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BLOOD METABOLIC PROFILE AND SOME OF HORMONES CONCENTRATION IN EWES DURING DIFFERENT PHYSIOLOGICAL STATUS

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Abstract

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The aim of this paper is to determine the influence of physiological status on blood metabolic profile and some of hormones concentration in the blood of ewes. Investigations were carried out on 45 Tsigai ewes (15 pregnant ewes on the 10th day prior to lambing, 15 lactating ewes on the 20th day of the lactation and 15 non-pregnant ewes) during winter feeding season. The ewes were average 3 years old and healthy. Ewes were fed meadow hay and 300 g of feed mixture. Higher concentrations of Cl, urea, total bilirubin, AST and GGT activities were determined in the blood ewes in lactation compared to high pregnant and non pregnant ewes. The opposite trend was observed for concentration glucose, hemoglobin, T3, RBC and ALT activity. In the blood pregnant ewes were determined lower concentrations of glucose and urea in relation to non-pregnant ewes and higher concentrations of total protein, albumin, LDL-cholesterol, hematocrit, leptin and insulin in relation to the ewes in lactation. In the ewe's blood in lactation were determined higher concentrations of K, a number of PLT and increased LDH activity in relation to pregnant ewes. Higher concentrations of triglycerides and lower PLT were determined in blood pregnant ewes in relation to non-pregnant ewes. Changes in concentrations of biochemical indicators (glucose, cholesterol, triglycerides, urea), T3, leptin and insulin levels indicate an energy deficiency of ewes in early lactation. The results obtained blood indicators point out justification of the blood metabolic profile in the assessment of food status ewes and ensuring good health in very demanding physiological states - high pregnancy and lactation.

Key words: ewe, blod metabolic profile, physiological status, thyroid hormones, leptin, insulin, nutritional status

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Introduction

Blood metabolic profile (BMP) is a set of diagnostic procedures that are based on determining the various indicators in the blood of animals (Van Saun, 2000). The most common indicators in the blood of animals used in the preparation of the BMP are biochemical and hematological parameters. BMP is used in assessing nutritional status and animal health (Herdt et al., 2000; Antunović et al., 2009). Significant variations in the blood metabolic profile depend on many, genetic and non-genetic factors. One of the important factors is physiological status which affects on concentration of indicators in blood that are involved in the development of the blood metabolic profile (Antunović et al., 2002; Roubies et al., 2006). However, most research does not include all important biochemical and hematological parameters as well as some of hormones (leptin, insulin and thyroid hormones) in blood of ewes. Leptin is a 16-kDa non-glycosylated protein and it is considered a hormone as regulated body weight by maintaining the balance between food intake and energy expenditure through signaling to the brain the changes in stored levels (Friedman and Halaas, 1998). The thyroid hormones maintain the homeostasis of energy and protein metabolism, thermoregulation, growth and productivity parameters (Huszenicza et al., 2002). High pregnancy and lactation, especially in the early stages, are very demanding physiological state of the organism when nutritional requirements are increased (Goff and Horst, 1997). During lactation the mammary gland secretary cells utilize 80% of the circulating metabolites in the blood for milk synthesis (Karapehlivan et al., 2007). In the understanding of the food status of ewes, particularly the energy supply of ewes, leptin concentrations are used (Altman et al., 2005) and thyroid hormones (Todini, 2007).

The aim of this study is to determine the influence of physiological status on blood metabolic profile and concentrations of leptin and thyroid hormones in the blood of ewes.

Material and Methods

This study was conducted during 2009 at the family farm Ursic (Croatia, located 35 km southeast of Osijek, TX (42.150^0 N; long 52.647^0 E). This area is located within the Baranja region and has an evaluation of approximately 91 m. Investigations were carried out on 45 Tsigai ewes (15 pregnant ewes on the 10th day prior to lambing, 15 lactating ewes on the 20th day of the lactation and 15 non-pregnant ewes) during winter feeding season. The ewes were average 3 years old and healthy. The mean body weights and standard deviation of pregnant ewes were 88.25 ± 9.91 kg, for lactating ewes 74.00 ± 11.28 kg and for non-pregnant ewes 76.60 ± 10.11 kg. The body condition scores (BCS) of ewes (1= emaciated to 5 = obese) was evaluated by two trained technicians according Russel (1991). The mean BCS and standard deviation of pregnant ewes were 3.10 ± 0.74, for lactating ewes 2.48 ± 0.65 and for non-pregnant ewes 2.86 ± 0.38. The ewes were fed meadow hay and 300 g of feed mixture. Water and a vitamin-mineral mixture were provided ad libitum to all ewes for the entire trial. Pregnancy was detected by ultrasound techniques (standard device Tringa 300, PIE Medical, Netherlands).

Blood was collected from the jugular vein into both serum Vacutainer tubes (Venoject®, Sterile Terumo Europe, Leuven, Belgium) and ethylenediamine tetra-acetic acid (EDTA) after feeding ewes. The EDTA tubes were inverted several times to ensure adequate mixing of the blood with anticoagulant. After that, the serum was separated by centrifugation (10 min) at 3000 revolutions/min. Within the blood serum there have been found the concentrations of the electrolytes (Ca-calcium, P-inorganic phosphorus, K-potassium, Na-sodium and Cl-chloride), concentrations of the biochemical indicators (urea, glucose, total proteins, albumin, cholesterol, HDL-cholesterol, LDL- cholesterol, triglycerides, creatinine and bilirubin total) and enzyme activity (ALT-alanine aminotransferase, AST-aspartate aminotransferase,

CK-creatine kinase, GGT-ã-glutamyl transferase and LDH-lactate dehydrogenase). All electrolytes and biochemical values were determined with Olympus System Reagents (OSR), manufactured and distributed by Olympus Diagnostic GmbH (Irish Branch), Lismeehan, Ireland, manufactured for Olympus Diagnostic GmbH, Hamburg, using OLYMPUS AU 600 apparatus.

Determination of haematological indicators (number of white blood cells-WBC, red blood cells RBC, number of platelet-PLT and content of haemoglobin, hematocrit, mean erythrocyte volume-MCV, average content of haemoglobin in erythrocytes-MCH, and mean concentration of haemoglobin in erythrocytes -MCHC) in the whole blood of ewes was carried out on 3 diff hematology analyzer SYSMEX pocH-100iV. From whole blood GSH-Px activity was determined using commercial Ransel kit on Spectrophotometer JENWAY 6305.

Concentrations of total T_3 and T_4 in blood serum were determined by means of duplicate determinations using commercial kits for clinical use in humans (Abbott Laboratories, USA) by Imx-Abbott immunoanalyser. Methods for determination of T_3 and T_4 were MEIA (Microparticle Enzyme Immunoassay) and FPAI (Fluorescence Polarization Immunoassay). Sensitivity of the assay was less than 0.4 nmol/L (T_3) and 12.8 nmol/L (T_4). Mean recovery rates were 98.6%. The ratio between the T_3: T_4 is determined by dividing these two values for each animal separately.

Serum leptin concentrations were analyzed with a specific enzyme immunoassay that has been validated for ovine samples (Sauerwein et al., 2004). The intra-assay coefficient of variation was 6.3%, the inter-assay coefficient of variation was 13.9%, and the limit of detection was 0.3 ng/mL. Serum insulin concentrations were analyzed by MEIA (Microparticle Enzyme Immunoassay) with AxSYM Insulin Reagent Pack (2D01-21) on Abbott AxSYM Systems (Axis-Shield Diagnostic, Ltd, Dundee, UK). The sensitivity of AxSYM Insulin assay was calculated to be better than or equal to 1.0 μU/mL.

Animals used in this study were maintained in facilities approved by the Croatian Association for Accreditation of Laboratory Animal Care, and in accordance with current regulations and standards issued by the Croatian Ministry of Agriculture, Forestry and Water Management.

The results were statistically evaluated by using Duncan's multiple range tests (Statistica, 2008). Differences were considered as significant at the level of 0.05 or less.

Results

In Table 1 are shown average values of electrolytes in the blood of ewes in different physiological states. Analyzing the concentration of electrolytes, biochemical and hematological indicators as well

Table 1

Influence of physiological states of ewes on blood electrolytes

Parameter	Physiological status			Reference range*
	Non-pregnant Mean ± s	Pregnant Mean ± s	Lactating Mean ± s	
Ca, mmol/L	2.55 ± 0.01	2.55 ± 0.06	2.59 ± 0.07	2.88-3.20
P-inorganic, mmol/L	1.68 ± 0.20	1.63 ± 0.25	1.61 ± 0.52	1.62-2.60
K, mmol/L	5.54^{ab} ± 0.33	5.39^{a} ± 0.61	5.83^{b} ± 0.40	3.90-5.40
Na, mmol/L	156.00^{A} ± 3.09	153.20^{A} ± 2.04	162.30^{B} ± 2.21	139.00-152.00
Cl, mmol/L	102.40^{A} ± 3.21	111.90^{A} ± 1.20	115.80^{B} ± 0.92	95.00-103.00

*- Kaneko et al. (1997), $^{A, B}$- means with different superscript letters differ significantly ($p<0.01$);
$^{a, b}$- means with different superscript letters differ significantly ($p<0.05$); s –standard deviation

Table 2
Influence of physiological status of ewe on blood biochemical parameters

Parameter	Physiological status			Reference range*
	Non-pregnant Mean ± s	Pregnant Mean ± s	Lactating Mean ± s	
Glucose, mmol/L	$3.86^{A} \pm 0.32$	$3.51^{B} \pm 0.30$	$3.26^{C} \pm 0.37$	2.78-4.44
Urea, mmol/L	$6.02^{a} \pm 0.52$	$5.70^{a} \pm 0.44$	$6.80^{b} \pm 0.46$	2.86-7.14
Cholesterol, mmol/L	1.59 ± 0.24	1.85 ± 0.53	1.57 ± 0.28	1.35-1.97
HDL-cholesterol, mmol/L	0.98 ± 0.11	1.00 ± 0.09	1.09 ± 0.23	-
LDL-cholesterol, mmol/L	$0.55^{a,b} \pm 0.16$	$0.73^{a} \pm 0.41$	$0.40^{b} \pm 0.08$	-
Triglyceride, mmol/L	$0.17^{a} \pm 0.05$	$0.25^{b} \pm 0.09$	$0.19^{a,b} \pm 0.06$	0.0-0.2
Creatinine, µmol/L	$88.40^{a} \pm 6.28$	$87.90^{a} \pm 7.11$	$79.20^{b} \pm 11.71$	50-109
Total proteins, g/L	$74.97^{a,b} \pm 3.87$	$76.86^{a} \pm 4.78$	$72.50^{b} \pm 4.50$	60-79
Albumine, g/L	$30.31^{a,b} \pm 1.31$	$30.93^{a} \pm 0.64$	$28.60^{b} \pm 3.80$	24-30
Total bilirubin, µmol/L	$2.40^{A} \pm 0.52$	$2.60^{a} \pm 0.52$	$3.10^{B,b} \pm 0.57$	1.71-8.55

*Kaneko et al. (1997); $^{A, B, C}$- means with different superscript letters differ significantly ($p<0.01$);
$^{a, b}$- means with different superscript letters differ significantly ($p<0.05$); s –standard deviation

Table 3
Influence of physiological status of ewe on blood enzymes activity

Enzyme, U/L	Physiological status			Reference range*
	Non-pregnant Mean ± s	Pregnant Mean ± s	Lactating Mean ± s	
AST	$102.20^{a} \pm 15.48$	$97.20^{A} \pm 11.15$	$114.20^{B,b} \pm 10.50$	60-280
ALT	$19.80^{a} \pm 2.53$	$19.60^{a} \pm 3.81$	$15.70^{b} \pm 3.68$	6-20
GGT	$46.80^{A} \pm 9.40$	$45.90^{A} \pm 10.63$	$63.50^{B} \pm 4.03$	20-52
CK	102.40 ± 36.92	89.50 ± 26.29	83.00 ± 22.52	106-168
LDH	$422.10^{a,b} \pm 46.77$	$396.70^{a} \pm 48.86$	$469.20^{b} \pm 81.69$	238-440

*Kaneko et al. (1997); $^{A, B}$ - means with different superscript letters differ significantly ($p<0.01$);
$^{a, b}$- means with different superscript letters differ significantly ($p<0.05$); s –standard deviation

as concentrations of hormones (leptin, insulin and thyroid hormones) in the blood of ewes at different physiological stages are visible significant changes in most of mentioned parameters (Tables 2, 3, 4 and Figure 1). Higher concentrations of Cl, urea, total bilirubin and AST and GGT activities were determined in the blood of ewes in lactation compared to high pregnant and non-pregnant ewes. The opposite trend was observed for glucose, hemoglobin, T3, RBC and ALT activity. In the blood pregnant ewes were determined lower concentrations of glucose and urea in relation to non-pregnant ewes and higher concentrations of total protein, albumin, LDL-cholesterol, hematocrit, leptin and insulin in relation to ewes in lactation. Higher concentrations of triglycerides and lower PLT were determined in blood pregnant ewes in relation to non-pregnant ewes. In the blood of ewes

Table 4

Influence of physiological status of ewes on blood hematological parameters

Parameter	Physiological status			Reference value*
	Non-pregnant Mean ± s	Pregnant Mean ± s	Lactating Mean ± s	
WBC (x 10^9/L)	11.97 ± 5.35	10.45 ± 4.34	10.49 ± 2.32	9-15
RBC (x 10^{12}/L)	$11.02^{A,a}$ ± 0.74	12.10^{b} ± 1.45	10.11^{B} ± 1.33	9-15
Hemoglobin (g/L)	120.44^{a} ± 7.62	$131.00^{A,b}$ ± 12.03	111.40^{B} ± 11.97	90-150
Hematocrit, %	$0.46^{A,B}$ ± 0.04	0.51^{A} ± 0.05	0.44^{B} ± 0.05	-
MCV, fL	42.19 ± 2.11	42.05 ± 2.31	43.55 ± 2.01	28-40
MCH, pg	10.97 ± 0.73.	10.91 ± 0.63	11.04 ± 0.51	9-12
MCHC, g/L	259.56 ± 8.02	259.45 ± 10.76	253.90 ± 9.68	-
PLT (x 10^9/L)	428.75^{a} ± 137.07	252.36^{b} ± 142.7	411.10^{a}± 109.78	500

*Kramer (2000); $^{A, B}$ - means with different superscript letters differ significantly ($p<0.01$);
$^{a, b}$- means with different superscript letters differ significantly ($p<0.05$); s –standard deviation

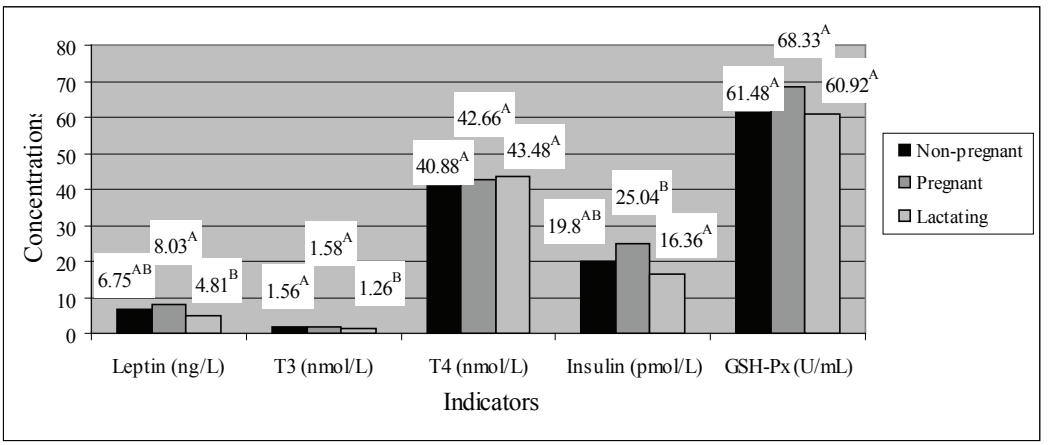

Fig. 1. Influence of physiological status of ewes on blood hormones and GSH-Px concentration

in lactation were determined higher concentrations of K, a number of PLT and higher activity LDH in relation to pregnant ewes.

Discussion

Changes in the concentrations of electrolytes in the blood of ewes, particularly in lactating ewes were partly different from the reference values for ewes (Kaneko et al., 2008). These differences could be associated with an increased requirements for intensive growth of fetus in high pregnancy due to increased synthesis of milk in lactation. The lowest concentrations of Na and K were detected in the ewes in a high stage of pregnancy. To similar results have come Antunoviæ et al. (2004). Azab and Abdel-Maksoud (1999) reported that concentration of Na increased ($P>0.05$) in goat blood at 3 and 4 weeks postpartum, as well as decreased of K concentration during late pregnancy.

Decreased blood glucose concentrations in lactating ewes have to be considered as a result

of constant energy loss with the milk. Specifically, these changes suggest that the combination of increased utilization of glucose for milk lactose synthesis and the low intake of nutrients during investigation was insufficient to maintain blood glucose homeostasis (Pambu—Gollah et al., 2000). Current findings are consistent with earlier report in lactating ewes (Roubies et al., 2006) and in lactating mares (Heidler et al., 2002). Low glucose levels in high pregnancy are associated with fetus development and mobilization of maternal glucose to fetal blood circulation (Jacob and Vadodaria, 2001). These findings are in support with insulin concentrations (Graph 1). Greater urea concentration in lactating ewes can also be a result of catabolizing muscle protein when large amounts of body reserves are mobilized. This is accordance with BCS and body weights of ewes. Caldeira et al. (2007) concluded that ewes with lower BCS can have greater urea concentration. Similar results have obtained Whitney et al. (2009). The pregnant ewes have had statistically higher concentrations of total proteins and albumin in blood comparing to the lactating ewes. Similar concentrations of total proteins in blood of the pregnant ewes and those in lactation have been found by Karapehlivan et al. (1997). Decrease of total protein and albumins over the lactation could be explained by a rapid extraction of immunoglobulin from the plasma during the last few months of pregnancy when colostrums' is being formed in the mammary gland (Kaneko et al., 2008). The highest concentrations of triglycerides and LDL-cholesterol in the blood of the ewes during late pregnancy comparing to the non-pregnant ewes can be explain with a consequence of a heavier transport of the lipoproteins or energy deficiency in a meal. Similar results have been detected by Nazifi et al. (2002). Decreased triglycerides and cholesterol concentrations in early lactation are consistent with an increased energy requirement and negative energy balance. These results are in agreement with those of Karapehlivan et al. (2007). In this investigation we have detected the increase of the total bilirubin in the blood of the lactating ewes and those in pregnancy compared to the non-pregnant ones. This could be the reason of the increased liver metabolism in those stages (Kaneko et al., 1997).

The increase in ALT activity, AST, and GGT in the blood of ewes in lactation indicated an increase in hepatic metabolism. Current findings in blood of lactating ewes are consistent with earlier reports (Antunoviæ et al., 2004). Significantly higher LDH activity was observed in the blood of ewes in lactation compared to pregnant ewes. Changes in activities of these enzymes may be related to reduced dry matter intake around parturition, may lead to hepatic lipidosis to alter the normal function of the liver (Greenfield et al., 2000).

On the unsatisfactory supply of ewes in early lactation with energy show, accept lower concentrations of glucose and hormones (T_3, leptin and insulin) in blood serum. The results of the present study showed that mean values of serum T_3 were significantly affected by the physiological status. Significantly lower ($P<0.01$) concentrations of T_3 were obtained in the blood of lactating ewes, compared to non-pregnant and pregnant ewes. T4 concentration were in opposite trend but differences had no significant ($P>0.05$). Similar results of T_3 and T_4 levels in ewes were determined by Karapehlivan et al. (2007). Lower blood T_3 concentration could reduce the rate of oxidation and the rate of continuous breakdown and formation of protein and fat in the most, if not all mammary tissue (Riis and Madsen, 1985). Todini et al. (2007) explain that during gravidity in all mammalian species increased thyroid activity and blood hormone levels.

The physiological status, in present study, had significant effect on the serum concentration of leptin and insulin. Significantly lower ($P<0.01$) concentrations of serum leptin and insulin were obtained in the blood of lactating ewes if compared to pregnant ewes. In animals, plasma leptin levels are closely correlated with nutritional status and body condition score (Chilliard et al., 2005). Serum leptin is sensitive to energy balance and is reduced

during periods of negative energy balance in ewes (Tokuda et al., 2002). Lactation in many species is associated with marked loss energy through the milk, which can not be fully compensated by food intake (Maèajova et al., 2004). During pregnancy, particularly in later pregnancy, plasma leptin levels increasing (Tamura et al., 1998), and decreased sharply after delivery. This is in accordance with some trials with lactating cows (Block et al., 2001) when plasma leptin concentrations were reduced by 50% after parturition. Leptin is synthesized in the placenta at comparable or greater levels than in adipose tissue and it may function as a growth factor for the fetus, signaling nutritional status from the mother to her offspring in human (Masuzaki et al., 1997). These findings strongly suggested that in the present study is expressed energy deficit in lactating ewe's meals (early lactation). Lower plasma leptin levels in lactating ewes (early lactation) compared with late pregnancy ewes determined by Erhardt et al. (2001) and McFadin et al. (2002).

The lowest blood insulin levels determined in lactating ewes ($P<0.01$) in comparison with pregnant ewes. In investigation Hatfield et al. (1999) determined greater ($P<0.01$) concentration of insulin in non-lactating than in lactating ewes. The insulin level in the blood of cows is low during the initiation period of lactation (Accrosi et al., 2005). This is accordance with investigation Weekes (1986) who conclude that level of insulin secretion is subject to homeorrhetic inhibition in catabolic states such as lactation.

Decline in the number of RBC in the blood of ewes in the early lactation are similar to those of Azab and Abdel-Maksoud (1999) in goats. Decline in the number of RBC and WBC in goats in early lactation (3 weeks) compared to pregnant goats found Iriadam (2007). The low number of WBC during pregnancy and the increase at parturition and early lactation is probably a response to uterine involution. The results agree with those in goats (Mbassa and Poulsen, 1991). A rise in RBC volume during later pregnancy causing increased volume of water during advanced pregnancy (Kataria et al., 2002). Increased hemoglobin content in later pregnancy ewes are probably due to higher demand for oxygen and the requirements of higher metabolic rate for pregnancy. Increase in hemoglobin content during pregnancy confirms the results by El-Sharif and Assad (2001). The fall of hemoglobin in the blood of goats in lactation were found Azab and Abdel-Maksoud (1999). The MCV, MCH and MCHC remain constant during investigation.

Conclusion

Effect of physiological status significantly manifested on the blood metabolic profile and concentrations of hormones (leptin, insulin and thyroid hormones). Changes in biochemical indicators and the concentration of leptin, insulin and T_3 indicate the energy deficit of ewes in early lactation. Therefore, it is recommended development of the blood metabolic profile of ewes and these hormones in assessing the nutritional status and ensuring good health states in very demanding physiological conditions - high pregnancy and lactation.

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