



EFFECT OF SEASONAL VARIATIONS ON ELECTROPHORETIC PATTERN OF SERUM PROTEINS AND SOME BIOCHEMICAL INDICES IN OSSIMI EWES AND THEIR NEONATAL LAMBS

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Summary

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This study aimed to observe the impact of seasonal variations on the behaviour of serum proteins electrophoretic patterns by the Hellabio Agarose Gels and the changes of some metabolic indices in Ossimi ewes and their lambs (15–30 days postpartum). Serum samples from 20 ewes and their lambs during each season (summer and winter) were used in this study. Temperature-humidity index (THI) in winter was 72 (comfort), but a severe stress was shown in July, when the THI exceeded 90. Blood albumin decreased by 7.31% and 9.92% in summer, but the contrary was shown for α -globulins in ewes and lambs: α 1-globulins increased by 52.94 % and 22.22 %, α 2-globulins by 27.85 % and 93.02 % and albumin/globulin (A/G) ratio by 13.11% and 20% in summer, compared with winter. The values of β -globulins and γ -globulins did not differ between seasons. Ewes had higher values for protein fractions than lambs, but the reverse was true for A/G ratio. Summer serum glucose increased by 16.16% and 13.42%, urea by 16.16% and 25.78% and creatinine by 21.49% and 26.63%, whereas summer calcium (Ca) decreased by 6.81% and 5.18% and inorganic phosphorus (P) by 12.68% and 13.89% in ewes and their lambs respectively compared with winter. Lambs had higher values of serum glucose, Ca and P than their mothers, however the opposite was true for urea and creatinine. The presented results indicate that hot environment in summer season causes changes in serum protein electrophoretic patterns and metabolic indices in Ossimi ewes and their newborn lambs, which indicates an adaptive performance of these animals to seasonal variations and heat stress.

Key words: ewes, neonatal lambs, proteins electrophoresis, season

INTRODUCTION

Animals exhibit seasonal cycles of physiological functions. These seasonal rhythms reflect the adaptive mechanism to cope with seasonal fluctuations in the environ-

ment (Mohapatra *et al.*, 2019). The physiological responses of the animals to environmental stress during the winter and summer, and their energy balance showed

that seasonal heat and cold stress have profound effects on serum biochemical parameters (Romo-Barron *et al.*, 2019; Rashamol, *et al.*, 2020). The physiologists usually define such stress as a biological cost of adaptation against the stressor (Marai *et al.*, 2007; Al-Dawood 2017; Collier *et al.*, 2019; van Wettère *et al.*, 2021), while from the point of view of pathologists, it is a deviation from normal and is considered an environmental disease (Constable *et al.*, 2017).

Proteins are the main and most abundant constituents of blood serum or plasma, having many essential physiological functions (Tóthová *et al.*, 2016). The electrophoretic pattern of serum proteins and its interpretation are related to differences observed among various animal species, as well as among different groups of animals (Nagy *et al.*, 2015). Because the total proteins comprise albumin and globulins, it is more important to know which protein fraction is altered (Tóthová *et al.*, 2016). Changes in serum proteins can be indicative of health problems and may provide important diagnostic information to clinicians in confirming the diagnosis of various abnormalities and diseases (Cerón *et al.*, 2010 ; Tóthová *et al.*, 2018). Several factors, including non-pathological and pathological conditions, may influence the concentrations of proteins in the serum, thus the entire profile of serum proteins (Tóthová *et al.*, 2016; 2018; Nagy *et al.*, 2020). However, studies dealing with changes in the serum protein pattern and in concentrations of protein fractions in ewes and their lambs during the neonatal period are still limited. Acclimatisation engages also behavioural, hormonal and metabolic changes that are part of either homeostasis or homeorhetic mechanisms used by the animals to survive in a new physiological

state (Constable *et al.*, 2017; Li *et al.*, 2018). The response to heat load and the heat induced change in homeorhetic modifiers alters post-absorptive energy, lipid and protein metabolism, impairs liver function, endangers the immune response and decreases reproductive performance (Collier *et al.*, 2019).

The access to quick ancillary tests to aid the diagnostic process has been traditionally limited. However, over the past decades, diagnostic technologies have improved substantially, producing several accurate tests to establish a better diagnosis, a quick and informed therapeutic plan and to determine the prognosis (Abuelo & Alves-Nores, 2016). Blood biochemical parameters reflect the health, the metabolic status of an animal and the function of organs which are widely used in clinical situations (Ribeiro *et al.*, 2018).

The complexity of global climate changes with the heating of the global environment raised , “heat stress” problem in Egypt (Marai *et al.*, 2007; Rabie, 2020). So, the aim of the present study was to throw some light on the effect of seasonal variations, especially the impact of summer vs winter stress on the electrophoretic pattern of serum proteins and some blood biochemical indices in ewes and their neonatal lambs during the first month postpartum.

MATERIALS AND METHODS

Study area and meteorological data

This study was conducted in the Agriculture Research Station, in the eastern part of Assiut governorate (27° 10' 51.46" N and 31° 11' 1.25" E), Egypt, in July 2020 representing the summer season and January 2021 representing the winter season. The meteorological data during the study period were obtained from the local

weather station. These data were used to calculate the temperature-humidity index (THI) according to the following formula: $THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$, where T = air temperature (°C) and RH = relative humidity (%) as per Igono *et al.* (1992).

Animals

The study was carried out on ewes and their newly born lambs of Ossimi breed. Forty multiparous ewes (3–4 years old, 40–45 kg) were selected at the first month postpartum. The herd consisted of 66 ewes, of them 37 lambed in winter and 23 in summer. The selection was based to include a maximum number of ewes with a close age, weight and parity. Further, the selection was done depending on the balance of lambing within a as narrow time period (15 days) as possible. The selected animals were allocated into two equal groups (20 each) according to the season. Each ewe had a single lamb, so the number of lambs per group was 20. Sheep were housed indoors with outdoor access, so they were exposed to seasonal changes in environmental conditions. All born lambs were housed with their mothers and allowed to suck freely on their dams during the neonatal period. Animals were apparently healthy, free from internal and external parasites according to the routine laboratory examinations and were in good physical condition. All the animals had free access to water and to good quality Barseem Hegazi (*Medicago sativa*) and alfalfa hay. They were supplemented with 200 g per animal per day concentrates (oats 23%, corn 36%, barley 38% and mineral and vitamin 3%).

Sampling

Blood samples were taken in the early morning before feeding from all selected

ewes and their lambs at both seasons by jugular vein puncture in 10 mL tubes without anticoagulant to obtain clear serum. The collected blood were left till formation of compact clot. Serum was separated by centrifugation at $3000 \times g$ and clear non-haemolytic sera were stored at $-20^{\circ}C$ until analysis.

Laboratory analyses

The electrophoretic pattern of serum proteins was analysed by zone electrophoresis on an agarose gel using the electrophoresis system with HellaBio diagnostic kits (HellaBio, Thermi, Greece) according to the procedure described by the manufacturer. After electrophoresis, the stained gels strip (Fig. 1) were scanned using densitometer optical scanning system (520–600 nm) and evaluated according to light transmission principles and conversion into an optical density curve. The computer software programme Phoresis 5.50 (Sebia Corporate, France) was used to visualise the bands as peaks (electrophoretogram). According to the obtained optical density, the area under each peak was evaluated and the relative concentrations (%) of individual zones were calculated as percent of the total serum proteins. Consequently, the absolute concentrations (of each band were derived from percentage and q total serum protein concentrations (Jeppsson *et al.*, 1979). The ratios of albumin to globulins (A/G) were also calculated.

Concentrations of total serum protein, glucose, urea, creatinine, calcium (Ca) and inorganic phosphorus (P) were measured colorimetrically by using Bio diagnostic kits according to the procedure outlined by the manufacture's instructions.

Statistical analysis

ANOVA was used to evaluate the influence of season and the differences between ewes and lambs using a software (SPSS, Chicago, IL, Version.10). Means were compared with comparison-wise standard error rate after significant F-test. Differences between summer and winter were estimated by means of Student's t-test. Significance level was set at $P < 0.05$.

RESULTS

Meteorological data

In the studied area, the average maximum ambient temperatures in July was by 145.9 % higher (17.85 vs. 43.89 °C) and the relative humidity was by 19.97%

lower (61.45 vs. 49.18 %) than that recorded in winter. By applying the THI scales, it was noticed that the winter period value was less than 72 indicating a non-stressor zone lying within the comfort and safe region, but a severe stress was shown in July, where the THI exceeded 90 and an emergent risk was evident.

Serum proteins electrophoretical patterns

Using Hellabio Agarose Gels for protein electrophoresis, serum proteins in ewes and their lambs were fractionated into 5 bands: albumin, α_1 -globulins, α_2 -globulins, β -globulins and γ -globulins (Fig. 1, 2).

Fig. 3 illustrates the mean total serum protein and protein fractions values in ewes and their lambs during summer and winter season. Albumin decreased by

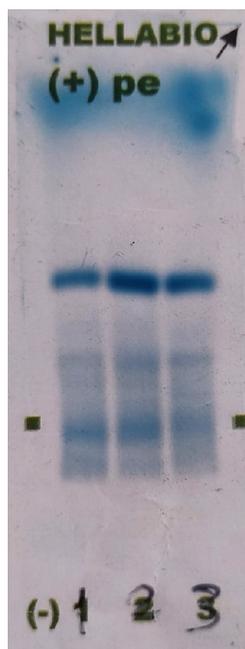


Fig. 1. Representative strip of Hellabio Agarose Gels.

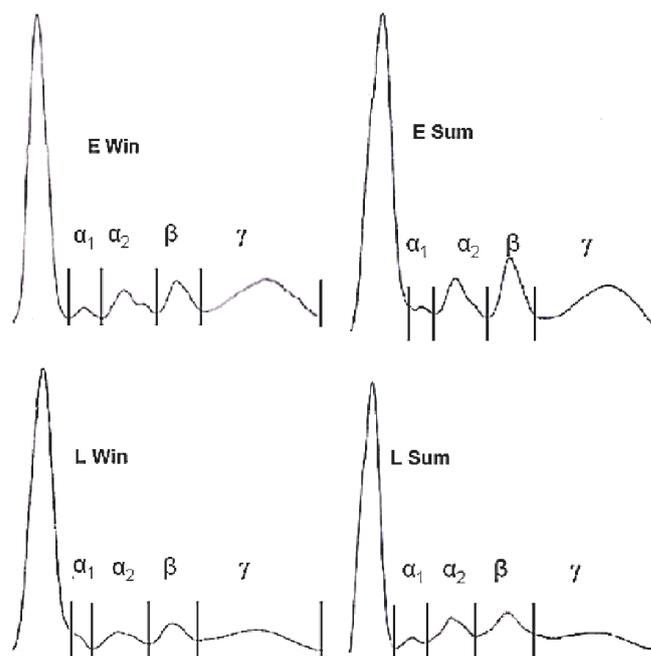


Fig. 2. Blood serum electrophoretic patterns using Hellabio Agarose Gels for protein electrophoresis during summer and winter season in ewes (E Sum, E Win) and their lambs (L Sum, L Win).

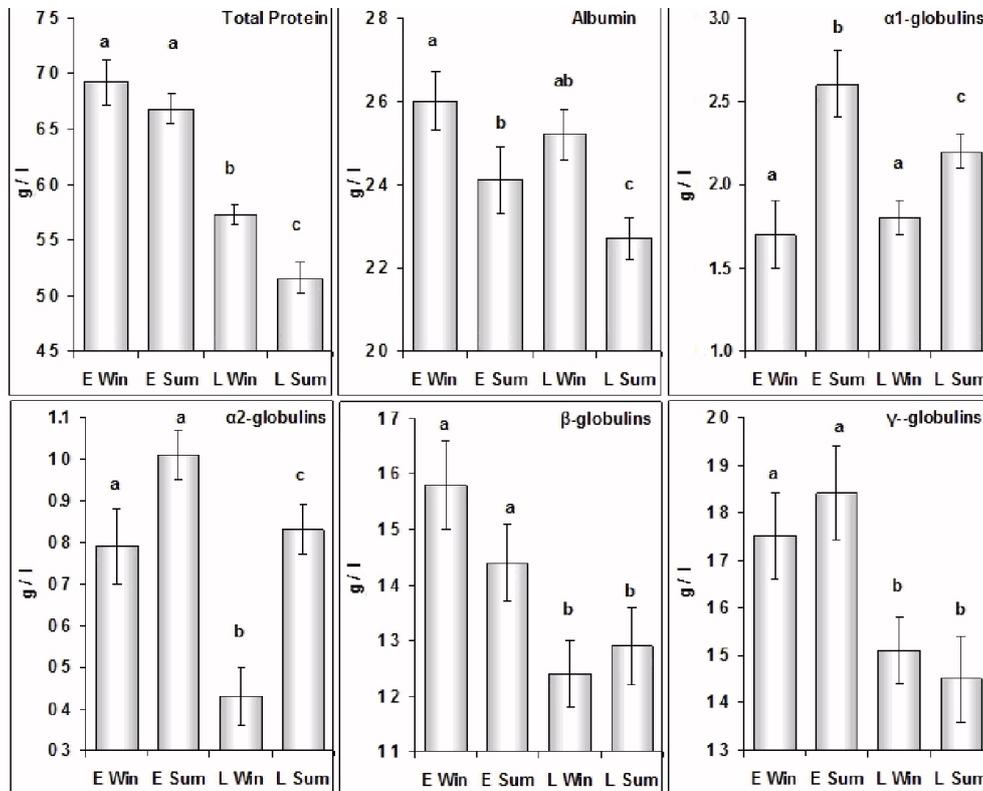


Fig. 3. Mean values (\pm SEM; n=20) of serum total protein and protein fractions (g/L) using Hellabio Agarose Gels for protein electrophoresis during summer and winter season in ewes (E Sum, E Win) and their lambs (L Sum, L Win). ^{a, b, c}: Values with different superscripts are significantly different at $P \leq 0.05$.

7.31% and 9.92% in summer compared with winter in ewes and lambs, respectively. Ewes had higher serum albumin than lambs during the summer. The contrary was shown for the values of α -globulins, where α 1-globulins increased in summer by 52.94% and 22.22% and α 2-globulins: by 27.85 % and 93.02% in ewes and lambs, respectively compared with winter. Ewes had higher α -globulins than lambs (Fig. 3). The values of β -globulins and γ -globulins did not differ between seasons, but ewes had higher values than their lambs. Total protein concentrations did not vary between

summer and winter, but was higher in ewes than in lambs. Consequently, the calculated A/G ratio was lower in summer and decreased by 13.11% and 20% in ewes and lambs, respectively. The lambs had higher A/G values compared to ewes.

Biochemical analysis data

Concentrations of serum glucose, urea, creatinine, calcium and inorganic phosphorus are shown in Fig. 4 and 5. Season had a significant effect on the estimated parameters. Summer serum glucose increased by 16.16% and 13.42%, urea by 16.16% and 25.78% and creatinine by

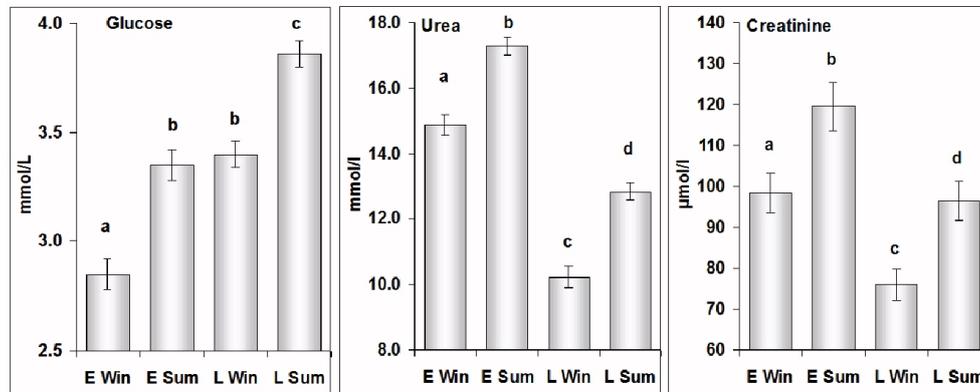


Fig. 4. Mean concentrations (\pm SEM; n=20) of serum glucose, urea and creatinine during summer and winter season in ewes (E Sum, E Win) and their lambs (L Sum, L Win). ^{a, b, c, d}. Values with different superscripts are significantly different at $P \leq 0.05$.

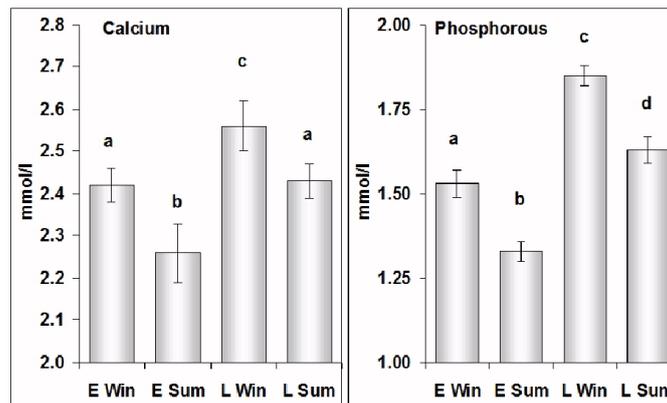


Fig. 5. Mean concentrations (\pm SEM; n=20) of serum calcium and inorganic phosphorus during summer and winter season in ewes (E Sum, E Win) and their lambs (L Sum, L Win). ^{a, b, c, d}. Values with different superscripts are significantly different at $P \leq 0.05$.

21.49% and 26.63% in ewes and their lambs respectively compared with the values recorded in winter season. On the other hand, summer concentrations of serum calcium decreased by 6.81% and 5.18% and those of inorganic phosphorus by 12.68% and 13.89% in ewes and their lambs compared with winter levels. The lambs had higher values of serum glucose, calcium and inorganic phosphorus than

their mothers, whereas the reverse was true for urea and creatinine (Fig. 4 and 5).

DISCUSSION

Livestock in open pasture are exposed to uncontrolled climatic changes. Daily temperature patterns are a net result of both heat loss to the environment and heat production by the animal (Romo-Barron *et al.*, 2019). Heat loss to the environment

can be severely restricted under conditions of high temperature and humidity (Marai, *et al.*, 2007; Rashamol *et al.*, 2020). THI is commonly used as an indicator of the degree of climatic stress on animals where a THI of 72 and below is considered as no heat stress (cool), 73–77 as mild heat stress (HS), 78–89 as moderate and above 90 as severe (Ravagnolo *et al.*, 2000). So the THI is a commonly used index for defining thermal comfort ranges in farm animals (Igono *et al.* 1992; Wojtas *et al.*, 2014).

In this study, the THI in winter was found to be 62.83. According to the THI scales cited by Igono *et al.* (1992), the winter group was reared in the thermoneutral environment. However, THI in summer was 96.16. According to the THI scales, the summer group was already reared in severe heat stress environment (risky zone). In Egypt, Hady *et al.* (2018) reported that THI values ranged from 79.74 to 90.4 during summer period. Further, Khalil & Omran (2018) found that July and August months recorded the highest THI values while January and December months recorded the lowest values of THI.

Agarose gels provide higher reproducibility of results and improved performance and clarity of the electrophoretic pattern (Fayos *et al.*, 2005). Using Hel-labio Agarose Gels for protein electrophoresis, 5 serum protein bands were identified in ewes and their lambs: albumin, α 1-globulins, α 2-globulins, β -globulins and γ -globulins. Similar results were obtained by Keay & Doxey (1984) and Piccione *et al.* (2011a,b). Variations in the serum protein profile and shifts in albumin and globulin concentrations may occur under pathological and physiological conditions (Constable *et al.*, 2017). Environmental temperature, relative humidity and conse-

quently the THI have a considerable influence on farm animals, causing changes in feed intake, metabolism and heat balance (Romo-Barron, *et al.*, 2019). Animal age and the physiological state are also important factors that may affect the concentrations of the different serum protein fractions or their electrophoretic pattern (Constable *et al.*, 2017).

The obtained values for serum proteins and their electrophoretic pattern in this study lie within the normal ranges (Eckersall, 2008). In the current study albumin decreased by 7.31% and 9.92% in summer compared with winter in ewes and lambs, respectively and ewes had higher summer serum albumin than lambs. Albumin is the most abundant protein found in blood plasma or serum, and essential part of the blood biochemistry profile. Serum albumin is the major negative acute-phase protein. The synthesis of positive acute-phase proteins is markedly increased during stressed conditions. These reactions require a great amount of amino acids. Thus, albumin synthesis is downregulated and amino acids are used mainly for the synthesis of the positive acute-phase proteins (Evans, 2002). Catabolism of albumin in muscle, liver, and kidney differs between animals and consequently the turnover of albumin reflects the body size (Aldred & Schreiber, 1993). In the severe stress zone, plasma albumin was decreased in Rahmani sheep with a consequent reduction in total protein levels. The significant reduction in sheep seems to be due to dilution of these proteins, decrease protein synthesis as a result of the depression of anabolic hormonal secretion and the increase in the catabolic hormones as glucocorticoids and catecholamins (Habeeb *et al.*, 1992; Marai *et al.*, 2007). Also, albumin might be filtered and redistributed into the extravascular spaces dur-

ing thermal stress due to its high osmotic sensitivity and relatively lower molecular mass and size than other protein fractions (Eckersall, 2008), resulting in reduction in the circulating portion. The re-absorption and recycling of circulating urea from the blood to the rumen in tropical ruminants utilised by the microflora for protein synthesis might be an additional factor (Igbokwe, 1997).

In contrast to albumin, α -globulins increased in summer in comparison to winter – $\alpha 1$ -globulins increased by 52.94% and 22.22% and $\alpha 2$ -globulins by 27.85% and 93.02% in ewes and lambs, respectively. Ewes had higher α -globulins than lambs. Piccione *et al.* (2011a) reported similar seasonal observations on α -globulins in sheep. The α -globulins fraction includes two important acute phase proteins (serum amyloid A and haptoglobin), which increase in animals exposed to HS, and may be useful sensitive markers in monitoring HS in small ruminants (Eckersall, 2008; Al-Dawood, 2017). In fact, the environmental conditions represent an important trigger for the shift in acute phase proteins (Iliev & Georgieva, 2018).

The values of β -globulins and γ -globulins did not differ between seasons in the current work. The most important proteins included in β -globulin fractions are the complement and the C-reactive protein, which like the acute phase proteins are involved in the environmental stress response (Iliev & Georgieva, 2018). The γ -globulin fraction is principally composed of immunoglobulins (Tóthová *et al.*, 2016). It seems that that these two fractions have a different liver production according to breed and environmental changes (Tóthová *et al.*, 2016; 2018). In the adult, the lymphoid tissues produce immunoglobulins as a response to anti-

genic stimulation, but in the neonatal period these are provided exclusively by colostrum so that the passive immunity can be acquired (Jackson & Elsawa, 2015). In sum, our results showed that the mean serum concentrations of total proteins, albumins and globulins were significantly affected by the age. The values were lower in lambs compared to their dams. Similarly, Roubies *et al.* (2006) and Antunović *et al.* (2012) found that the total proteins increased with age, something they attributed to an increase in the globulin fractions.

The obtained values in this study for biochemical indices lie within the normal ranges cited by Kaneko *et al.* (2008); Braun *et al.* (2010) and Carlos *et al.* (2015) for glucose, urea and creatinine. Season had a significant effect on the estimated parameters. Serum glucose increased by 16.16% and 13.42%, urea by 16.16% and 25.78% and creatinine by 21.49% and 26.63% in ewes and their lambs respectively in summer compared with the values recorded in winter.

Neuro-endocrine regulation is one of the principal adaptive responses shown by the animals in extreme stress conditions (Collier *et al.*, 2019). Activation of the hypothalamic-pituitary-adrenal axis and the consequent increase in plasma glucocorticoid concentrations are perhaps the most important responses of animals to stressful conditions (Habeeb *et al.*, 1992; Marai *et al.*, 2007). Furthermore, the thyrotropin-releasing hormone (TRH), thyroid stimulating hormone (TSH) and thyroid hormones (T3 and T4) are recognised to have a significant role in thermoregulation and metabolic response in livestock animals and play an important role in the animal's adaptation to environmental changes (Afsal *et al.*, 2018). The changes in the ambient temperature suppresses the

activity of thyroid hormone. So, Joy *et al.* (2016) identified these hormones to be the stress indicators for assessing the heat tolerance in the farm animals. Generally, lower activity of the thyroid glands helps to reduce the metabolic energy, with a consequent coping of animals to extreme environmental conditions (Collier *et al.*, 2019).

Similar to our results, glucose levels increase during HS in sheep (Al-Haidary *et al.*, 2012). Under severe heat stress the hyperglycaemic action of cortisol is most likely required to provide the expected increase in glucose utilisation. Under high temperature, energy demand of sheep should increase because of higher respiratory rate. The results of the present study showed higher serum glucose in lambs compared with their mothers. This could be attributed to a combination of increased absorption of glucose in the lambs and increased losses in lactating ewes, due to milk production, where glucose is necessary for the synthesis of lactose and the low essential nutrition (Pambu-Gollah *et al.*, 2000). There is also evidence that the intestinal capacity for sugar absorption in sheep declines with age (Vinardell, 1987).

During the summer season, the levels of blood urea nitrogen (BUN) and creatinine (Cr) were significantly increased. It may be due to reduced blood flow toward kidneys during heat stress condition. The increase in BUN due to heat stress may indicate that kidneys experience reduced blood flow during heat stress conditions; heat stress is known to cause peripheral vasodilation to expel body heat and reduce the blood flow to the internal organs (Srikandakumar *et al.*, 2003). Similar findings of increased BUN and Cr during summer season have been reported by (Indu *et al.*, 2014; Rathwa *et al.*, 2017). Fan *et al.* (2018) found that

metabolites, including urea, and creatinine were upregulated 1.24- to 1.70-fold ($P < 0.01$) in the heat-stressed ruminants as compared with the non-heat stressed group. In contrast with glucose, increased BUN and creatinine in dams than their born lambs were noticed. Our results are in accordance with previously reported data (Antunović *et al.*, 2012; Carlos *et al.*, 2015). The mean values of BUN were independent of age (Carlos *et al.* 2015), but dependent of reproductive stage as they were significantly higher in lactating ewes. The latter might be attributed to the higher dietary proteins intake during lactation than late pregnancy, due to increased requirements. Creatinine is formed by the degradation of phosphocreatine for energy release in the skeletal muscle. Serum creatinine is proportional to the muscle mass and the development of animals (Samra & Abcar, 2012).

On the other hand, the present work showed that concentrations of serum calcium decreased by 6.81% and 5.18% and inorganic phosphorus by 12.68% and 13.89% in ewes and their lambs respectively in summer compared with those obtained in winter. Rai *et al.* (1983) and Nazifi *et al.* (2003) reported that serum calcium and inorganic phosphorus showed a decreasing trend in experimental thermal stress of sheep. This might be due to the fact that animals kept at the lower temperature grew faster, consumed more feed and had a higher feed conversion ratio than those at the higher temperature (Constable *et al.*, 2017). Hence, they had higher serum calcium and phosphorus levels.

Lambs had higher values of serum calcium and inorganic phosphorus than their mothers. The age effect was evident – serum Ca concentrations of young lambs were significantly higher than those of the

adult ewes. Comparable results were obtained by Baiomy (2010). Moreover McDowell (1992) reported that young lambs with high Ca requirements absorbed Ca at higher rate and greater efficiency than mature sheep. With time, serum Ca concentration progressively decreased a few days after parturition. Serum P concentrations followed the same trend as for Ca. Roubies *et al.* (2006) and Antunović *et al.* (2012) reported similar results. A possible explanation of the present findings is that they result from the intense osteoblast activity that is normally observed in growing animals (Rosol & Capen, 1997). Furthermore, younger animals absorbed dietary Ca more efficiently and achieved much higher maximum rates of absorption for both Ca and P than the older ones (Braithwaite, 1975).

CONCLUSIONS

Hot environment during the summer season caused changes in serum protein electrophoretic patterns and metabolic indices in Ossimi ewes and their newborn lambs, indicating an adaptive performance of these animals to heat stress. Therefore, such stress should be avoided to the best possible extent to prevent changes in serum biochemical parameters which may cause disease and compromised production and reproduction efficacy.

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