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Reference intervals and physiological variations of the macro-mineral plasma concentrations in Ouled Djellal ewes

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Abstract. *This study aimed to establish values and reference intervals of macro-mineral plasma concentrations, as well as the variations of these variables depending on the physiological stages of Ouled Djellal ewes, reared in the Northeast of Algeria. Six plasma macro-mineral elements: calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), and chlorine (Cl), were analyzed in blood samples belonging to one hundred thirty-four (n=134) healthy Ouled Djellal ewes (2-5 years old). Obtained levels were statistically analyzed using Reference Value Adviser software in order to establish the reference intervals. In addition, a follow-up study using thirty (30) ewes was carried out to determine the variations of these same parameters depending on the animals' physiological stages. Animals were subjected to blood sampling during seven periods of their reproductive cycle (dry period; early gestation; mid-gestation; late gestation; early lactation; mid-lactation; after weaning). Reference intervals values for the studied macro elements were: Ca (66 -106.7 mgl/L), P (30.5-85 mg/L), Mg (17-32 mg/L), Na (132.6-165 mEq/L), K (3.8-6.2 mEq/L) and Cl (98-123.9 mEq/L). The follow-up study showed significant variations of these macro-elements according to the animal's reproductive stages. In late gestation, both calcium and magnesium levels increased significantly; however, they showed a significant decrease in early lactation. Phosphatemia was significantly higher in both dry and empty ewes. The lowest values of sodium and potassium were recorded at the beginning of lactation. Chlorine plasma levels decreased very significantly in mid-lactation. Such results provide baseline information that permits monitoring the impact of various mineral disturbances in this breed and checking the animal's health status depending on its physiological stage.*

Keywords: gestation, lactation, macro-minerals, Ouled Djellal ewes, plasma, reference value

Introduction

Eighty percent (80%) of all mineral elements in the animal's body constitute macro-minerals that play various anatomical and physiological roles: they form part of tissue and organ structures, they play essential roles in physicochemical balances, cellular exchanges, and energy transport. Furthermore, some of these macro elements are co-activators of many enzymatic and hormonal systems (Meschy, 2010).

In sheep, a healthy status and optimum performances depend on the availability of minerals in sufficient and appropriate quantity. However, due to various food interactions and metabolic processes, the sufficient concentrations of these elements in feed do not guarantee their total availability for the animal. For example, in pregnant sheep, even with diets containing enough Ca and Mg, an imbalance in other minerals can cause a deficiency in these two minerals (Hocking Edwards et al., 2018).

Minerals levels in blood plasma and other body fluids also depend on other factors such as intestinal physiology, metabolic use, homeostasis, and excretion. According to studies on ruminants, inadequate ingestion or availability of minerals resulted in reduced productivity (Khan et al.,

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2005; Khan et al., 2007). Also, in ruminants, the critical fetal development and parturition processes depended on satisfying calcium and magnesium requirements. Clinical deficiencies of these two elements (Ca and Mg) contributed to both ewe and lamb mortality (Master et al., 2017; Hocking Edwards et al., 2018).

In sheep, data on blood or body fluids minerals concentrations are usually used as a diagnostic tool for assessing various disorders (Khan et al., 2008); hence, establishing reference values and intervals for major minerals is essential. Throughout the years, data on the reference intervals for blood parameters in sheep had been determined by many authors (Desco et al., 1989; Ramos et al., 1994; Dubreuil et al., 2005; Dimauro et al., 2008; Kaneko et al., 2008). Nevertheless, these reference intervals have been developed as universal data for sheep as a species and not for a specific breed; these reference intervals were collected over a long and unknown interval of time; and have not been verified for local use in a particular breed under defined conditions (Al-Jbory and Al-Samarai, 2016; Shek-Vugrovečki et al., 2017). Determining reference values in animals living in different environmental conditions remains essential to interpret the animal's health status correctly.

Ouled Djellal breed is the most numerous indigenous sheep breed in Algeria. The study of its biochemical parameters had been approached by some authors (Deghnouche et al., 2011; Boudebza et al., 2014). However, data on the mineral blood parameters in this breed remained scarce, and no studies, to our knowledge, have been carried out on reference intervals. Thus, the main objective of this study was to propose values and reference intervals for plasma macro-minerals concentrations in Ouled Djellal ewes, using Reference Value Advisor V.2.1 software. The impact of these minerals on the animal's reproductive stages was also analyzed.

Material and methods

Ethical statement for the study

The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and were approved by the Ethics Committee of the Institute of Veterinary Sciences of Constantine - Algeria.

Husbandry and feeding

One hundred thirty-four ewes of Ouled Djellal breed - Hodna variety were used in this study. Animals belonged to a state-owned pilot farm located in the Northwest of Constantine province (Eastern Algeria). The study region was subject to the Mediterranean climate, characterized by irregular rainfall and prolonged summer drought. Mating season preparation (April) consisted of feed supplementation (flushing: 3-4 weeks before mating), distributed according to the body condition score of the ewes. The diet consisted of barley hay and concentrates (barley and wheat bran). Mating was usually done around the beginning of May. At the beginning of their gestation (May-June), ewes grazed exclusively on natural pastures during the day, while in the evening, they just received hay without any other supplementation.

Ewes in their mid-gestation (June-July) were fed with barley and field faba bean. During the end of gestation and the beginning of lactation periods (August-September), animals remained in stalls and fed concentrate consisting of a mixture of barley (85%) and faba bean (15%) in the morning, while in the evening, they were served barley hay and faba beans. In their dry period coinciding with the beginning of spring (March), animals received *ad libitum* hay, morning and evening, while they grazed the rest of the day. During all periods, water was distributed *ad libitum,* and "salt bricks" for licking were also provided.

Selection of animals

In order to establish the reference intervals (RI), two main criteria were used to include animals in our study: Ouled Djellal of the breed and the good health. Animals considered in "good health" state were those presenting a good general body condition score, with normal clinical examination the day of the sampling and no declared pathology in the previous seven days. Also, animals should not have been subjected to any medical treatment during the previous month.

The study included 134 healthy Ouled Djellal ewes, aged between 2 and 5 years at different physiological stages (gestation, lactation, and dry period).

In order to study the influence of the female reproductive status on macro-minerals plasma concentrations, thirty (30) ewes were subjected to blood sampling during seven periods of their production cycle, starting from the mating period and up to three weeks after weaning: (**Dry period 1**:1 week before mating; **Early gestation**: 4 weeks of gestation; **Mid gestation**: 10 weeks gestation; **Late gestation**: 1 week before lambing; **Early lactation**: 1 week of lactation; **Mid lactation**: 8 weeks of lactation; **Dry period 2**: 3 weeks after weaning).

Blood sampling

Samples were collected in the morning at 7.00 am before feed distribution. Blood was taken from the jugular vein into heparinized vacuum tubes Venoject®. Immediately after blood sampling, the plasma was separated by centrifugation at 3000 rpm/15min. The obtained plasma was divided into two aliquots and stored at -20°C until analyzed at the Biochemical Laboratory of Constantine University Hospital.

Biochemical analysis

Calcium, phosphorus, and magnesium were analyzed using the ADVIA®1800 Chemistry System (Siemens Healthcare, Frankfurt am Main, Germany), while potassium, sodium, and chlorine were analyzed using the Dimension RxL Max Integrated Chemistry System (Siemens Healthineers, Germany). Colorimetric methods were used for analysis of calcium (Henry et al., 1974), phosphorus (Fisk and Subbarow, 1925), magnesium (Burtis and Ashwood, 1994). Sodium, potassium, and chloride were determined electrochemically (Tietz, 1986).

Statistical analysis

According to the American Society of Veterinary Clinical Pathology (ASVCP) recommendations, data analysis was carried out related to reference interval guidelines for veterinary species (Friedrichs et al., 2012). The software Reference Value Advisor V.2.1 (Geffré et al., 2011) was used to determine descriptive statistics (mean, median, SD, minimum and maximum values), outlier analysis (Dixon–Reed and Tukey tests), and reference intervals (RI). Suspected values were conserved. Values classified as outliers were either eliminated from the analysis if they were aberrant observations (biologically implausible) or retained if they were credible. The distribution normality was assessed by examining the histogram and QQ-plots and confirmed with the Anderson-Darling test. Determinations of RI with 90% CI (Confidence Interval) of reference limits were generated using parametric and non-parametric methods. However, when fewer than 120 samples were available, and the distribution was not Gaussian, the bootstrap method was used to determine 90% CI. In order to determine the effect of the physiological stages on biochemical blood parameters one-way analysis of variance (ANOVA) was carried out using STATISTICA 10 software (2014 evaluation version). *P* values less than 0.05 were considered significant.

Results

Table 1 showed plasma macro-minerals reference intervals (RI) established from 134 sampled Ouled Djellal ewes. In our data, not all variables had the same sample size, which came as a result of eliminating damaged samplings, anomalous results provided by the laboratory, and the presence of outliers.

Table 1. Reference intervals of macro-mineral plasma concentrations for Ouled Djella ewes, descriptive statistics, number of outliers, and 90% confidence interval for lower and upper limits of reference

	Ca	P	Mg	Na	K	Cl
Unit	mg/L	mg/L	mg/L	mEq/L	mEq/L	mEq/L
N	130	133	131	100	102	101
Mean	85.3	57	22.9	152	5.0	113.2
SD	9.7	14.3	3.7	8.3	0.6	6.8
Median	85.7	54.6	22.2	154.0	5.0	115.0
Min	61	25.3	16.3	131	3.6	95
Max	111.2	96.5	33.8	167.0	6.6	126.0
RI* (LRL-URL)	66 -106.7	30.5-85	17-32	132.6-165	3.8-6.2	98-123.9
90% CI LRL*	61-69.5	25.3-35.5	16.3-17.9	131-136.1	3,7-4	95-100.1
90% CI URL*	103.1-111.2	80.8-96.5	29.9-33.8	163-167.0	6-6.3	122.5-126.0
Outliers	4	1	3	2	0	1
Method	NP	NP	NP	NP*	P	NP*

N: number of animals in the sample. SD: standard deviation. Min: minimum value. Max: maximum value. RI: reference interval.LRL: lower reference limit. URL: upper reference limit. P: parametric. NP: non-parametric. NP*: The confidence intervals of the limits of the non-parametric reference interval were determined using a bootstrap method.

Table 2 showed the obtained reference intervals of Ouled Djellal ewes and those from relevant literature. In general, RI obtained for Ouled Djellal ewes was more comprehensive than those reported in the relevant literature. Also, for all plasma macro-mineral parameters analyzed in this study, results revealed that the low limits reference intervals were below the lower limits of the general reference values.

Like the case with other authors, the reference intervals for plasma calcium concentrations obtained for Ouled Djellal ewes (Table 2) were lower than the reference intervals noticed by Kaneko et al. (2008).

Table 2. Reference intervals of macro-mineral plasma concentrations in Ouled Djellal ewes and those from relevant literature

Variable	Ouled Djellal ewes (n =134)	Baumgartner et Pernthaner (1994) (n=70)	Dubreuil et al. (2005) (n =83)	Kaneko et al.* (2008)
Ca mg/L	66 -106.7	80-104	92-106	115-129
P mg/L	30.5-85	28-75	38-62	50-73
Mg mg/L	17-32	17-29	21-27	22-28
Na mEq/L	132.6-165	-	141.9-151.8	139-152
K mEq/L	3.8-6.2	-	4.95-7.25	3.90-5.4
Cl mEq/L	98-123.9	-	108.3-117.1	95-103

*: Textbook literature

Table 3 showed the mineral plasma concentrations obtained for the different categories of ewes. Table 4 shows the variations of these concentrations during the different physiological stages of the animals.

All the macro-minerals plasma concentration showed significant variations. Calcemia and magnesemia were significantly higher in pregnant sheep compared to lactating and dry ones, whereas plasma phosphorus was significantly higher in dry sheep compared to the other categories. Plasma (Na) and (Cl) concentrations were significantly lower in lactating ewes. The plasma potassium level was significantly higher in dry and empty ewes.

Table 3. Mean (M ± SD) of macro-mineral plasma concentrations of pregnant, lactating and dry Ouled Djellal ewes

	Pregnant ewes	Lactating ewes	Dry ewes	*P*-value		
				a	b	c
Ca mg/L	91.3±13.3	76.1±12.1	83.5±12.1	0.00002	0.0002	0.02
P mg/L	54.5±13.7	49.3±12.9	61.2±15.2	NS	0.007	0.01
Mg mg/L	24.7±4.90	20.4±5.97	19.8±3.64	0.00002	0.00002	NS
Na mEq/L	147.6±6.60	136.8±13.06	146.4±7.12	0.00002	NS	0.00002
K mEq/L	4.36±0.54	4.19±0.63	4.81±0.59	NS	0.00008	0.00002
Cl mEq/L	113.5±6.02	102.8±13.80	108±5.73	0.00002	0.0006	0.01

a: (pregnant vs lactating). b: (pregnant vs dry). c: (lactating vs dry).

At the end of gestation, both calcium and magnesium showed a very significant increase (P<0.00003), followed by a very significant drop at early lactation. Mg levels increased significantly in the middle of lactation, then dropped significantly (*P*<0.00004) three weeks after weaning.

Plasma phosphorus did not show significant changes between the mating period through the middle of lactation. However, three weeks after weaning, its level increased significantly (*P*<0.03).

The lowest values of kalemia and natremia were recorded at early lactation. Despite its increasing trend, plasma chlorine levels did not show a significant variation during the different stages of gestation. However, it decreased very significantly during early and mid-lactation periods (*P*<0.0002).

Table 4. Macro-mineral plasma concentrations depending on physiological stages in Ouled Djellal ewes

	Ca (mg/L)	P (mg/L)	Mg (mg/L)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)
Dry period1	$83.9±12^{a}$	59.4±16.5	$21±2.37^{a}$	$147±9.49^{ac}$	$5.03±0.58^{a}$	$110.5±6.89^{ac}$
Early gestation	$89.7±11^{a}$	56.5±13.1	$22.8±3.54^{ab}$	$148±1.73^{a}$	$4.33±0,29^{b}$	$113±2.65^{a}$
Mid gestation	$82.5±11.91^{ac}$	54.3±15.9	$25.4±5.65^{b}$	$152.6±6.45^{a}$	$4.84±0.49^{ab}$	$113.9±5.55^{a}$
Late gestation	$100.9±10.05^{b}$	$52.8±12.2^{a}$	$28.7±3.14^{abc}$	$143.3±6.86^{ac}$	$4.02±0.48^{bc}$	$113.4±8.43^{a}$
Early lactation	$74.53±12.56^{c}$	51.18±13.02	$17.56±6.14^{c}$	$132.7±17.02^{b}$	$3.77±0.53^{bc}$	$103.5±15.9^{b}$
Mid lactation	$80.5±9.95^{ac}$	47.4±13.22	$23.3±4.20^{a}$	$141±4.66^{c}$	$4.61±0.41^{ab}$	$101.3±5.19^{bc}$
Dry period 2	$82.8±12.5^{ac}$	$64.4± 12.08^{b}$	$17.7±4.49^{c}$	$145.5±2.67^{ac}$	$4.56±0.51^{ab}$	$105.8±2.91^{bc}$

[a,b,c,d] means with different letters mean that they differ significantly *P*<0.05.

Discussion

Using plasma samples from 134 healthy Ouled Djellal ewes, the reference intervals of six macro-minerals plasma concentrations (Ca, P, Mg, Na, K, Cl) were established.

Reference intervals obtained in our study were different from those reported in relevant textbook literature.

Such textbook reference intervals were established in veterinary laboratories in Europe or the United States and concerned animals under good husbandry conditions in temperate climates, which could explain their differences with those established in developing countries. Differences could be attributed to genetic factors, climate factors (hot temperatures), nutritional factors (quality and quantity of feed), water availability, electrolyte losses in sweat, parasitism (Pritchard et al., 2009).

According to Dimauro et al. (2008), separating the animals according to their age, sex, parity, and lactation stage increased the precision of reference intervals. Indeed, many authors indicated significant differences in hematological and biochemical blood parameters according to factors such as the diet (Caldeira et al., 2007), the season (Šimpraga et al., 2013), and husbandry conditions (Pennisi et al., 2010). Significant differences were also noticed when it concerned the sex of the animals (Al-Jbory and Al-Samarai, 2016), their age (Roubies et al., 2006), and their reproductive status (Piccione et al., 2009; Antunović et al., 2011; Deghnouche et al., 2013).

In our study, the two observed significant fluctuations in calcemia concerned its increase at the end of gestation and its decrease at early lactation, while it remained relatively stable during the other stages. Higher calcemia levels in pregnant sheep were also reported by other studies (Antunović et al., 2004; Moghaddam and Hassanpour, 2008). The fall in plasma (Ca) levels at parturition could be considered as an alarm signal to the parathyroid gland to release more (PTH) hormone in order to meet the increased (Ca) requirements for the maintenance of lactation (Haenlein, 1991). It was worth mentioning that other authors did not note any significant influence of the physiological stage on calcemia (Roubies et al., 2006; Gürgöze et al., 2009; Deghnouche et al., 2013).

Phosphorus concentration values did not vary significantly during gestation; they were lower during lactation and higher during dry periods. According to Yokus et al. (2004), lactation decreased the serum levels of this mineral compared to gestation. In comparison, Alonso et al. (1997) observed elevated phosphatemia in Merino ewes at the beginning of gestation compared to those in lactation. The increased losses of this macro-element in milk phospholipids could explain this situation. In contrast, other authors (Antunović et al., 2004; Roubies et al., 2006; Deghnouche et al., 2013) reported higher values in lactating ewes compared to pregnant and dry ones. Moreover, Mohamed Elsir and Abdalla Mohamed (2010) showed a significant decrease in phosphatemia at the end of gestation and a significant increase during parturition.

Concerning plasma Mg levels, Underwood and Suttel (1999) set a deficiency limit at 0.2 mmol/L (5 mg/L) and a toxicity threshold at 1.6 mmol/L (39 mg/L). Serum levels of this mineral showed a significant difference between gestation and lactation in sheep (Kulcu and Yur, 2003; Dar et al., 2014) and goats (Kadzere et al., 1996). Our results agreed with those reported by Sansom et al. (1982), who pointed out a high serum Mg concentration in ewes at the end of gestation and a decrease at parturition and at three weeks of lactation.

Also, in agreement with our results, Roubies et al. (2006) reported higher Mg serum levels in lactating sheep than empty and dry ones.

Our results indicate a fall in magnesemia following the onset of lactation and the 3rd week after weaning. This last period coincides with the beginning of springtime and the growth of young grass, which is deficient in magnesium but also presents a high content in soluble potassium and nitrogen and low content in sodium and energy. Consumption of such grass decreases the amount of Mg provided by the diet and triggers the mobilization of lipid reserves due to its low energy content. Knowing that the enzymatic systems involved in lipolysis require magnesium as a cofactor, we witness an intense uptake of blood magnesium by adipocytes (Meschy, 2010). A situation complicated by the very limited magnesium reserves of the body (Suttle, 2010).

The combination of these factors could partly explain the low plasma magnesium concentration recorded during this period. Similarly, the decline in magnesemia at the onset of lactation could be linked to the stress of parturition and lactation and the frequently negative energy balance affecting animals during this period, forcing them to rely upon lipo-mobilization and therefore consumption of circulating magnesium.

Although sodium and chlorine perform their functions, they are often considered together because of their functional interrelations and their mode of supply (often provided as sodium chloride). In our study, plasma sodium levels increased in the first half of gestation but not in an effective manner. However, these levels showed a significant decrease in late gestation until mid-lactation. In their study on Tadmit ewes Boulfekhar and Brudieux (1980) reported that plasma (Na) variations during gestation were very limited, with an average level around 140 mmol/L and a significant increase recorded on the 45th day after the mating period.

Nevertheless, Mohamed Elsir and Abdalla Mohamed (2010) noted a significant decrease in serum (Na) levels during gestation. The authors attributed this decrease to changes in the renal regulation of water and electrolyte balances. Indeed, gestation alters renal excretion (Na) through the modification of renal perfusion and the glomerular filtration rate.

During the end of the gestation period, the low natremia observed in Ouled Djellal ewes could be explained by the increase in urinary losses of (Na) due to the increase in progesterone levels during this period (Boulfekhar and Brudieux, 1980; Benyounes et al., 2006; Riester and Reincke, 2015). Indeed, according to Laidlaw et al. (1962), progesterone has an aldosterone-like action in the renal tubules. The authors reported an increase in sodium excretion following progesterone administration.

The augmentation of fetal needs of this element and its accumulation in fetal tissues could explain the decrease of natremia during gestation.

Our results indicate a significant decrease in plasma (Na) and (Cl) concentrations during lactation. In their study, Mohamed Elsir and Abdalla Mohamed (2010) described the same tendency for (Na) during this period. This recorded decrease is probably linked to the loss of these elements in colostrum and milk. Indeed, in mammals, the aqueous colostrum phase contains high concentrations of these two major minerals (Na and Cl) (Mayer and Fiechter, 2012; AL-Hadithy et al., 2012).

In our study, potassium levels were significantly elevated in both dry and empty sheep. Results reported by other studies showed a gradual increase in potassium concentrations with the advance of gestation, with a peak recorded in the middle of this period, followed by a downward trend towards the end of it. The authors explained these changes by the antagonistic effect of progesterone and aldosterone. (Antunović et al., 2011; Mohamed Elsir and Abdalla Mohamed, 2010).

Yildiz et al. (2005) reported a high serum (K) level during gestation compared to other stages. In their study on Tadmit sheep, Boulfekhar and Brudieux (1980), reported that, as for sodium, the plasma concentrations of potassium increase significantly on the 45th day of gestation and remain constant until the 17th day before parturition. Afterwards they tend to decline significantly until the seventh day of the prepartum. The decrease in the plasma concentration of (K) during lactation could be explained by its export in the milk and the other electrolytes (Na and Cl).

Conclusion

Compared to reference interval values in other sheep breeds, considerable differences were recorded in Ouled Djellal ewes concerning the six studied macroelements (Ca, P, Mg, Na, K, Cl). Recorded differences could be attributed to many factors: genetics, climate, nutrition, water availability, electrolyte losses in sweat, parasitism. The plasma concentrations of these same macro-minerals presented variations depending on the reproduction stages of the animals. Both calcium and magnesium levels increased significantly in late gestation but showed a significant decrease in early lactation. Phosphorus levels were significantly higher in both dry and empty ewes. Sodium and potassium were at their lowest levels at the beginning of lactation, while chlorine plasma levels decreased significantly in mid-lactation. Our study is a contribution to establishing reference intervals in Ouled Djellal ewes. Further studies are necessary to enrich our findings by using larger animal herds and establishing appropriate references for each physiological stage of the animal. Such valuable data would allow a more accurate assessment of the animal's health status and improve the clinical diagnosis and the treatment of mineral imbalances in this breed.

Conflicts of interest

The authors have declared no conflict of interest.

References

Al-Hadithy HAH, Al-badawi NM and Mahmoud MM, 2012. Status of some serum electrolytes concentrations in Iraqi Awassi sheep. International journal of advanced biological research, 2, 540-544.

Al-Jbory WA and Al-Samarai FR, 2016. Some hematological reference values estimated by the reference values advisor in the Iraqi Awassi sheep. Comparative Clinical Pathology, 25, 1155-1162.

Alonso AJ, de Teresa R, García M, González JR and Vallejo M, 1997. The effects of age and reproductive status on serum and blood parameters in merino breed sheep. Zentralbl Veterinarmed, 44, 223-31.

Antunović Z, Novoselec J, Sauerwein H, Speranda M, Vegara M and Pavic V, 2011. Blood metabolic profile and some of hormones concentration in ewes during different physiological status. Bulgarian Journal of Agricultural Science, 17, 687-695.

Antunović Z, Speranda M and Steiner Z, 2004. The influence of age and the reproductive status to the blood indicators of the ewes. Archives Animal Breeding, 47, 265-273.

Baumgartner W and Pernthaner A, 1994. Influence of age, season, and pregnancy upon blood parameters in Austrian Karakul sheep. Small Ruminant Research, 13, 147-15.

Benyounes A, Iamrani F, Melo De Sousa N, Sulon J, Folch J, Beckers JF and Guellati MA, 2006. Suivi de la gravidité chez la brebis *Ouled Djellal* par dosage de la protéine associée à la gestation et de la progestérone. Revue d'élevage et de médecine vétérinaire des pays tropicaux, 56, 65-73.

Boudebza A, Bensegueni A, Abdeldjelil MC and Belatreche C, 2014. Some blood biochemical parameter changes in Ouled Djellal ewes during lactation and dry period. Annals of Biological Research, 5, 42-45.

Boulfekhar L and Brudieux R, 1980. Peripheral concentrations of progesterone, cortisol, aldosterone, sodium and potassium in the plasma of the Tadmit ewe during pregnancy and parturition. Journal of Endocrinology, 84, 25-33.

Burtis CA and Ashwood ER, 1994. Tietz text book of clinical chemistry, 2nd edition. Philadelphia, PA, WBSaunders: 1913. Carpenter JW, 2012. Exotic animal formulary, 4th edition, St. Louis, MO, Elsevier, p. 744.

Caldeira RM, Belo AT, Santos CC, Vazques MI and Portugal AV, 2007.The effect of long-term feed restriction and over-nutrition on body condition score, blood metabolites and hormonal profiles in ewes. Small Ruminant Research, 68, 242-255.

Dar AA, Jadhav RK, Dimri U, Khan AA, Khan HM and Sharma MC, 2014. Effects of physiological status and seasonal variation on plasma mineral profile of sheep in Kashmir valley. Academic Journal Scientific Research and Essays, 9, 69-76.

Deghnouche K, Tlidjane M, Meziane T and Touabti A, 2011. Influence du stade physiologique sur divers paramètres biochimiques sanguins chez la brebis *Ouled Djellal* des zones arides du Sud-Est algérien. Revue de Médecine Vétérinaire, 162, 3-7.

Deghnouche K, Tlidjane M, Meziane T and Touabti A, 2013. Influence of physiological stage and parity on energy, nitrogen and mineral metabolism parameters in the *Ouled Djellal* sheep in the Algerian Southeast arid area. African Journal of Agricultural Research, 8, 1920-1924.

Desco M, Cano MJ, Duarte J, Rodriguez F and Del Canizo JF, 1989. Blood biochemistry values of sheep (*Ovis Aries Ligeriensis*). Comparative Biochemistry and Physiology, 94 A., 717-719.

Dimauro C, Bonelli P, Nicolussi P, Rassu SP, Cappio-Borlino A and Pulina G, 2008. Estimating clinical chemistry reference values based on an existing data set of unselected animals. The Veterinary Journal, 178, 278-281.

Dubreuil P, Arsenault J and Belanger D, 2005. Biochemical reference ranges for groups of ewes of different ages. Veterinary Record, 14, 156, 636-638.

Fisk CH and Subbarow Y, 1925. The colorimetric determination of phosphorus. Journal of Biological Chemistry, 66, 375-400.

Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J, 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Veterinary Clinical Pathology, 41, 441-453.

Geffré A, Concordet D and Braun JP, 2011. Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Veterinary Clinical Pathology, 40, 107-112.

Gürgöze SY, Zonturlu AK, Ozyurtlu N and Icen H, 2009. Investigation of Some Biochemical Parameters and Mineral Substance During Pregnancy and Postpartum Period in Awassi Ewes. Kafkas Universitesi Veteriner Fakultesi Dergisi, 15, 957-963.

Haenlein GFW, 1991. Advances in the nutrition of macro and microelements in goats. In: Proceedings of the Seventh Annual Conference on Goats Production, pp. 290-320, Monterrey, Mexico.

Henry RJ, Cannon DC and Winkerman JW, 1974. Clinical chemistry, principles and technics, 2nd edition, Harper and Row, Hargestown, p. 646.

Hocking Edwards JE, Masters DG, Winslow E, Hancock S, Thompson AN, Refshauge G, McGrath SR, Robertson SM, Bhanugopan MS and Friend MA, 2018. Calcium and magnesium status of pregnant ewes grazing southern Australian pastures. Animal Production Science, 58, 1515-1521.

Kadzere CTL, Lewelyn CA and Chivandi EA, 1996. Plasma progesterone, calcium, magnesium and zinc concentrations from oestrus synchronization to weaning in indigenous goats in Zimbabwe. Small Ruminant Research, 24, 21-26.

Kaneko JJ, Harvey JW and Bruss ML, 2008. Fluid, Electrolyte, and Acid-Base Balance. In: Clinical Biochemistry of Domestic Animals, 17, 529-554, 6th ed. Elsevier/Academic Press, Amsterdam.

Khan ZI, Ashraf M, Ahmad I, Jaz Javed I and Valeem EE, 2008. A comparative study on mineral status of blood plasma of small ruminants and pastures in Punjab, Pakistan. Pakistan Journal of Botany, 40, 1143-1151.

Khan ZI, Hussain A, Ashraf M, Valeem EE and Javed I,

2005. Evaluation of variation of soil and forage minerals in pasture in a semiarid region of Pakistan. Pakistan Journal of Botany, 37, 921-931.

Khan ZI, Ashraf M and Hussain A, 2007. Evaluation of macro mineral contents of forages: Influence of pasture and seasonal variation. Asian Australasian Journal of Animal Sciences, 6, 908- 913.

Kulcu R and Yur F, 2003. A study of some serum mineral levels before and during lactation period of sheep and cattle. Biological Trace Element Research, 92, 275-279.

Laidlaw JC, Ruse JL and Gornall AG, 1962.The influence of estrogen and progesterone on aldosterone secretion. Journal of Clinical Endocrinology and Metabolism, 22, 161-171.

Masters D, Hancock S, Refshauge G, Robertson S, Bhanugopan M, Friend M and Thompson A, 2017. Mineral status of reproducing ewes grazing vegetative cereal crops. Animal Production Science, 58, 2049-2060. https://doi.org/10.1071/AN16530

Mayer HK and Fiechter G, 2012. Physical and chemical characteristics of sheep and goat in Austria. International Dairy Journal, 24, 57-63.

Meschy F, 2010. Nutrition minérale des ruminants domestiques. Editions Quae, p. 208.

Moghaddam G and Hassanpour A, 2008. Comparison of blood serum glucose, beta hydroxybutyric acid, blood urea nitrogen and calcium concentrations in pregnant and lambed ewes. Journal of Animal and Veterinary Advances, 7, 308-311.

Mohamed Elsir E and Abdalla Mohamed A, 2010. The mineral profile in Desert ewes (Ovis aries): effect of pregnancy, lactation and dietary supplementation. American Eurasian Journal of Agricultural & Environmental Sciences, 7, 18-30.

Pennisi P, Biondi L, Casella S and Piccione G, 2010. Effects of housing systems on physiological and energetic parameters in comisana ewes. Journal of Environmental Biology, 31, 857-860.

Piccione G, Caola G, Giannetto C, Grasso F, Calanni Runzo S, Zumbo A and Pennisi P, 2009. Selected biochemical serum parameters in ewes during pregnancy, post-parturition, lactation and dry period. Animal Science Papers and Reports, 27, 321-330.

Pritchard JC, Burn CC, Barr ARS and Whay HR, 2009. Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. Research in Veterinary Science, 87, 389-395.

Ramos JJ, Verde MT, Marca MC and Fernandez A, 1994. Clinical chemical values and variations in Rasa Aragonesa ewes and lambs. Small Ruminant Research, 13, 133-139.

Riester A and Reincke M, 2015. Progress in primary aldosteronism: mineralocorticoid receptor antagonists and management of primary aldosteronism in pregnancy. European Journal of Endocrinology, 1, 172 23-30.

Roubies N, Panousis N, Fytianou A, Katsoulos PD, GiadinisN and Karatzias H, 2006. Effects of Age and Reproductive Stage on Certain Serum Biochemical Parameters of Chios Sheep Under Greek Rearing Conditions. Journal of Veterinary Medicine, 53, 277-281.

Sansom BF, Bunch KJ and Dew SM, 1982. Change in plasma calcium, magnesium, phosphorous and hydroxyproline concentrations in ewes from twelve weeks before until three weeks after lambing. British Veterinary Journal, 138, 393-401.

Shek-Vugrovečki A, VOJTA A and Šimpraga M, 2017. Establishing reference intervals of haematological and serum biochemical variables in Likapramenka sheep. Veterinarski Arhiv, 87, 487-499.

Šimpraga M, Smuc T, Matanovic K, RadinL,Shek-Vugrovečki A, Ljubicic I and Vojta A, 2013. Reference intervals for organically raised sheep: Effects of breed, location and season on hematological and biochemical parameters. Small Ruminant Research, 12, 1-6.

Suttle NF, 2010. Mineral Nutrition of Livestock, 54-182, 4th Edition. CABI Publishing, Wallingford, UK.

Tietz NW, 1995. Text book of clinical chemistry (eds. C.A. Curtis, L.M . Silverman and R.H. Christensen), 3rd Edition, pp. 523-524.

Underwood MEJ and Suttle NF, 1999. The Mineral Nutrition of Livestock, 3rd edition. CABI Publishing, Wallingford, UK.

Yildiz A, Balikci E and Gurdogan F, 2005. Serum mineral levels at pregnancy and postpartum in single and twin pregnant sheep. Biological Trace Element Research, 107, 247-254.

Yokus B, Cakir DU, Kanay Z, GultenT and Uysal E, 2004. Effects of seasonal and physiological variations on the serum major and trace element levels in sheep. Biological Trace Element Research, 101, 241-255.