



Biochemical and Endocrine Profiling of Bosnia and Hercegovina's Native Pramenka Sheep

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ABSTRACT

The aim of this investigation is to determine changes in concentrations of biochemical and metabolic hormones in the blood of Pramenka sheep. The study included 117 sheep of Pramenka breed monitored during three different periods (July, August, September) from Livno and Travnik (Vlašić) areas. The following biochemical parameters were determined in sheep blood samples: glucose, cholesterol, triglycerides, total protein, albumin, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), were performed by spectrometric analyzer (Kodak Ektachem, analyzer DT 60 II, DTSC Modul and DTE Modul). In sheep blood serum determined the following hormones by ELISA values: triiodothyronine, thyroxine, cortisol, and insulin. Hormonal status of test animals was relatively stable during periods of sampling, but significant differences have been confirmed based on locality, with the exception of cortisol. The lack of statistical significance of differences for cortisol between areas of Livno and Travnik can be due to stress caused by collecting blood samples which have equally increased cortisol levels and "concealed" all other factors that affect its level in the serum. High concentrations of thyroid hormones, particularly in sheep from the locality of Livno, can be a sign of decreased appetite, which is one of the earliest disorders in conditions of heat stress. In both areas, high insulinemia was determined as well, possibly as a result of feeding which significantly changed the hormonal regulation of metabolism in lactation process and led towards stimulation of the endocrine pancreas.

Keywords: blood, biochemical, hormones, sheep

Sheep with its multifaceted utility (for meat, wool, and milk) plays an important role in the Bosnian and Herzegovina agrarian economy. Biochemical determination of serum constituents can provide valuable information as relating to nutrition, sex, age and physiological status of the animal (Al-Fartosi *et al.*, 2010). Biochemical parameters responsible for various body functions and its deficiency result in impairment of functions induce structural and physiological abnormalities. It is well known that

variations existing biochemical constituents with regard to the sampling procedure, analytical techniques, physical factors, environmental conditions or variations in the breed and to know some advantages for these parameters evaluations to the animal body. The complex process of maintaining the constancy of the internal environment depends on homeostatic and homeorhetic mechanisms, which are trying to subordinate the intensity of metabolic processes to the needs of the body, depending on conditions



such as pregnancy and lactation. Hormones as bioactive substances, which are able to regulate metabolic process even when they are present in small amounts, have the most important role in this process. Hormones, acting on different organs and organ systems, affect the balance between homeostatic and homeorhetic mechanisms, directing nutrients towards metabolic processes which have priority. Start of lactation represents a milestone in which endocrine system redirects to another homeorhetic process (Savić, 2010). Metabolic hormones such as insulin and thyroid hormone (T3 –triiodothyronine and T4 - thyroxine) play an important role in the metabolism of animals. The significance of thyroid hormone is in thermoregulation and homeostasis of metabolism of energy and proteins, while recent findings suggest that thyroid hormones are also involved in the metabolic response of animals to various changes (nutrition, environment) and participate in the regulation of ovarian function in ruminants (Huszenicza *et al.*, 2002). In most of the animal species, serum concentrations of thyroid hormones can vary widely due to internal or external factors including age, gender, the temperature of the environment, seasonal and daily rhythms of feeding (Čebulj-Kadunc *et al.*, 2003). In addition, those hormones present a significant role in the development and functioning of the nervous, reproductive and immune systems (Klimien *et al.*, 2008). As long as the animals consume sufficient amounts of energy precursors in the meal, regulatory mechanisms can influence and preserve a balanced metabolism. However, lack of energy consumed from the meals leads to a drop in the presence of thyroid hormone, which associates the presence of thyroid hormone with the nutritional status of animals (Antunović *et al.*, 2008). Tiirats (1997) pointed out that the activity of thyroid hormone is associated with homeostatic adaptation to changes in the energy supply of animals during lactation when the energy balance is negative, and milk production reaches its maximum. The reduced presence of thyroxine in serum is associated with a low level of insulin, indicating improved utilization of the substrate in the mammary gland (Antunović *et al.*, 2009).

Lactation is a very demanding period for animals and represents a significant metabolic effort. During this period, it is difficult to provide optimal nutritional requirements, and therefore it often leads to weight loss of

sheep in the early days of lactation. Antunović *et al.* (2008) highlight in their study a significant impact of the stage of lactation to the hormonal activity of the thyroid gland, especially presence of T3 hormone whose concentration can be taken as a very good indicator of the health status of the organism. In early lactation phases, animals, statistically, have the significantly lower presence of T4 compared to the later stages of lactation. Low concentrations of thyroid hormones are a consequence of metabolic burden during the growing milk production in the organism (Savić, 2010). Reduced secretion of T4 is the result of metabolic conversion to the negative energy balance during early lactation process, which is important in the process of adaptation of peripheral tissues to the increased metabolic demands of the mammary gland (Riis *et al.*, 1985).

The endocrine system precisely regulates the most delicate immune processes. Neuroendocrine and immune systems communicate via cytokines, peptide neurotransmitters and peptide hormones (Marković, 2004). The most important mediators of changes in thyroid function as the consequence of the impact of stress are opioid molecules, prolactin, growth hormone, glucocorticoids, somatostatin and certain cytokines (Cizza *et al.*, 1995). The sensitivity of the endocrine system of sheep towards stress depends on the type and severity of stress (physiological, physical and chemical stress). Sheep, as social animals, are very sensitive when isolated from the herd, which results in a high rise in cortisol and adrenocorticotrophic hormone (ACTH - Eng. Adrenocorticotrophic hormone) concentration level in plasma (Minton *et al.*, 1990). The stress of any kind will affect the increase in cortisol levels (Moolchandani *et al.*, 2008). Some animals secrete more stress hormones than others, which may be due to breed differences and metabolic activity.

MATERIALS AND METHODS

According to the goals set, research included two areas, Livno area (village Guber) in this work we used the name “Livno”, and area of Travnik (Vlasic), in this work we used the name “Travnik”. The animals were marked with the appropriate number of ear tags based on which we conducted sampling always on the same animals through different periods.

Animals and experimental design

Testing was performed on a total of 117 sheep from "Pramenka" breed from areas of Livno and Travnik (Vlasic). Blood samples were taken during the summer period (July-I, August-II, and September-III) when the diet of sheep was based on the summer pasture in the presence of large quantity of legumes, grasses, and forbs. 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. Blood samples were collected from the jugular vein using vacutainer tubes (BD Vacutainer, 6 ml) to determine concentrations of biochemical metabolites and hormones. All blood samples were centrifuged at LC 320, 3000 rpm for 10 min. Serum samples were harvested and stored at -20°C until the analysis process. The following biochemical parameters were determined in sheep blood samples: glucose, cholesterol, triglycerides, total protein, albumin, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), were performed by spectrometric analyzer with automated analysis carried out by the company Johnson & Johnson Clinical Diagnostics Kodak (Kodak Ektachem, analyzer DT 60 II, DTSC Modul and DTE Modul) using original commercial autoanalyzer test kits. At the "Institute of Physiology, Pharmacology and Toxicology" of the Veterinary Faculty University of Ljubljana, Slovenia the

value of metabolic hormones (triiodothyronine (T3), thyroxine (T4), cortisol and insulin) in the blood serum of sheep were determined by ELISA (T3, cortisol and insulin factory kits ACTIVE Cortisol ELISA (Diagnostic System Laboratories, Webster, USA) were used, and for the determination of T4 kits from manufacturer Demeditec Diagnostics Germany were used.

Statistical analysis

Statistical analysis was performed using the software package SPSS 15.00 (for biochemical parameters) and 21.00 (for metabolic hormones).

RESULTS AND DISCUSSION

The serum biochemical values of sheep obtained in this study are presented in Table (1, 2).

Concentrations of T3, T4, insulin, and cortisol hormones in serum of sheep from the area of Livno and Travnik are shown in Tables 1 and 2. Concentrations of T3, T4, and insulin were significantly different between the sampling areas (Table 1), whereas only for T3 statistically significant difference was determined ($p < 0.05$) between the sampling period for the Travnik area (Table 4). Statistically significant differences ($p < 0.01$) were found for T3 and T4 between Livno and Travnik in July (Table 5), while a statistically significant difference at the level of $p < 0.001$

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 ^{a*}	1.58±0.03 ^a	1.69±0.04 ^b
Cholesterol (mmol/l)	1.97±0.11 ^a	2.47±0.08 ^{b*}	1.77±0.06 ^a
Triglycerides (mmol/l)	0.302±0.015 ^a	0.351±0.013 ^{b*}	0.276±0.015 ^a
Total protein (g/l)	62.89±2.76 ^{a*}	63.11±1.04 ^{a*}	58.67±0.97 ^a
Albumin (g/l)	27.68±1.40 ^{a*}	29.68±0.45 ^{b*}	27.11±0.55 ^{a*}
Urea (mmol/l)	4.87±0.22 ^c	2.85±0.19 ^b	2.24±0.17 ^a
Aspartate aminotransferase (U/L)	140.84±8.33 ^{b*}	155.53±6.29 ^{b*}	110.47±3.54 ^a
Alanin aminotransferase (U/L)	44.29±1.90 ^{b*}	40.24±1.65 ^a	36.71±1.58 ^a
Gamma glutamyltransferase (U/L)	88.37±6.72 ^a	91.26±5.31 ^a	82.63±6.76 ^a
Alkaline phosphatase (U/L)	134.32±11.48 ^{a*}	134.37±15.72 ^a	119.16±11.43 ^{a*}
Lactate dehydrogenase (U/L)	1869.63±83.69 ^c	1646.89±42.33 ^b	1212.63±42.64 ^a

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 samne 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 ^a	1.57±0.03 ^a	1.81±0.04 ^{b*}
Cholesterol (mmol/l)	1.09±0.11 ^a	1.71±0.08 ^b	1.1±0.06 ^b
Triglycerides (mmol/l)	0.316±0.016 ^a	0.262±0.014 ^a	0.312±0.01 ^a
Total protein (g/l)	44.78±2.76 ^a	56.72±1.04 ^b	61.22±0.97 ^{c*}
Albumin (g/l)	18.90±1.37 ^a	26.60±0.44 ^b	25.75±0.54 ^b
Urea (mmol/l)	5.31±0.21 ^{b*}	4.73±0.18 ^{b*}	3.13±0.17 ^{a*}
Aspartate aminotransferase (U/L)	100.79±8.33 ^a	140.166.29 ^b	115.16±3.54 ^a
Alanin aminotransferase (U/L)	34.37±1.80 ^a	38.79±1.56 ^b	37.16±1.50 ^b
Gamma glutamyltransferase (U/L)	70.95±6.72 ^a	85.63±5.31 ^a	60.79±6.76 ^a
Alkaline phosphatase (U/L)	78.30±11.19 ^a	132.55±15.32 ^b	82.80±11.14 ^{ab}
Lactate dehydrogenase (U/L)	1149.05±81.57 ^a	1516.15±41.26 ^b	1311.75±41.56 ^a

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 samne periods of sampling different areas.

was determined for T3, T4, and insulin between sampling areas comparing aggregated samples. The concentration of cortisol was not significantly different between the areas of Livno and Travnik between periods of sampling and in summary.

The concentration of T3 hormones in sheep from the area of Livno has varied depending on the sampling period but without statistical significance of differences. The highest concentration of this hormone was found in July and the lowest in August-II (Table 3), which are higher values compared to the values stated by Nazif *et al.* (2008) in their research. Thyroid hormone secretion may have daily variations as well, where the highest value is in the morning and lowest at night. The activity of T4 significantly differed depending on sampling periods, the lowest concentration being recorded in August and the highest in September but without statistically significant differences (Table 3).

Highly statistical significant differences in concentrations of T3 and T4 hormones were found between Livno and Travnik in July-I (Table 5), and statistically significant in August-II and September (Table 5). The differences in the concentration of hormones T3 and T4 between sampling areas may be due to a change in diet in given areas but also to significant differences in climatic conditions and changes

Biochemical serum parameters have common use in the

clinical assessment of domestic animals. Determination and monitoring of metabolic profile parameter values may show whether homeostatic mechanisms can maintain blood composition in physiological limits under different conditions of animal husbandry (Prodanović *et al.*, 2012). Hypoglycemia and mild hypoproteinemia and hypoalbuminemia of the examined animals may be a logical consequence of any nutritional, primarily energy, the animal deficit in the lactation period as a metabolic highly demanding process that continues on a previously metabolic-challenging physiological state-pregnancy. Blood glucose concentrations, which show a broad range of domestic animals, are regulated by the hypoglycaemic and hyperglycaemic hormones (Durak *et al.*, 2015). Blood glucose levels are also known to be related to genetic predisposition. Glucose concentrations may alter with the secretion of catecholamines, and may also increase secondarily, as a result of the stress of muscle and liver enzymes induced by myopathy and hypoxia (Durak *et al.*, 2015). The findings of hypoglycemia in both areas, with a tendency to increase glycemia at the end of the experiment, maybe the logical consequence of the nutritional energy deficit of the sheep after two metabolic highly demanding physiological states of pregnancy and lactation, earlier in terms of findings at the very end of lactation. Glucose is an energetic and plastic precursor of many and most important milk constituents. The condition of hypoglycemia may also indicate a possible

Table 3: Concentrations of hormones in serum sheep from the Livno area by period of sampling

Hormones/Sampling periods	I sampling	II sampling	III sampling	p
T ₃ (nmol/l)	4,25	3,74	4,01	—
T ₄ (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 serum sheep from the Travnik area by period of sampling

Hormones/Sampling periods	I sampling	II sampling	III sampling	p
T ₃ (nmol/l)	2,26 ^a	2,597 ^b	2,26 ^a	*
T ₄ (nmol/l)	119,9	122	112,48	—
Insulin (pmol/l)	100,77	91,53	133,13	—
Cortisol (nmol/l)	469	554,83	519,24	—

The mean values in the same row of different fonts differ significantly, where * p < 0.05. I, II, III - represent sampling periods: July, August and September.

Table 5: Statistically significant differences in the concentration of hormones between the Livno and Travnik by sampling periods and collectively

Hormones/Sampling periods	LI/II	LII/III	LIII/III	L/T collectively
T ₃ (ng/dl)	**	*	*	***
T ₄ (μg/dl)	**	*	*	***
Insulin (μIU/mL)	*	*	—	***
Cortisol (μg/dl)	—	—	—	—

L - sampling area LIVNO; T - sampling area TRAVNIK. I, II, III - sampling periods: July, August and September *p < 0,05; **p < 0,01; ***p < 0,001.

disorder of carbohydrate metabolism or, in turn, reduced intake of foods rich in carbohydrates (starvation). The condition of hypoglycemia may also indicate a possible disorder of carbohydrate metabolism or, in turn, reduced intake of foods rich in carbohydrates (starvation). Most of the carbohydrates in the progenitors of the survivors are degraded to lower fatty acids; the glucose resorption from the digestive tract is insignificant, and this is also the reason for the physiologically low glycemia in survival. The results of this study show that in some animals there was a mild hypoproteinemia in blood serum which can be considered as a lack of protein in the diet or poor quality pasture in the summer period on Vlasica and Livno. This

is supported by the results of Jovanović *et al.* (1983), which noted that the largest proteinemia in sheep blood serum was early in spring because the pasture is richest in proteins at this period of the year. The activity of some plasma enzymes may increase under physiological factors (muscle activity, hormones) and the presumption is that in physiological conditions, the minimal permeability of cell membrane might be changed reversibly. After muscle activity, transport, feed and lodging changes or after a combination of mentioned factors, an increase in serum aminotransferases may appear. Causes of enzyme activity changes in blood serum may be a disorder in the permeability of cell membrane, increased or decreased



or synthesis, disorders in secretion or disorder, in the elimination of enzymes from circulation. Increase in alkaline phosphatase (AP) in the blood of young lambs may be related to a higher activity of osteoblasts, because of intensive development of bone tissue (Antunović *et al.*, 2008). Some enzymes, especially and alanine aminotransferase (ALT), aspartate aminotransferase (AST) are good indicators of serum protein deficiency which is a result of changes in functions in specific organs. In the liver, AST, ALT, and GGT (gamma-glutamyl transferase) show high activity and are most often determined if there is a suspicion of acute and chronic liver disease (Hrković-Porobija *et al.*, 2017). The serum ALT, AST, GGT, and LDH values, which were close to or above the upper physiological limits, could indicate the necessarily compensatory intensification of metabolic processes as a response to the organism, primarily the liver, to a negative energy balance. Aspartate aminotransferase is an enzyme that is found in the liver and heart muscle and plays an important role in the metabolism of amino acids. The average value of γ -glutamyl transferase (GGT) in the blood serum samples of sheep was slightly elevated compared to reference values. Increased activity of this enzyme in clinically healthy sheep can be considered as a consequence of the intensification of metabolic processes and a response of the body to the negative energy balance (Stevanović *et al.*, 2015). Also, the increased activity of this enzyme in clinically healthy sheep can be a consequence of moderate oxidative stress, related to the increased degradation of glutathione (Hodžić *et al.*, 2011). Higher cholesterol concentrations measured in the II Livno sampling period (Table 1) may also be associated with intensified metabolic liver activity while simultaneously increasing the activity of the dairy gland. Start of lactation is characterized by negative energy balance, which requires reallocation of nutrients in the body and under the influence of an endocrine change in activity of almost all cells in the body happens in order to provide the optimum amount of nutrients for the needs of the mammary gland (Horvat, 2012).

Many studies have reported seasonal variations in the activity of the thyroid gland and thyroid hormone concentrations in the blood. These variations of hormones are especially important in grazing and free ranged animals, whose primary physiological functions (eating, reproduction, hair growth) are of highly seasonal character.

This is the case with small ruminants which are bred in the traditional way. Such variations in the concentration of hormones allow the animals to adjust their metabolic status to different environmental conditions, variations in needs and availability of nutrients, as well as homeopathic changes during different physiological stages. Values of the concentration of hormones in the blood are characterized by high variability, which is very important in any study. On the other hand, the values obtained in certain studies are not comparable due to the large difference between tested animals and their conditions, as well as due to differences in testing methods.

The decrease of energy level in meals leads to the decreased presence of thyroid hormones, which indicates that the activity can be correlated with the nutritional status of the animals. Antunović *et al.* (2009) examined the activity of hormones T3 and T4 in sheep breed of "Cigaja" where they found that concentrations of T3 and T4 were the lowest in the first period of lactation, when the milk production is on highest level, and slowly rising later on, which is probably related to increased nutritional needs due to high milk production and severe energy deficit. Yokuşu *et al.* (2006) indicate in their studies that reproductive status and seasonal variations have to be examined together so that we can properly interpret serum levels of hormones. In adult sheep, more than 99.9% of T4 and 99.5% of T3 in the circulation are bound to plasma proteins. The only unrestricted fraction of hormones is responsible for the biological activity, while hormones related to proteins serve as usable reserves, reducing the effects of thyroid secretion, but also as a protection against a sudden increase in secretory activity (Todini, 2007).

In the serum of sheep from the area of Travnik, the concentration of T3 hormone significantly differed through sampling periods (Table 4) but has been consistent with the results from the research of Nazifi *et al.* (2008). The highest concentration of T3 hormone was found in August, while during other two sampling periods the concentration of this hormone has had the same value (Table 4). The concentration of T4 in the serum of sheep from the area of Travnik had no significant variation between sampling periods. The lowest concentration was determined in September (Table 4). In July and August determined concentrations of this hormone (Table 4), were higher compared to the ones stated in research done by Yokuşu *et al.* (2006) but were lower than the values found in the

serum of sheep from the area of Livno (Table 4). Values of T4 hormone in both areas of sampling were significantly greater than values found by other authors (Nazif *et al.*, 2008; Antunovic *et al.*, 2009).

It seems that the action of thyroid hormones depends on the type, season and interactions with other regulatory factors. Interaction with other factors is highly important, primarily prolactin (Todini, 2007), as well as the local activity of insulin (Puchala *et al.*, 1998). Gender-related differences have been spotted in other mammals as well and are related to the different influence of sex steroid hormones. Differences in levels of total T4 can be explained by the reduction of catabolism thyroxine binding globulin caused by estrogen (Todini, 2007). Heat stress can affect the activity of thyroid in an inhibitory manner, where the concentration of hormone T4 in the serum of sheep from the area of Livno, during the period of intense heat stress (month of July) was lower compared to other two sampling period. However, it is still an open question whether the reduction in the concentration of thyroid hormone is a sign of adaptation of the organism to high environmental temperature, or a sign of decreased appetite, which is one of the earliest disorders in terms of heat stress. Studies conducted by Oldenbroek *et al.* (1989) suggest a positive correlation between levels of thyroid hormones in the blood and energy balance and a negative correlation between their levels and milk. The reduction in milk production in the conditions of high temperature may be due to the reduced synthesis of thyroid hormones. This reduces the metabolic heat production. Regulation of T3 concentration in the peripheral blood takes place independently of the T4 concentration. Changes in the level of T4 in serum that are related to energy balance and metabolism indicate the level of secretion of thyroid changes under the influence of TSH (Riis and Madsen, 1985) and the balance with extrathyroidal activation or inactivation of T4 (Horvat, 2012).

To restore thyroid activity a longer period of time is required, which reduces thyroid activity during the summer period compared to its activity in the winter (Horvat, 2012). It is known that the needs for lactating ruminant tissue for insulin reduce, which can cause a temporary increase in blood glucose levels and thereby stimulate the production of milk (Antunovic *et al.*, 2009). During starvation, its concentration decreases, while the normally fed animals concentration increases (Sutton *et*

al., 1989). Increased sympathetic activity leads to reduced secretion of insulin.

The entry of glucose into the cells of the mammary glands is independent of insulin. Period early lactation is characterized by insulin resistance (Sladojević, 2012), or the state in which the sensitivity of peripheral tissues to insulin decreased. Insulin levels in the serum of sheep from the area of Livno showed some variation by sampling periods, but without statistical significance (Table 3). The lowest concentration was found in July, while in August and September increased concentrations are a very high value compared to the values of other authors (Antunović *et al.*, 2011). Insulin is a major anabolic hormone that promotes the construction of the body (mitogenic effect) and prevents the breakdown of tissue and metabolic substrates other than glucose (metabolic effect). The concentration of insulin in the blood of ruminants is closely related to the diet of animals (Sladojević, 2012). High values of insulin may be the result of disorders of the liver and its reduced gluconeogenesis. Nutrition significantly changing hormonal regulation of metabolism in animals and lactating through insulin significantly affects the redistribution of energy resources during lactation. Larger amounts of easily digestible carbon hydrates in meals, can lead to stimulation of the endocrine pancreas and maintain high insulin levels. Changes in the concentration of insulin throughout the day may be a result of changes in the intensity of the synthesis and secretion of insulin and are directly dependent on a diet (Radojičić *et al.*, 2007).

In the area of Travnik insulin levels varied depending on the sampling periods (Table 4). The concentration of insulin in the blood tends to increase when the sheep are fed with concentrated food. This is a result of increasing concentration of propionate and butyrate in the rumen contents, which are made during degradation process of carbohydrates from food by means of microflora (Radojičić *et al.*, 2007). Cortisol, a corticosteroid hormone produced in the adrenal cortex, is often referred to as the “stress hormone” because it is participating in the stress response of the organism. In the area of Lvno cortisol levels found were had high values (Table 3), compared to the values of other authors (Doubek *et al.*, 2003).

Fluctuations in cortisol levels can be attributed to stress, which was associated with the expulsion of sheep to pasture. Stress, caused by moving of sheep to a new



environment could cause an increase in cortisol levels. The high concentration of cortisol in the analyzed serum samples of sheep from both sampling areas (Table 3 and 4) may be the consequence of thermal stress which may partly explain the higher values of cortisol in sheep from the area of Livno (Jenko, 2009). During the summer months, the animals are exposed to heat stress due to high ambient temperatures that are sometimes over 35 degrees. With the increase in external temperature above 250 degrees intake of dry matter in a meal when endogenous regulatory mechanisms try to compensate for this lack of gluconeogenesis induced elevated levels of cortisol. In domestic animals, including sheep, endogenous circadian biorhythm of adrenocorticotrophic hormone and cortisol was established, which regulates suprachiasmatic nucleus of the hypothalamus. The highest values were measured in the morning, and the lowest in the evening (Jenko, 2009). If during lactation higher level of cortisol in relation to insulin is established and maintained, high lactation will be maintained for a longer period (Horvat, 2012).

In order to maintain normal body temperature, food intake is reduced to 35%, milk production is lowered by 10-20%, plasma glucose levels fall, butterfat percentage is lowered and reproduction capability drops (Rhoads *et al.*, 2009; Brouček *et al.*, 2009). Acute exposure to elevated temperature and isolation with the lambs as well as one of the important factors for the increase in cortisol (Jenko, 2009). The concentration of cortisol can be different for each individual because some animals secrete more stress hormones than others, possibly due to the metabolic activity. Of all domestic animals, sheep are the most sensitive to the effects of stress (Moolchandani *et al.*, 2008). The sensitivity of sheep endocrine system to stress depends on the type and severity of stress (physiological, physical and chemical stress). By monitoring changes in hormonal status and metabolic profile the degree of stressful reactions in the body can be determined. This is especially important during chronic stress to which the animals are exposed in conditions of intensive breeding.

The influence of cortisol increases blood glucose levels which is a result of increases in the volume of gluconeogenesis. Exposure of sheep to prohibitions and isolation stress causes an increase in the concentration of ACTH and plasma cortisol (Minton *et al.*, 1990). Minton *et al.* (1990) found growing levels of cortisol with lambs as a response to isolation stress, where exposure to an

elevated temperature of environment or isolation triggers the activity of the adrenal gland, and this is one of the important factors contributing to elevated levels of cortisol which is the result of isolation stress. Moolchandani *et al.* (2008) have also found a statistically highly significant of the effect of stress on cortisol levels with a tendency to increase. The concentration of cortisol levels in serum of sheep from the area of Livno had a downward trend as the end of lactation approached (Table 5). Jenko (2009) states in his study that the highest cortisol levels were found in the spring and summer while in the autumn period there is a decrease in concentration, which is consistent with our results from the Livno area (Table 3). Looking at circadian biorhythm (morning sample) and periods of our sample it is possible that in addition to stress, these two factors had a significant impact on the concentration of serum cortisol levels of sheep from the area of Livno.

CONCLUSION

The examination of the basic parameters of metabolism as well as the established difference between the sheep and the two areas did not indicate significant differences in relation to the available literature data. The established differences between the examined areas are most likely to be the result of animal feeding conditions. Hormonal status of most of the tested animals was relatively stable for periods of sampling, but significant differences were detected between the localities of Livno and Travnik (Vlasic), which in addition to stress can be the result of different botanical composition with regard to a variety of altitudes.

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