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EVOLUTION OF BLOOD METABOLIC PROFILE AND ANTIOXIDANT ENZYMES ACTIVITIES IN EWES DURING DIFFERENT PHYSIOLOGICAL STATUS

Alexandra PĂDURARIU, Stelian Vasile DĂRĂBAN, Vioara MIREȘAN

University of Agricultural Science and Veterinary Medicine of Cluj-Napoca, Faculty of Animal Science and Biotechnology, 3-5 Manastur Street, 400372, Cluj-Napoca, Romania

Corresponding author email: vioara.miresan@usamvcluj.ro

Abstract

Blood metabolic profiles are widely used to monitor health, reproductive and nutritional status. In the last few years, the evaluation of oxidative stress level of the organism has contributed increasingly as a complementary tool in the evaluation of metabolic status. The aim of this paper was to investigate the possible influence of age and physiological status on blood metabolic profile: Hb, PCV, TP, Creatinine, Glucose, TL, Cholesterol, Triglycerides, ALT and AST in ewes. We also measured two antioxidant enzymes, GPx and SOD to evaluated the oxidative stress level and establish the relationship between those and variation of biochemical parameters. The results showed that all the blood parameters determined in serum, were within or close to normal range value for healthy ewes and were significantly ($P<0.05$) affected by the age and variations of physiological status. A strong correlation ($P<0.01$) was observed in lactation period between antioxidant enzymes and lipid profile. Taking the results together suggests that age and physiological status have to be taken into consideration for a correct interpretation of the serum chemistry values of sheep.

Key words*: age, blood metabolic profile, ewes, oxidative stress, physiological status.*

INTRODUCTION

Sheep keeping was, is and will continue to be part of Romanian agriculture because there is still a great tradition of and experience with sheep production with adapted local and multipurpose breeds (Ilișiu et al., 2013; Wojtas et al., 2014). Among Merino breeds exploited in Romania, Merino of Cluj represents about 0.23% of the total sheep population of the country and had mixed characteristics: fine wool-meat-milk. This breed presents the following morpho-productive traits and it is the one adapted to agro-pedo-climatic conditions of Transylvania, especially to hill zones with a more accentuated raining level, unsuitable for other breeds with fine wool (Dărăban et al., 2009). The availability of information on haematological and biochemical parameters is essentials to research conducted with an aim to increase yields in sheep production (Dönmez et al., 2016). Blood metabolic profile is a set of diagnostic procedures that are based on determining the various indicators in the blood of animals and is affected by the internal and external environment (Oramari et al., 2014; Sharma et al., 2015). For example, age, nutrition, sex, genetics, physiological status, housing, environmental factors, starvation and stress are known to affect haemato-biochemical parameters (Opara et al., 2010; Elzein et al., 2016). One of the important factors is physiological/reproductive status which affect on concentration of indicators in blood that are involved in the development of the blood metabolic profile (Antunovic et al., 2011; Alkudsi et al., 2015). In sheep, the peripartum and early lactation periods are especially critical and present considerable physiological challenges to homeostasis by imposing significant metabolic stressors that may contribute to the onset of diverse disorders (Castillo et al., 2005; Celi, 2010). Also, pregnancy is a period when oxidative stress can be expected due to a high energy demand and increased oxygen requirement (Mohammadi et al., 2016). In the last few years, the detection of free radical damage and protection against it has become increasingly important in clinical medicine as a complementary toll in the evaluation of the metabolic status (Castillo et al., 2005). There are numerous studies on the

effects of oxidative stress during different phases of the reproduction cycle on biochemical parameters in domestic animal species (Roubies et al., 2006). Although, sufficient literature is available on the haemato-biochemical profiles of sheep breeds like Merino (Wojtas et al., 2014; Alhidary et al., 2015; Chauhan et al., 2014) during different reproductive/physiological phases but literature on Merino of Cluj sheep is scarce.

The aim of this study was to investigate the possible influence of age and physiological status on the blood metabolic profile in ewes. We also were evaluated two antioxidant enzymes, Glutathione peroxidase (GPx) and Superoxide dismutase (SOD), with a view to establishing oxidative stress level in ewes during different physiological status.

The parameters were measured in different age groups, to evaluate the effect of gestation, parturition and lactation as a form of stress on Merino of Cluj ewes. Relationship between antioxidant status markers and other biochemical parameters were also investigated. Establishment of reference value was also maintained as an object of this study. For that reason only age and physiological status were evaluated as variables, maintaining all other parameters unchanged.

MATERIALS AND METHODS

This study was conducted during one year at the Research and Development Station for Sheep of the University of Agricultural Science and Veterinary Medicine from Cluj-Napoca (Figure 1).

Figure 1. Research and Development Station for Sheep USAMV Cluj-Napoca (46°46'30.5"N 23°32'43.6"E)

From the flock at the station, 60 estrus synchronized clinically healthy 2-6 years old Merino of Cluj ewes, with a body weight of 45.0 ± 2.30 kg (ranging from 39.0 to 47.0 kg) were used in the trial (Figure 2).

Figure 2. Merino of Cluj ewes

To determine the effect of age of the animals on the normal ranges of the serum biochemical parameters, the sheep were assigned to one of three groups. The first group (PP) was consisted of 20 primiparous ewes aged less than 2 years old, the second group (MP_1) of 20 multiparous ewes aged 2-4 years old and the third group (MP_2) of 20 multiparous ewes aged more than 4 years old. To determine the effect of physiological status of the animals on the blood metabolic profile blood samples were taken in every 3 months over one year and the experiment started at the beginning of autumn. The periods were as follows: early gestation (EG), Post-partum (*Pp*), mid of lactation (ML) and end of lactation (EL). To understand the possible variations on the serum chemistry, sheep raised under the uniform pasture conditions.

To facilitate the restraint and limit variations related to food intake and stress, the blood samples were taken by puncture of the jugular vein with a 21G needle in the early morning before feeding. 4 ml of blood was collected using sterilized needles and plastic syringe in vacutainer plastic blood collection tubes with Li-Heparin (Figure 3). Blood samples were stored at 0-4°C and transported to the university's biochemical analysis laboratory.

From the blood samples we evaluated 12 parameters: Haemoglobin (Hb), Packed Cell Volume (PCV), Total Protein (TP), Glucose, Creatinine, Total Lipids (TL), Cholesterol, Triglycerides, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), GPx and Superoxide dismutase (SOD).

Figure 3. Blood sampling

Hematological parameters, Hb and PCV, were analyzed on the same day as the outrage of the blood samples, and the biochemical parameters were analyzed in the following days. For this, it was necessary to divide the blood samples into two aliquots microcentrifuge tubes and kept frozen at -20°C till further analysis. One aliquot was used for estimation of Hb concentration and hematocrit (PCV) while the other was used for plasma separation. Plasma was separated from the blood by centrifugation at 1200 rpm at room temperature for 5 min. Plasma samples were used to estimate biochemical parameters.

The haematological parameters were determined as follows: Hb was determined by the colorimetric method and the hematocrit value was determined by the Janetzki capillary microhaematocrit method (Kádár, 2002). Haemoglobin is dosed at room temperature by an End-Point colorimetric reaction, and the extinction is read in the visible range at a wavelength equal to 546 nm. The intensity of the colour is directly proportional to the amount of haemoglobin in the sample. The PCV is measured after centrifugation by determining the fraction of total blood volume in a microhematocrit tube that is occupied by erythrocytes (Figure 4).

All biochemical parameters were detected using the commercial available kits. The analyses were carried out according to the manufacturer's instructions. Data were measured on UV/VIS spectrophotometer Screen-Master Touch (λ=340 - 620 nm), (Figure 5). Glucose, PT, cholesterol and triglyceride were dosed by an End-Point colorimetric reaction in the visible range (λ 450-620 nm). Creatinine was dosed by an enzymatic reaction using the Fixed-time method in the UV at 340 nm. The enzymes, ALT, AST and also antioxidant enzymes, GPx and SOD, were dosed at a wavelength of 340 nm in UV by the kinetic method involving the reading of the enzymatic reaction at 37ºC (Kádár, 2002).

Figure 4. The Janetzki capillary microhaematocrit method

Figure 5. Spectrophotometer Screen-Master Touch

All statistical analysis was performed using Graph Pad Prism 7 and Microsoft Office Excel. Mean values, standard errors, minimum and maximum value were calculated for each parameter. To compare the variables according to physiological status in each group was performed by using the one way ANOVA test. In order to explain the probable interactions between various the physiological status Tukey-honest significantly different (HSD test) was used for multiple comparisons of the variables. Statistical significance was established at $P<0.05$. To determine the significance of interactions between antioxidant

status and other biochemical parameters, each period, Pearson correlation was performed.

RESULTS AND DISCUSSIONS

Haematological diagnostic techniques have become an essential part of the minimum data base to monitor and evaluate health and nutritional status of animals. Blood composition is not static, but rapid changes may occur as a response to various physiological events triggered by stress (Polizopoulou, 2010; Badawi & AL-Hadithy, 2014). The influence of the physiological status and the age of the sheep on the haematological parameters in the blood were presented at Figures 6 and 7. The analyses performed demonstrated that, in the Merino of Cluj ewes included in the study, Hb level and PCV value were within the physiological ranges determined in general for sheep (Etim, 2015; Ghergariu et al., 1985) with one exception.

The Hb value for all three groups was lower than the references values during the gestation period (Figure 6). The same things was observed by other authors when they studied the influence of temperature, altitude and landform on some hetological parameters in sheep (Wojtas et al., 2014; Titaouine & Meziane, 2015).

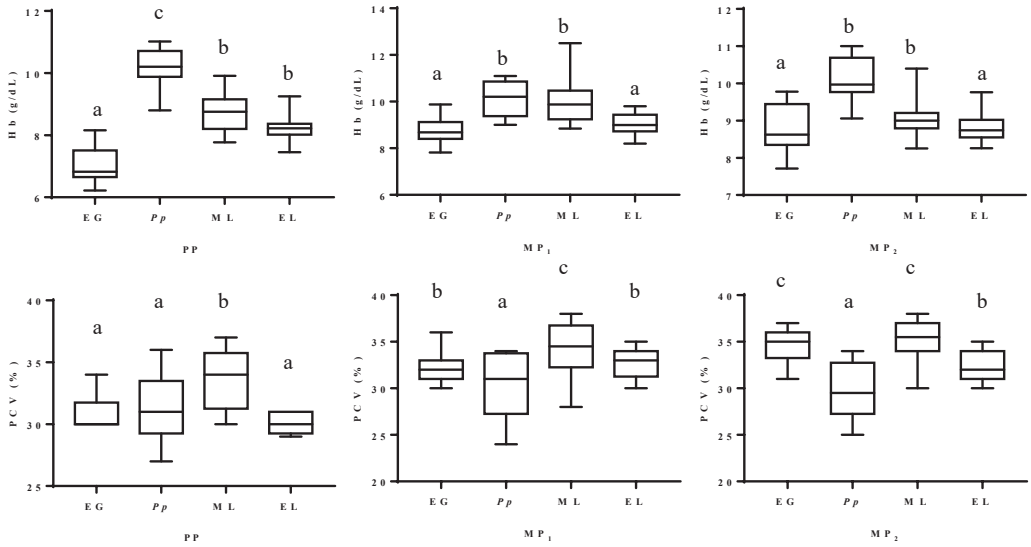

Figure 6. The influence of physiological status on hematological parameters in blood of the Merino of Cluj ewes during the study

Hb=Haemoglobin; PCV=Packed Cell Volume, PP=primiparous ewes aged <2 years old; MP_1=multiparous ewes aged >4 years old; MP_2=multiparous ewes aged 2-4 years old; EG=Early gestation; *Pp*=Post-partum; ML=Mid of lactation; EL=End of lactation.
All values are given as mean and min and max value (n = 20).
a, b, c Means with the same letter do not differ significantly at the 5% level (Tukey's HSD test).

The results also indicated that a significant age difference (P<0.05) for Hb and PCV (Figure 7). The values of Hb (7.02 ± 0.13 g/dl) and PCV (29.75 ± 0.67%) were significantly lower (P<0.05) in primiparous than multiparous sheep, in contrast other researchers who reported a significantly higher haematological values in young than adult sheep (Oramari et al., 2014). Effects of the seasonal variations or age on the hematological parameters were observed on the other ruminant, like buffalo (Enculescu at al., 2017), goats (Piccione et al., 2014; Guzmán & Callacná, 2013) and cattle (Yokus et al., 2006). The Hb values of Merino of Cluj sheep revealed significantly higher (P<0.05) during *Post-partum* period than other reproductive phases and the PCV values were significantly higher (P<0.05) in mid of lactation than other periods (Figure 6).

These results are in agreement with the earlier reports (Antunovic et al., 2011; Sharma et al., 2015). Significantly higher (P<0.05) Hb and PCV concentration in the pregnant ewes and *Post-partum* period are probably due to

increased demand for oxygen and the requirements of higher metabolic rate for pregnancy. According to other study, PCV is involved in the transport of oxygen and absorbed nutrients, and increased PCV shows a better transportation (Erisir et al., 2009). The Hb and PCV subsequently decreased during lactation, which might be attributed to the hemodilution effect resulting from an increase in plasma volume and/or increasing water mobilization to mammary gland through the vascular system (Sharma et al., 2015).

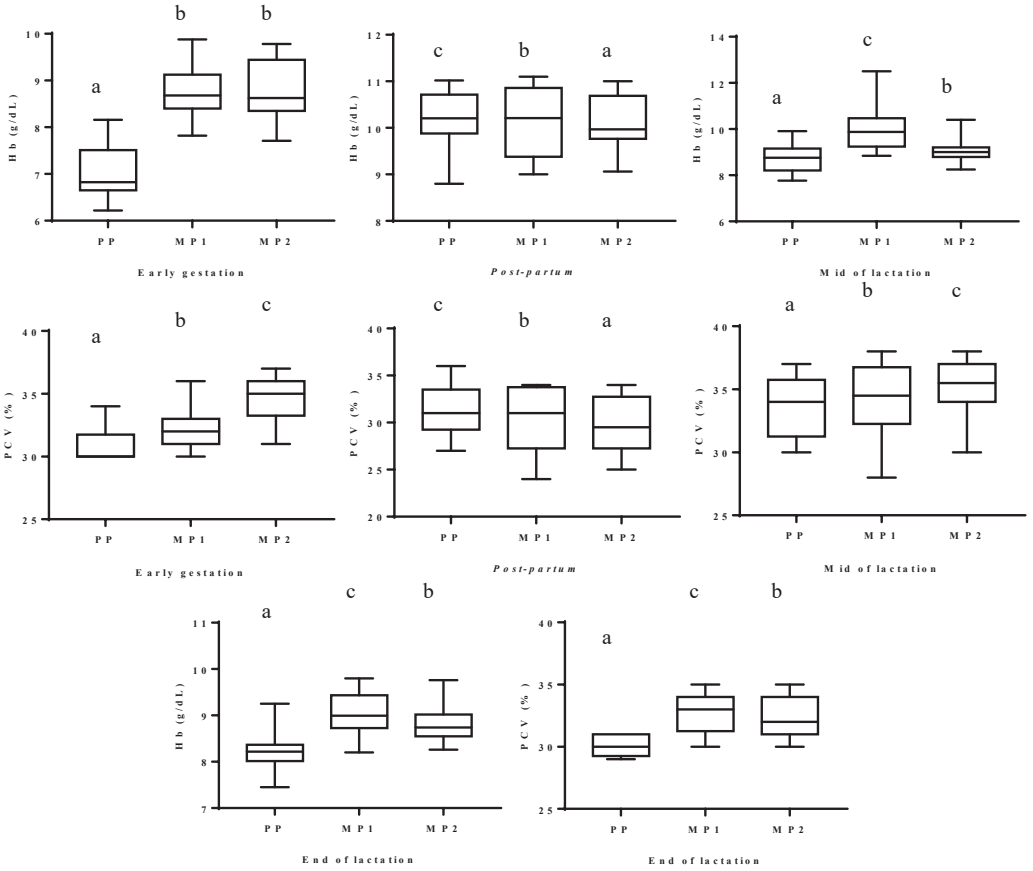

Figure 7. The influence of age on haematological parameters in blood of the Merino of Cluj ewes during the study
Hb=Haemoglobin; PCV=Packed Cell Volume; PP=primiparous ewes aged <2 years old; MP_1=multiparous ewes aged >4 years old; MP_2=multiparous ewes aged 2-4 years old;
All values are given as mean and min. and max. value (n = 20).
a, b, c Means with different letter differs significant (P<0.05) (Tukey's HSD test)

The blood metabolic profile of the Merino of Cluj sheep during different physiological status for each age group was summarized at Table 1. In the current study all the biochemical parameters were within or close to normal range values for healthy ewes (Ghergariu et al., 1985; Kádár, 2002) except TL, cholesterol and triglyceride, who were slightly higher. Hyperlipidemia is found in ketosis of ruminants or other liver disorders and hypercholesterolemia accompanied by increased triglyceride is observed under stressful conditions to which the animal is subjected (Safsaf et al., 2014). Animal reproduction is also negatively affected by the lack of lipids in the body, as it damages the vital cellular phenomena and negatively affects the cell permeability (Kádár, 2002). In our study this increase of total lipids, cholesterol and triglyceride was small and certainly not pathological and was due to the good

maintenance of ewes in the gestation period. Significantly higher (P<0.05) levels of cholesterol were recorded during gestation in present study which might be due to its role in ovarian steroidogenesis. Similar results were also reported by other researcher (Alkudsi et al., 2015; Safsaf et al., 2014; Piccione et al., 2009). The data of serum triglyceride are globally in accordance with total lipids and cholesterol level. Decline in the triglyceride in the blood of ewes in the *Post-partum* period are similar to those of other researcher in goats (Alkudsi et al., 2015; Safsaf et al., 2014; Piccione et al., 2009). Pregnancy and lactation are physiological states considered to modify metabolism in animals and induce stress (Mohammadi et al., 2016).

Major adaptations in maternal physiology and metabolism are required for successful pregnancy outcome (Gürgöze et al., 2009). The values of glycemia were significantly lower (P<0.05) in *Post-partum* (63.67 ± 0.37 mg/dl) than other periods four all three groups. Increased blood glucose concentrations in lactating ewes have to be considered as a result of good maintenance.

Table 1. Mean and standard error of blood metabolic profile of the Merino of Cluj ewes during different physiological status for each age group

Biochemical parameter	Group n=20	Physiological status			
		Early gestation	*Post-partum*	Mid of lactation	End of lactation
Total Protein (g/dL)	PP	$6.31 \pm 0.05^{a,A}$	$7.02 \pm 0.07^{b,A}$	$6.38 \pm 0.07^{a,A}$	$6.47 \pm 0.06^{a,A}$
	MP_1	$6.40 \pm 0.07^{a,B}$	$7.07 \pm 0.05^{b,A}$	$6.40 \pm 0.07^{b,A}$	$6.93 \pm 0.07^{a,C}$
	MP_2	$6.29 \pm 0.03^{a,A}$	$7.21 \pm 0.03^{c,B}$	$6.62 \pm 0.08^{b,B}$	$6.82 \pm 0.09^{b,B}$
References value		6.0 – 7.9 (Fielder, 2015); 6.0 – 6.5 (Ghergariu et al., 1985; Kádár, 2002)			
Creatinine (mg/dL)	PP	$0.73 \pm 0.02^{a,A}$	$0.87 \pm 0.01^{b,B}$	$0.89 \pm 0.01^{bc,A}$	$0.91 \pm 0.01^{c,C}$
	MP_1	$0.75 \pm 0.01^{a,B}$	$0.87 \pm 0.01^{b,B}$	$0.90 \pm 0.01^{b,B}$	$0.87 \pm 0.01^{b,A}$
	MP_2	$0.77 \pm 0.01^{a,C}$	$0.84 \pm 0.01^{b,A}$	$0.90 \pm 0.01^{c,B}$	$0.88 \pm 0.01^{c,B}$
References value		1.2 – 1.9 (Fielder, 2015; Ghergariu et al., 1985) 0.5 – 2.0 (Kádár, 2002)			
Glucose (mg/dL)	PP	$84.06 \pm 0.48^{b,B}$	$63.67 \pm 0.37^{a,A}$	$82.37 \pm 1.79^{b,C}$	$83.12 \pm 3.02^{b,A}$
	MP_1	$79.18 \pm 0.61^{b,A}$	$68.40 \pm 1.66^{a,C}$	$79.51 \pm 1.36^{b,B}$	$82.04 \pm 2.61^{b,A}$
	MP_2	$78.69 \pm 0.15^{b,A}$	$64.67 \pm 0.48^{a,B}$	$74.94 \pm 1.11^{b,A}$	$88.25 \pm 2.34^{c,B}$
References value		50.0 – 80.0 (Fielder, 2015, Kaneko et al., 2008]); 30.0 – 60.0 (Ghergariu et al., 1985, Kádár, 2002)			
Total Lipids (mg/dL)	PP	$497.1 \pm 0.47^{c,A}$	$182.7 \pm 1.46^{a,A}$	$382.7 \pm 5.48^{b,A}$	$342.4 \pm 10.5^{b,A}$
	MP_1	$499.3 \pm 0.52^{c,B}$	$209.0 \pm 5.27^{a,B}$	$384.1 \pm 8.43^{b,A}$	$366.5 \pm 7.20^{b,C}$
	MP_2	$499.2 \pm 0.35^{c,B}$	$203.5 \pm 4.09^{a,B}$	$406.2 \pm 8.64^{bc,B}$	$356.9 \pm 8.09^{b,B}$
References value		382.0 (Ghergariu et al., 1985, Kádár, 2002)			
Cholesterol (mg/dL)	PP	$196.1 \pm 0.35^{c,B}$	$62.45 \pm 1.57^{a,A}$	$92.21 \pm 2.02^{ab,A}$	$128.4 \pm 3.63^{b,C}$
	MP_1	$196.6 \pm 0.91^{c,B}$	$65.39 \pm 1.90^{a,C}$	$99.15 \pm 2.46^{ab,B}$	$126.5 \pm 3.22^{b,B}$
	MP_2	$195.7 \pm 0.37^{c,A}$	$63.19 \pm 0.56^{a,B}$	$99.27 \pm 1.55^{ab,B}$	$122.7 \pm 2.35^{b,A}$
References value		52.0 – 76.0 (Fielder, 2015, Kaneko et al., 2008); 64.0 – 108.0 (Ghergariu et al., 1985; Kádár, 2002)			
Triglyceride (mg/dL	PP	$241.5 \pm 0.70^{c,A}$	$34.69 \pm 1.37^{a,A}$	$125.1 \pm 6.79^{b,A}$	$146.2 \pm 9.33^{b,A}$
	MP_1	$245.1 \pm 0.18^{c,B}$	$36.21 \pm 1.60^{a,C}$	$124.3 \pm 6.92^{b,A}$	$147.2 \pm 6.71^{b,A}$
	MP_2	$245.3 \pm 0.13^{c,B}$	$35.45 \pm 0.50^{a,B}$	$127.9 \pm 5.93^{b,B}$	$150.8 \pm 4.57^{b,B}$
References value		NA			
ALT (U/L)	PP	$21.01 \pm 0.46^{c,A}$	$13.17 \pm 0.11^{a,B}$	$16.48 \pm 0.39^{b,A}$	$22.29 \pm 0.34^{c,B}$
	MP_1	$21.05 \pm 0.59^{c,A}$	$11.98 \pm 0.22^{a,A}$	$16.64 \pm 1.01^{b,A}$	$21.96 \pm 0.92^{c,A}$
	MP_2	$21.95 \pm 0.59^{b,B}$	$12.01 \pm 0.17^{a,A}$	$19.21 \pm 0.65^{b,B}$	$22.11 \pm 0.62^{b,B}$
References value		6.0 – 20.0 (Kaneko et al., 2008); 26.0 – 34.0 (Fielder, 2015); 20.0 – 40.0 (Kádár, 2002)			
AST (U/L)	PP	$95.35 \pm 0.86^{b,A}$	$70.95 \pm 0.75^{a,A}$	$75.57 \pm 3.26^{a,C}$	$101.6 \pm 3.65^{b,C}$
	MP_1	$99.73 \pm 3.16^{b,B}$	$73.67 \pm 0.96^{a,C}$	$69.28 \pm 2.06^{a,A}$	$97.72 \pm 2.69^{b,A}$
	MP_2	$95.09 \pm 1.55^{c,A}$	$72.30 \pm 0.85^{a,B}$	$82.09 \pm 3.32^{b,B}$	$98.34 \pm 1.94^{c,B}$
References value		60.0 – 280.0 (Fielder, 2015; Kaneko et al., 2008); 25.0 – 510.0 (Kádár, 2002)			

ALT=Alanine aminotransferase; AST=Aspartate aminotransferase; PP=primiparous ewes aged <2 years old; MP_1=multiparous ewes aged >4 years old; MP_2=multiparous ewes aged 2–4 years old;; NA=Not available.

All values are given as mean ± standard error. In this study, measured variables was compared with both according to the periods in same group (Tukey's HSD test) and between the groups.

^{a,b,c} Means with different superscripts within a row differs significant (P<0.05)

^{A,B,C} Means with different superscripts within a column differs significant (P<0.05)

Specifically, these changes suggest that the combination of increased utilization of glucose for milk lactose synthesis and the high intake of nutrients during investigation was sufficient to maintain blood glucose homeostasis (Antunovic et al., 2011). Current results are consistent with earlier report in lactating ewes (Mohammadi et al., 2016; Roubies et al., 2006) and lactating goats (Elzein et al., 2016).

A highly significant difference was noted between the groups (P<0.05). Total plasma proteins showed a decreasing trend from lactation to gestation period, which might be due to the preparation of reproductive system during pregnancy (growth of uterus) which requires large quantity of protein during pregnancy (Mohammadi et al., 2016; Mireșan et al., 2003). The highest value of total protein (7.21 ± 0.03 g/dl) was registered in the second group (MP_2) in *Post-partum* period, and the lowest value was observed in early gestation for all three groups. The decrease of protein concentration after parturition is due to using protein for colostrum production.

Similar results were also reported in sheep (Antunovic et al., 2011; Gürgöze et al., 2009) in goats (Opara et al., 2010) and cattle (Yokus et al., 2006). The values of TP were significantly lower (P<0.05) in primiparous then multiparous sheep. The physiological status, in present study, had significant effect (P<0.05) on the serum concentration of TP and creatinine.

Creatinine content of serum was statistically significant higher (P<0.05) in multiparous ewes in mid of lactation (0.90 ± 0.01 mg/dl) than primiparous in early gestation (0.73 ± 0.02 mg/dl). The quantity of creatinine formed each day depends on the total body content of creatinine, which turn depends on dietary intake, rate of synthesis of creatinine and muscle mass (Piccione et al., 2009) In other study the serum creatinine concentration decreased during lactation and increased during post-weaning (Gürgöze et al., 2009).

In these investigation activities of enzymes in the blood of the Merino of Cluj sheep were in physiological limits any gestation and lactation dependent changes. The effects of pregnancy in serum AST and ALT activity levels are somewhat controversial. In a few studies, an increase in AST and ALT activities have been found at *Post-partum* (Elzein et al., 2016; Antunovic et al., 2011) while some researchers (Opara et al., 2010; Wojtas et al., 2014) determined a decreased in that Gürgöze et al. (2009) observed an increase in AST and ALT levels during pregnancy. However, in some published studies, serum AST activity levels do not change during pregnancy and the *Post-partum* period (Yokus et al., 2006; Wojtas et al., 2014). The lowest value (11.98±0.22 U/L) were statistically significant (P<0.05) in *Post-partum* period in MP_1 and the highest significant (P<0.05) value (22.11±0.62 U/L) was noted in MP_2 in end of lactation. Similar results were also reported (Gürgöze et al., 2009; Elzein et al., 2016). Activity of ALT and AST can be recommended as a reliable liver status criterion. The increase in ALT activity and AST in the blood of ewes in lactation indicated an increase in hepatic metabolism. Current results in blood of lactating ewes are consistent with earlier reports (Antunovic et al., 2011).

The organisms are well-equipped with a network of substances capable of counteracting oxidative attack, enzymatic antioxidants, including SOD and GPx, and are the main form of intracellular antioxidant defense (Celi, 2011). Figures 8 and 9 shows the influence of physiological status and age on the oxidative status markers during the study.

SOD and GPx are high molecular weight enzymatic antioxidants that work together in preventing oxidative damage. SOD is responsible for dismutation of superoxide radicals into hydrogen peroxide, whereas GPx is responsible for the removal of hydrogen peroxide (Chauhan et al., 2015; Yuksel et al., 2015). Also, GPx has been used as an indicator of selenium status in animals (Celi, 2010; Chauhan et al., 2014).

It is well known that reactive oxygen metabolites are produced continuously by normal metabolic processes, but the rate of production may be increased markedly under diverse conditions of increased metabolic demand. The metabolic requirements imposed on colostrum production sheep and the onset of lactation far exceed the requirements of the fetus (Castillo et al., 2005).

These individual adaptations, especially after lambing, lead to inter-individual variation in metabolic activities, with variable tissue

consumption of O_2, and hence variable lipoperoxide production (Erisir et al., 2009). The marked individual variations observed in GPx and SOD around lambing may explain the lack of statistical significance. In fact, these individual variations probably reflect not only individual metabolic adaptations but also variations in the physical effort of lambing, which is likely to generate free radicals and thus lipid peroxidation, since increased demand for energy activities mitochondrial respiration in the skeletal musculature and increases oxygen uptake by muscles (Castillo et al., 2005; Erisir et al., 2009; Chauhan et al., 2015). In fact, we found GPx concentrations peaked after lambing, in parallel with SOD concentrations suggesting that the antioxidant system, can cope efficiently with lipoperoxide production, during this critical period and thus protect against oxidative stress, the cause of several reproductive diseases (Castillo et al., 2005). Moreover, the placental environment is one of enhanced oxidative stress that induces protective mechanisms against free radicals as gestation progresses. The plasma free radical trapping and antioxidant potential are able to counteract oxidative stress in normal pregnancy. GPx pregnant sheep protection systems adapted very early to maintain a stable balance (Erisir et al., 2009).

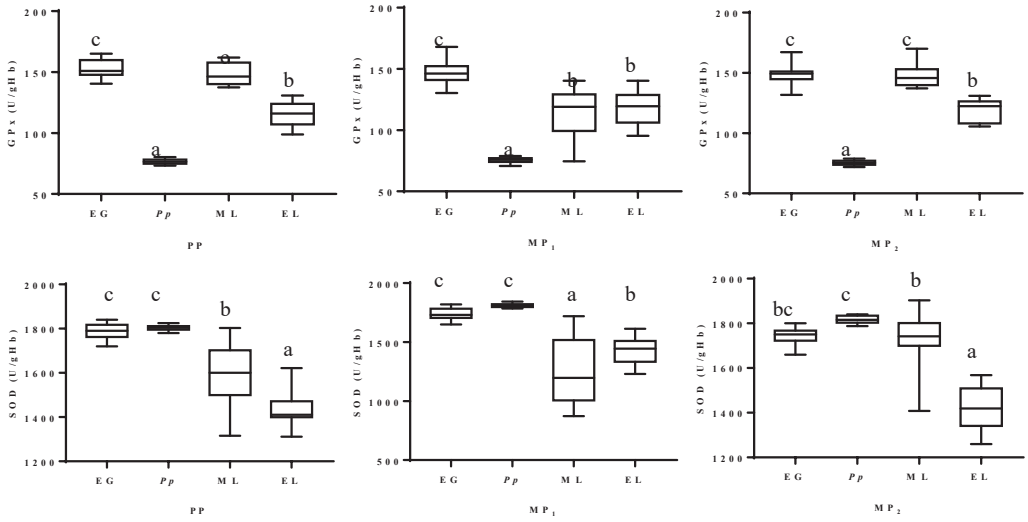

Figure 8. The influence of physiological status on antioxidant enzymes in the blood serum of the Merino of Cluj ewes during the study

GPx=Glutathione peroxidase; SOD=Superoxide dismutase; PP=primiparous ewes aged <2 years old; MP_1=multiparous ewes aged >4 years old; MP_2=multiparous ewes aged 2-4 years old; EG=Early gestation; *Pp*=Post-partum; ML=Mid of lactation; EL= End of lactation.
All values are given as mean and min and max value (n = 20).
a, b, c Means with different letter differs significant (P<0.05) (Tukey's HSD test

During milking period, we observed a gradual increase of oxidative processes, which is probably related to the larger amount of thyroid hormones during the first month of lactation sheep.

These hormones induce lipolysis and intensify the basal metabolism, increasing O_2 consumption, with subsequent excess of free radicals. The high values of GPx and SOD at the end of milking period testify the compensative response of the organism to oxidative stress (Piccione et al., 2006; Piccione et al., 2008). Mean GPx and SOD levels obtained in multiparous and primiparous ewes at all stages were higher than in previous reports of lactating ewes (Erisir et al., 2009) but were in agreement with other researchers (Piccione et al., 2008), who likewise observed a progressive decline in antioxidant activity as lactation progresses, probably due to the depletion of fat-soluble antioxidants by milk (Castillo et al., 2005).The value of GPx and SOD obtained in all three groups can be considered a reflection of the physiological

balance, especially during reproductive cycle. Considering that these metabolic changes are in agreement with previous studies (Sgorlon et al., 2006; Chauhan et al., 2014). However, our mean values of GPx and SOD cannot be compared with previous studies for several reasons: most earlier work was undertaken in sick animals, different management and environmental conditions (Sgorlon et al., 2006; Alhidary et al., 2015; Chauhan et al., 2014), or used different protocols during investigations and the different methods employed (Gaál et al., 2008), so that direct comparison are difficult and previous studies generally considered tissues other than plasma (Piccione et al., 2006; Lipko-Przybylska & Kankofer, 2012; Yuksel et al., 2015).

Our results must therefore be considered in the light of the available literature on the effects on antioxidant status of the homeostasis changes that appear in reproductive cycle of ewes, and the relationships between antioxidant status and other metabolic parameters during the study (Castillo et al., 2005; Erisir et al., 2009).

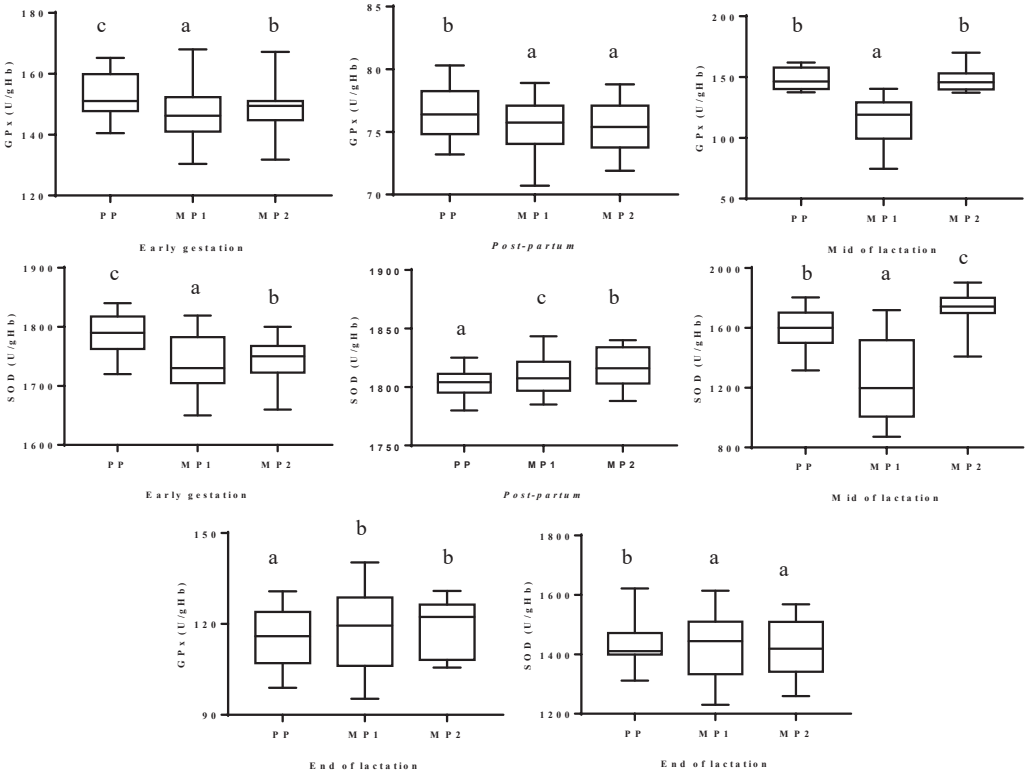

Figure 9. The influence of age on antioxidant enzymes in the blood serum of the Merino of Cluj ewes during the study
GPx=Glutathione peroxidase; SOD=Superoxide dismutase; MP_1=multiparous ewes aged >4 years old; MP_2=multiparous ewes aged 2-4 years old; PP=primiparous ewes aged <2 years old;
All values are given as mean and min and max value (n = 20).
a, b, c Means with different letter differs significant (P<0.05) (Tukey's HSD test)

Table 2 summarizes the correlations observed between GPx and SOD and metabolic parameters in primparous and multiparous ewes for each period. In the primiparous ewes SOD showed a positive correlation with creatinine (P<0.05), TL (P<0.01) and triglyceride (P<0.01) in lactation period. Multiparous ewes in end of lactation showed a negative correlation with Hb (P<0.05), TP (P<0.01), creatinine (P<0.01) and cholesterol (P<0.05). The possibility that metabolic activity may determine oxidant status is supported by various correlations detected depending on the physiological condition.

Table 2. Pearson correlation between anti-oxidant status indicators and other metabolic indicators in each group during different physiological status

		Physiological status							
		Early gestation		*Post-partum*		Mid lactation		End of lactation	
		SOD	GPx	SOD	GPx	SOD	GPx	SOD	GPx
Hb	PP	0.24	-0.16	0.37	0.06	-0.17	0.16	-0.38	**-0.52***
	MP_1	-0.15	-0.10	0.45	0.07	0.32	**0.48***	**-0.46***	0.00
	MP_2	-0.18	0.07	0.38	0.24	-0.28	-0.13	-0.02	0.26
PCV	PP	0.18	-0.13	0.12	**-0.49***	-0.33	0.10	-0.10	-0.32
	MP_1	-0.41	-0.17	0.13	-0.24	0.24	0.22	-0.44	-0.03
	MP_2	0.23	0.06	0.43	0.07	-0.36	**-0.53***	-0.14	0.19
Total Protein	PP	0.26	0.30	-0.17	-0.03	0.14	0.26	-0.03	0.22
	MP_1	0.43	0.11	0.07	-0.34	-0.35	-0.29	**-0.59****	0.02
	MP_2	-0.21	-0.14	-0.14	0.07	0.26	0.13	0.16	-0.14
Creatinine	PP	0.15	0.15	**0.54***	**0.46***	0.08	-0.19	0.00	-0.29
	MP_1	-0.16	-0.26	0.00	0.09	0.09	-0.17	**-0.63****	-0.12
	MP_2	-0.08	0.00	0.26	0.16	0.05	0.16	0.18	**-0.54***
Glucose	PP	-0.40	-0.34	0.35	0.11	0.43	0.00	0.12	-0.34
	MP_1	-0.22	**0.54***	-0.03	-0.03	-0.01	0.30	0.00	-0.44
	MP_2	0.44	-0.36	0.30	0.01	-0.05	-0.12	0.23	0.05
Total Lipids	PP	-0.25	-0.07	0.02	0.14	-0.29	-0.01	**0.64****	0.09
	MP_1	0.09	0.05	0.20	0.14	-0.16	0.01	**0.55***	**-0.63****
	MP_2	0.17	-0.14	-0.13	**0.46***	-0.17	-0.18	0.27	-0.09
Cholesterol	PP	0.21	-0.17	0.34	-0.20	-0.07	-0.23	-0.25	-0.25
	MP_1	0.09	0.32	**0.52***	0.14	**-0.50***	-0.43	0.32	-0.42
	MP_2	0.32	-0.14	-0.16	0.01	-0.25	-0.02	**-0.46***	0.00
Triglycerides	PP	0.21	-0.29	0.29	-0.29	-0.33	0.39	**0.59****	0.39
	MP_1	-0.22	-0.19	**0.55***	-0.14	**0.46***	0.36	0.35	-0.02
	MP_2	0.26	-0.18	-0.04	-0.32	-0.28	0.25	0.05	0.36
ALT	PP	0.30	0.24	0.28	0.17	**-0.59****	0.14	-0.09	-0.38
	MP_1	0.07	-0.09	0.00	**0.47***	0.03	**-0.46***	-0.22	**0.54***
	MP_2	**-0.47***	-0.39	0.19	0.00	0.32	-0.23	-0.21	0.04
AST	PP	0.38	0.00	-0.12	0.16	0.19	-0.21	**-0.47***	-0.22
	MP_1	0.21	-0.19	0.37	-0.41	0.01	-0.11	0.03	-0.08
	MP_2	0.21	-0.35	-0.42	-0.17	**0.52***	-0.26	-0.09	0.23

ALT=Alanine aminotransferase; AST=Aspartate aminotransferase; GPx=Glutathione peroxidase; SOD=Superoxide dismutase; PP=primiparous ewes aged<2 years old; MP_1=multiparous ewes aged>4 years old; MP_2=multiparous ewes aged 2–4 years old;
*P<0.05; **P<0.01.

It is evident from above, that all correlation coefficients among different hematological and serum biochemical which were calculated are expected. Such results are in agreement with those reported earlier by Oramari et al. (2014).

A different pattern was observed in the lactation period, lipoperoxide production showed negative correlations with serum glucose and AST levels, while after lambing lipoperoxide concentrations showed strong positive correlations with lipid metabolism indicators. The relationship between blood lipid and triglyceride in the peripartum period has been well documented and the increase in the oxidative metabolism implies peroxidation of fatty acids leading to formation of lipid peroxides. This antioxidant role acquires great importance if we consider the negative correlation observed between GPx and cholesterol. In fact, cholesterol metabolism requires cytochrome P-450, which is an important source of reactive oxygen metabolites that consume antioxidants (Castillo et al., 2005).

CONCLUSIONS

In conclusions effect of physiological status and age were significantly manifested on the haematological parameters, biochemical indicators and antioxidant status in the blood serum of the Merino of Cluj ewes during the study. Increased metabolic activities due to lactation significantly affected certain biochemical parameters and correlation between the antioxidant enzymes and blood metabolic profile. Our data on GPx and SOD

concentrations suggest that several unknown factors, not only physiological status or age, determine oxidative stress risk around parturition, and these must be taken into account in pursuing research in this area. Taking the results together suggests that age and physiological status have to be taken in to consideration for a correct interpretation of the serum chemistry values of sheep. Therefore, it is recommended development of the blood metabolic profile of ewes and antioxidant enzymes in assessing the nutritional status and ensuring good health states in very demanding physiological conditions, pregnancy and lactation. Hence, the haematological and biochemical parameter values from this research can be used as normal reference to assess the health status of the Merino of Cluj ewes.

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