>a</sup>	119.16 ± 11.43 <sup>a*</sup>
Lactate dehydrogenase (U/L)	1869.63 ± 83.69 <sup>c</sup>	1646.89 ± 42.33 <sup>b</sup>	1212.63 ± 42.64 <sup>a</sup>
Calcium (mmol/l)	2.48 ± 0.08 <sup>a*</sup>	2.72 ± 0.03 <sup>b*</sup>	2.93 ± 0.04 <sup>c*</sup>
Phosphorus (mmol/l)	1.29 ± 0.06 <sup>c</sup>	1.12 ± 0.05 <sup>b</sup>	0.90 ± 0.06 <sup>a</sup>
Magnesium (mmol/l)	1.19 ± 0.039 <sup>a*</sup>	1.25 ± 0.023 <sup>a*</sup>	1.29 ± 0.028 <sup>a*</sup>

All values represent XX ± Sx. I, II, III represent sampling periods: July, August and September. a,b= values within a sampling area with different letters are statistically significant ( $p < 0.05$ ). \* = statistically significant ( $p < 0.05$ ) between same periods of sampling different areas

**Table 2.** Concentrations of serum biochemical parameters in sheep in the area of Travnik.

Biochemical parameters	Area "Travnik"		
	I sampling	II sampling	III sampling
Glucose (mmol/l)	1.62 ± 0.06 <sup>a</sup>	1.57 ± 0.03 <sup>a</sup>	1.81 ± 0.04 <sup>b*</sup>
Cholesterol (mmol/l)	1.09 ± 0.11 <sup>a</sup>	1.71 ± 0.08 <sup>b</sup>	1.1 ± 0.06 <sup>b</sup>
Triglycerides (mmol/l)	0.316 ± 0.016 <sup>a</sup>	0.262 ± 0.014 <sup>a</sup>	0.312 ± 0.016 <sup>a</sup>
Total protein (g/l)	44.78 ± 2.76 <sup>a</sup>	56.72 ± 1.04 <sup>b</sup>	61.22 ± 0.97 <sup>c</sup>
Albumin (g/l)	18.9 ± 0.137 <sup>a</sup>	26.60 ± 0.44 <sup>b</sup>	25.75 ± 0.54 <sup>b</sup>
Urea (mmol/l)	5.31 ± 0.21 <sup>b*</sup>	4.73 ± 0.18 <sup>b*</sup>	3.13 ± 0.17 <sup>a</sup>
Aspartate aminotransferase (U/L)	100.79 ± 8.33 <sup>a</sup>	140.16 ± 6.29 <sup>b</sup>	115.16 ± 3.54 <sup>a</sup>
Alanin aminotransferase (U/L)	34.37 ± 1.80 <sup>a</sup>	38.79 ± 1.56 <sup>b</sup>	37.16 ± 1.50 <sup>b</sup>
Gamma glutamyltransferase (U/L)	70.95 ± 6.72 <sup>a</sup>	85.63 ± 5.31 <sup>a</sup>	60.79 ± 6.76 <sup>a</sup>
Alkaline phosphatase (U/L)	78.30 ± 11.19 <sup>a</sup>	132.55 ± 15.32 <sup>b</sup>	82.80 ± 11.14 <sup>ab</sup>
Lactate dehydrogenase (U/L)	1149.05 ± 81.57 <sup>a</sup>	151615 ± 41.26 <sup>b</sup>	1311.75 ± 41.56 <sup>a</sup>
Calcium (mmol/l)	2.06 ± 0.08 <sup>a</sup>	2.45 ± 0.03 <sup>b</sup>	2.61 ± 0.05 <sup>c</sup>
Phosphorus (mmol/l)	1.19 ± 0.06 <sup>a</sup>	1.32 ± 0.05 <sup>ab*</sup>	1.56 ± 0.06 <sup>b*</sup>
Magnesium (mmol/l)	0.74 ± 0.038 <sup>a</sup>	0.89 ± 0.022 <sup>b</sup>	0.88 ± 0.027 <sup>b</sup>

All values represent XX ± Sx. I, II, III represent sampling periods: July, August and September. a,b= values within a sampling area with different letters are statistically significant ( $p < 0.05$ ). \* = statistically significant ( $p < 0.05$ ) between same periods of sampling different areas.

above the upper physiological limit, could be due to compensatory intensification of metabolic processes in response, primarily in the liver, in a negative energy balance (**Table 1** and **Table 2**). Season, age, animals, energy status are factors that have an impact on the test ALT, AST and GGT [10], which is most likely explanation for our findings for these enzymes.

Calcium levels were significantly higher in the Livno area (village Guber) (**Table 1**) in all three sampling period compared to the same periods in the area of mount Vlasic, which is probably a reflection of the different diet. In the area of Livno phosphorus concentrations show a tendency towards decreasing with increasing stage of lactation, while the area of mount Vlasic phosphorus concentrations were within the physiological range (**Table 1** and **Table 2**). The determined concentrations of magnesium in serum tested sheep show a statistically significant difference between the areas for all the data sampling.

### 3.2. Concentrations of Hormones

The hormonal status of the animals is relatively stable at experimental periods, but no statistically significant differences between sites with the exception of cortisol. In both areas of sampling revealed a high insulinemia, which could be due to diet that significantly change the hormonal regulation of metabolism during lactation (**Table 3** and **Table 4**).

### 3.3. Parameters of Sheep Milk

By comparing the values obtained for milk fat in relation to literature, were identified deviations from the values of the majority referred to in the literature. By increasing the number of lactation, there was an increase percentage of milk fat, which is most pronounced in the milk of any sheep in the field of mountain

**Table 3.** Concentrations of hormones in sheep in the area of Livno.

Hormones	I sampling	II sampling	III sampling	p
T <sub>3</sub> (nmol/l)	4.25	3.74	4.01	-
T <sub>4</sub> (nmol/l)	155.21	149.67	164.60	-
Insulin (pmol/l)	287.31	308.14	301.89	-
Cortisol (nmol/l)	602.28	623.53	530.83	-

I, II, III represent sampling periods: July, August and September.

**Table 4.** Concentrations of hormones in sheep in the area of Travnik.

Hormones	I sampling	II sampling	III sampling	p
T <sub>3</sub> (nmol/l)	2.26 <sup>a</sup>	2.597 <sup>b</sup>	2.26 <sup>a</sup>	*
T <sub>4</sub> (nmol/l)	119.9	122	112.48	-
Insulin (pmol/l)	100.77	91.53	133.13	-
Cortisol (nmol/l)	469.00	554.83	519.24	-

Mean values in the same row with different letter codes differ significantly. \*p < 0.05; I, II, III represent sampling periods: July, August and September.

Vlasic at the end of lactation period (**Table 5**). The protein content in the milk of sheep in the area of Livno had a declining trend towards the end of the lactation period, and on Mount Vlasic its concentration is varied with periods of sampling that can be linked to diet and stage of lactation. Lactose has proven to be the most stable parameter of milk with a slight variation in the area of Livno (**Table 5** and **Table 6**). Comparing median values of bioactive fatty acids sheep milk regardless of the sampling period (all samples collectively) determined the concentration of most fatty acids was higher in the milk of sheep from the area of mount Vlasic. A statistically significant difference of bioactive fatty acids in sheep milk from the area of Livno and the Vlasic mountain was found in C4:0, ARA, EPA, DHA and CLA fatty acids. The median value of most fatty acids in the milk of sheep from the area of Mount Vlasic showed variations between the sampling period. Metabolic hormones are to some extent affected the fatty acid composition of milk, primarily quantitatively more representative fatty acids (**Table 7**).

#### 4. Conclusion

In general though incomplete this is so far the most complete physiological characterization Dubska sheep. The metabolic profile of blood shows its ability to maintain homeostasis in terms of concurrent malnutrition and increased metabolic effort. Based on the variation of biochemical parameters in the blood serum, and their connection with variations of metabolites in milk can gain insight into the energy, protein and mineral supply of animals, and at the same time and in the health of the animals. Diseases associated with excessive consumption of food and non-compliance diet composition are becoming more common threat to health in younger and older age. An increasing number of

**Table 5.** The parameters of sheep milk from the area of Livno.

Sampling	Milk fat (%)	Lactose (%)	Protein (%)
I	9.34 ± 0.43	6.56 ± 0.27	4.30 ± 0.04
II	10.00 ± 0.46	6.11 ± 0.18	4.13 ± 0.06
III	9.96 ± 0.44	6.10 ± 0.18	4.42 ± 0.04

All values represent  $XX \pm Sx$ . I, II, III represent sampling periods: July, August and September. a, b = values within a sampling area with different letters are statistically significant ( $p < 0.05$ ) \*= statistically significant ( $p < 0.05$ ) between same periods of sampling different areas.

**Table 6.** The parameters of sheep milk from the area of Travnik.

Sampling	Milk fat (%)	Lactose (%)	Protein (%)
I	10.38 ± 0.38	6.23 ± 0.23	4.29 ± 0.04
II	10.15 ± 0.40	6.63 ± 0.15	4.24 ± 0.05
III	12.50 ± 0.38	6.04 ± 0.16	4.36 ± 0.04

All values represent  $XX \pm Sx$ . I, II, III represent sampling periods: July, August and September. a, b = values within a sampling area with different letters are statistically significant ( $p < 0.05$ ) \*= statistically significant ( $p < 0.05$ ) between same periods of sampling different areas.

**Table 7.** Median values of bioactive fatty acids in sheep's milk from Livno and Travnik area.

Fatty acid g/100 g FA	Livno	Travnik	p
C4:0	3.64	3.24	*
C6:0	1.75	1.73	
C8:0	1.32	1.37	
C10:0	3.69	3.83	
C18:0	9.02	8.77	
C18:1 cis-9	20.54	21.07	
C18:2 n-6	2.46	2.36	
C18:1 trans-11	2.73	2.89	
C18:3 n-3	1.83	1.87	
C20:4 n-6	0.21	0.27	*
C20:5 n-3 (EPA)	0.13	0.15	*
C22:6 n-3 (DHA)	0.11	0.14	*
C18:2 cis-9, trans-11 (CLA)	1.66	2.0	*

\*p &lt; 0.05.

nutritionists emphasize the negative implications of the consumption of foods of animal origin, especially of certain ingredients such as fatty acids because of their impact on human health. Given the importance of fatty acids in the human diet, are essential new knowledge about the structure and activities of the human body. Bioactive fatty acids have shown significant beneficial effects on human health, ranging from antiatherogenic and antineoplastic to immunostimulant. For that, and the fact that the acids secreted milk, such studies are essential in terms of improving the health quality of milk and dairy product design options with the preferred range of fatty acids.

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