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Comparison of electrolyte and acid-base balances of Dorper breed ewes between single and twin pregnancies[1]

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ABSTRACT.- Santarosa B.P., Dantas G.N., Ferreira D.O.L., Carvalho M.G., Rodrigues M., Pereira P.F.V, Silva A.A. & Gonçalves R.C. 2019. **Comparison of electrolyte and acid-base balances of Dorper breed ewes between single and twin pregnancies.** *Pesquisa Veterinária Brasileira 39(10):789-795.* Departamento de Clínica Veterinária, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Campus de Botucatu, Distrito de Rubião Júnior, Botucatu, SP 18618-970, Brazil. E-mail: biancasantarosavet@gmail.com

During pregnancy there are modifications in the metabolic profile of sheep that may predispose to the occurrence of metabolic disorders, of which pregnancy toxaemia (PT) is highlighted. Blood gas analysis is detects changes in acid-base and electrolyte balance effectively. The objectives of this study were to study the acid-base and electrolyte balance of sheep during gestation and in the immediate peripartum (up to 48 hours postpartum), comparing single gestation with twins. Sixty healthy sheep of Dorper breed, two to five years old were raised in a semi-intensive system and were divided in two experimental groups: Group 1: 30 ewes, with ultrasonographic diagnosis of single fetus gestation; Group 2: 30 ewes, with ultrasonographic diagnosis of twin pregnancy. The experimental moments were defined as: MI-immediately after artificial insemination (control); MG30 - 30 days of gestation; MG90 - 90 days of gestation; MG120 - 120 days of gestation; MG130 - 130 days of gestation; MG140 - 140 days of gestation; MP - lambing; MPP1 - 24h postpartum; MPP2 - 48h postpartum. At all times 1mL of blood was collected per jugular vein puncture for blood gas evaluation in a portable equipament (I-Stat®). The pH, carbon dioxide pressure (PCO_2), bicarbonate (HCO_3^-), base excess (BE), sodium (Na^+), potassium (K^+), chloride (Cl^-), ionized calcium (Ca^{+2}), lactate, anion gap (AG) were mensured and strong ion difference (SID) were calculed. This work showed that there were changes in acid-base and electrolyte balance in pregnant ewes, due to the decrease in BE, HCO_3^-, TCO_2 and increase of lactate and AG during gestation, but the pH remained normal and did not present any difference among moments in both groups. Comparing the groups, single-gestation ewes presented higher alkaline expenditure at delivery than twin-gestation, evidenced by lower levels of BE and HCO_3^-. Lower Na^+ levels were observed in prepartum; drop in K^+ values with advancing gestation; hyperchloremia and hypocalcemia during gestation according to the reference standards for

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species; decreased iCa^{+2} levels in the final third of gestation in both groups. It was concluded that the ewes of this study were healthy until the last moment analyzed (48h postpartum), although have shown greater consumption of the alkaline reserve in the peripartum, being more explicit in the single gestation animals. In addition, this situation can be physiological and result in increased energy demand during gestation, more exacerbated by peripartum.

INDEX TERMS: Electrolyte, acid-base, Dorper breed, ewes, twin pregnancies, blood gas analysis, metabolic diseases, pregnancy, small ruminants, sheep, ovine.

RESUMO.- [**Comparação dos equilíbrios eletrolítico e ácido básico de ovelhas da raça Dorper entre gestação única e gemelar.**] Durante a gestação ocorrem modificações metabólicas nas ovelhas que podem predispor a ocorrência de transtornos metabólicos, dos quais se destaca a toxemia da prenhez (TP). A hemogasometria é um exame que detecta alterações nos equilíbrios ácido-básico e eletrolítico de forma eficaz. Os objetivos deste estudo foram estudar os equilíbrios ácido-básico e eletrolítico de ovelhas durante a gestação e no periparto imediato (até 48 horas pós-parto), comparando-se gestação única com gemelar. Foram utilizadas 60 ovelhas criadas em manejo semi-intensivo, hígidas, da raça Dorper, com dois a cinco anos de idade. Foram constituídos dois grupos experimentais: Grupo 1: 30 ovelhas, com diagnóstico ultrassonográfico de gestação de feto único; Grupo 2: 30 ovelhas, com diagnóstico ultrassonográfico de gestação gemelar. Os momentos experimentais foram definidos como: MI - imediatamente após a inseminação artificial (controle); MG30 - 30 dias de gestação; MG90 - 90 dias de gestação; MG120 - 120 dias de gestação; MG130 - 130 dias de gestação; MG140 - 140 dias de gestação; MP - dia do parto; MPP1 - 24h pós-parto; MPP2 - 48h pós-parto. Em todos os momentos foi colhido 1mL de sangue por punção da veia jugular para avaliação hemogasométrica em aparelho portátil (I-Stat®). Foram analisados os parâmetros: pH, pressão de dióxido de carbono (PCO_2), bicarbonato (HCO_3^-), excesso de bases (EB), sódio (Na^+), potássio (K^+), cloreto (Cl^-), cálcio ionizado (iCa^{+2}), lactato, ânion gap (AG) e diferença de íons fortes (SID). Este trabalho mostrou que houve mudanças nos equilíbrios ácido-básico e eletrolítico nas ovelhas prenhes, pela diminuição do EB, HCO_3^- e aumento do lactato e AG no decorrer da gestação, porém o pH se manteve dentro da normalidade e não apresentou diferença ao longo dos momentos em ambos os grupos. Comparando os grupos, as ovelhas de gestação única apresentaram maior consumo da reserva alcalina no momento do parto do que as de gestação gemelar, evidenciado menores níveis de EB e HCO_3^-. Foram observados níveis mais baixos de Na^+ no pré-parto; queda dos valores de K^+ com o avanço da gestação; hipercloremia e hipocalcemia durante a gestação segundo os padrões de referência para espécie e diminuição dos níveis de iCa^{+2} no terço final da gestação nas ovelhas de ambos os grupos. Concluiu-se que as ovelhas deste estudo apresentaram-se saudáveis até o último momento analisado (48h pós-parto), embora tenham mostrado maior consumo da reserva alcalina no periparto, sendo mais evidente nos animais de gestação única. Apesar disso, essa ocorrência pode ser considerada fisiológica e consequência do aumento da demanda energética durante a gestação, mais exacerbada no periparto.

TERMOS DE INDEXAÇÃO: Equilíbrio eletrolítico, ácido básico, ovelhas, raça Dorper, gestação única, gestação gemelar doenças metabólicas, gestação, hemogasometria, pequenos ruminantes, ovinocultura, ovinos.

INTRODUCTION

The gestational period involves several anatomical, hormonal, and metabolic changes due to the preparation for delivery and early lactation. These changes may cause metabolic disorders such as pregnancy toxemia (PT), which affects ewes and goats in the third and final period of pregnancy (Campos et al. 2010, Souto et al. 2013, Souza et al. 2016).

Santos et al. (2011) studied PT in ewes and found metabolic acidosis explained by ketonemia due to excessive production of ketone bodies (acetoacetate, acetone, and beta-hydroxybutyrate), which is a consequence of lipoxidation. As these compounds have acidic characteristics, they increase the anion gap (AG) and reduce the concentration of bicarbonate (HCO_3^-), chloride (Cl^-), sodium (Na^+) and potassium (K^+). In addition, sheep and goats may present hypocalcemia and hypomagnesemia (Souto et al. 2013).

Blood gas analysis is a laboratory test that analyzes blood gases and acid-base balance quickly and conveniently. Many acid-base balance disorders bring about few disorders in the body because they are transient or properly compensated by the animal. However, in some situations, correcting this metabolic profile is essential for the individual's survival. Thus, the blood gas analysis provides data to inform diagnosis, help treatment, and establish a prognosis of several ruminant diseases such as PT (Ortolani 2003). Base excess values, for instance, are used to calculate the volume of bicarbonate to be infused in an animal with metabolic acidosis (Cosenza et al. 2015). González et al. (2012) found a decrease in blood pH, HCO_3^-, and base excess (BE), in addition to an increase in the anion gap [$(Na^+ + K^+) - (HCO_3^- + Cl^-)$] in goats experimentally induced PT, indicating that such parameters may identify early cases of the disease.

The acid-base status of extracellular fluid is affected directly by the concentration of strong cations (Na^+ e K^+) and strong anions (Cl^-, S^{-2}, and lactate). Measuring electrolytes such as Na^+, K^+ e Cl^- to calculate strong ion difference ($SID = [Na^+ + K^+] - Cl^-$) provides information on acid-base equilibrium, highlighting the influence of electrolytes on it (Constable 1999, 2003). The increase in plasma SID can be produced by increasing Na^+ and K^+ and/or decreasing Cl^-. According to this approach, this would result in increased pH and HCO_3^- - variables considered dependent - thus creating an alkalizing effect. The reduction in plasma SID has the opposite result, generating an acidifying effect (De Morais & Constable 2006).

Although many studies discuss the clinical and laboratory findings of sheep with PT, there are no studies in the literature analyzing the metabolic profile of sheep from conception to parturition and postpartum to identify the physiological processes of pregnancy in the acid-base and electrolyte balance, and subsequently to recognize changes that help inform early diagnosis of the disease. The aim of this study was

to analyze acid-base and electrolyte balance in Dorper ewes during pregnancy, parturition, and immediately postpartum, comparing single and twin pregnancies.

MATERIALS AND METHODS

The methods used in this study were approved by the Animal Research Ethics Committee (CEUA) of "Faculdade de Medicina Veterinária e Zootecnia" (FMVZ) of "Universidade Estadual Paulista" (Unesp), Botucatu Campus (Protocol 189/2014).

Healthy, hybrid Dorper ewes aged two to five years old that participated in an estrus synchronization protocol were selected. The long-term protocol (12 days) was used, in which the 0.33g progesterone vaginal implant (Cidr®, Zoetis) was placed on day 0 and kept for 12 days (D0 to D12). It was removed after this period, when 400 to 500IU of equine chorionic gonadotropin (eCG) (Novormon®, Zoetis) were intramuscularly administered (Bicudo & Sousa 2002) to each animal. After 48 hours (D14), fixed-time artificial insemination (FTAI) with frozen semen was performed by laparoscopy, and the first moment, considered as control moment (MI), was collected. After 30 days (MG30), the diagnosis of single or twin pregnancy (Santos et al. 2004) was performed with a portable ultrasound device (My LabTM30 Vet Gold Esaote®, Esaote Healthcare Brazil, São Paulo/SP, Brazil) with a 5.0MHz linear transducer for transrectal examination upon defining the experimental group in which the ewes were included.

Two experimental groups were created: Group 1, which included 30 ewes with an ultrasound diagnosis of single fetal pregnancy, and Group 2, which included 30 ewes with an ultrasound diagnosis of twin pregnancy. Empty animals were excluded from the experimental groups. For the other evaluation moments, a transabdominal ultrasound was performed with a 3.5 MHz convex transducer (Santos et al. 2004) to assess fetal viability. All ewes received deworming after parturition (Monepantel, Zolvix®, Novartis Animal Health) and multipurpose vaccine against Clostridioses (Symxan-T Polyvalent®, Merial) at 30 and 60 days before parturition.

The ewes were released in the morning and grazed during the day. The pasture used was Vaquero grass (cultivar *Cynodon dactylon*). In the late afternoon, the animals were enclosed in $12m^2$ collective pens in a masonry shed with rice straw bed, where they were fed with 0.5 kg/animal of maintenance feed and 1.0kg/animal of corn silage. In the shed, commercial mineral salt for ewes was available ad libitum (Ovinofós com Monensina®, Tortuga Companhia Zootécnica Agrária, Mairinque/SP, Brazil) in covered masonry troughs. The water came from an artesian well located in the farm, available *ad libitum* from automatic troughs. The total diet of the ewes was composed of pasture, corn silage, and feed made on the property based on corn meal, soybean meal, urea, mineral salt, and commercial seeds.

Experimental moments were defined as MI = immediately after FTAI (control); MG30 = 30 days of gestation; MG90 = 90 days of gestation; MG120 = 120 days of gestation; MG130 = 130 days of gestation; MG140 = 140 days of gestation; MP = day of delivery; MPP1 = 24 hours after delivery; MPP2 = 48 hours after delivery. All of the blood samples were collected by jugular vein puncture in the morning at the nine moments having the animals from both experimental groups been fasting. Blood was collected (1mL) in a polyethylene syringe heparinized with sodium heparin (Hemofol®, Cristália Pharmaceuticals Ltda., São Paulo/SP) fitted with a 30x8mm needle (BD®, BD Medical, Curitiba/PR, Brazil) and subsequently sealed with rubber. Immediately after collection, a blood gas analysis was performed on a portable pH, electrolyte, and blood gas analyzer (I-Stat®, Abbott Laboratories, Illinois, USA). $CG4^+$ and $CHEM8^+$ cartridges were used (I-STAT®, Abbott Laboratories, Illinois, USA). The $CG4^+$ cartridge measured the values of pH, carbon dioxide pressure (PCO_2), oxygen pressure (PO_2), and lactate for calculation of the values of HCO_3^-, TCO_2, BE, and oxygen saturation (sO2). The $CHEM8^+$ cartridge measured sodium (Na^+), potassium (K^+), chloride (Cl^-), ionized calcium (iCa^{+2}), and calculated the anion gap (AG). The Strong Ion Difference values [$SID = (Na^+ + K^+) - Cl^-$] (Constable 1999) were also calculated.

The statistical analysis was performed using the SigmaStat 3.5 software. For all parameters of blood gas analysis, except BE, a One-Way Repeated Measures ANOVA was performed to compare the evaluation moments within each group individually. When significance ($P<0.05$) was found, the Tukey test was performed to compare the means ($P<0.05$). At each moment, Student's t-test was also performed to compare the study groups ($P<0.05$). As they do not present a normal distribution, the nonparametric analysis was used for the data concerning BE. The Friedman test was performed with repeated measures to compare the evaluation moments within each group individually. When significance ($P<0.05$) was found, the Dunn test was used to compare the medians ($P<0.05$). At each moment, the Mann-Whitney test was also performed to compare the groups under study ($P<0.05$).

RESULTS AND DISCUSSION

None of the ewes presented clinical symptoms suggesting PT or any other disease. Thus, no treatment was administered in the property where the samples were collected. Only one sheep with a simple gestation required a cesarean section due to dystocia of maternal origin caused by failure of cervical dilation. Fifty-one out of the 59 normal deliveries were assisted, where fetal dystocia was the most common cause. The mean and standard deviation of the gestational period of the 60 ewes was 144.75±2.12 days of gestation, ranging from 140 to 149 days. The average birth weight of the 90 lambs was 3.29±0.94kg, with 42 females and 48 males. Most ewes calved at dawn or dusk, with only five sheep calving in the pasture in the daytime.

The blood pH (Table 1) of the ewes showed no difference between the moments and groups, and all the means were within the reference values for the species. Therefore, the animals remained healthy throughout their pregnancy, although changes were detected during the pregnancies in other acid-base balance parameters. In contrast with this study, Khatun et al. (2011) found alkaline blood pH in ewes at five stages of pregnancy, in addition to describing a difference between the first (14 to 57 days) and the last periods (121 to 140 days) analyzed, although no explanation was found for the fact.

The mean PCO_2 values (Table 1) of the ewes in this study were within the normal range for the species in both groups and at all times. There was a difference between the groups in two moments, with the mean of G1 higher than G2 in MG30 and MG120. The highest values in both groups were found in MI, which may be explained by the greater agitation of the animals during administration of FTAI, presenting hypercapnia compared to the other moments, when the sheep had already gotten used to collection. The lowest PCO_2 levels were found in MP for G1 and MG120 for G2; however, no clinical significance was found.

A difference in the BE medians (Table 2) was only found between the groups in MP, when the twin pregnancy group

Table 1. Venous blood pH and concentration of PCO_2 (mmHg) in the ewes included the two experimental groups (G1 and G2) at different times (M) of collection

M	pH		P	PCO_2		P
	G1	G2		G1	G2	
MI	7.38 ± 0.08	7.40 ± 0.05	0.439	41.4 ± 5.3^{a}	42.2 ± 3.9^{a}	0.580
MG30	7.38 ± 0.03	7.39 ± 0.04	0.292	38.2 ± 2.9^{abcA}	36.4 ± 2.7^{bcB}	0.025
MG90	7.37 ± 0.07	7.40 ± 0.04	0.109	40.6 ± 7.0^{ab}	37.8 ± 4.0^{bc}	0.105
MG120	7.39 ± 0.06	7.40 ± 0.04	0.402	38.6 ± 5.4^{abcA}	34.8 ± 3.7^{cB}	0.007
MG130	7.37 ± 0.05	7.39 ± 0.03	0.285	37.1 ± 4.4^{bc}	35.7 ± 3.8^{bc}	0.251
MG140	7.39 ± 0.08	7.39 ± 0.04	0.831	37.3 ± 5.7^{bc}	36.5 ± 4.5^{bc}	0.566
MP	7.35 ± 0.08	7.38 ± 0.05	0.094	36.2 ± 4.6^{c}	37.1 ± 4.1^{bc}	0.441
MPP1	7.37 ± 0.07	7.38 ± 0.06	0.418	37.8 ± 5.4^{abc}	37.9 ± 5.3^{bc}	0.931
MPP2	7.38 ± 0.08	7.37 ± 0.07	0.610	37.8 ± 4.0^{abc}	38.5 ± 3.1^{b}	0.515
P	0.195	0.651		<0.001	<0.001	

Data are shown as mean ± standard deviation; ^{a,b,c} Means followed by the same lower case letter in the columns did not differ statistically by the Tukey test (P>0.05); ^{A,B} Means followed by different upper case letters in the rows differed statistically by the t-test (P<0.05); blood pH = 7.32 to 7.54 (Kaneko et al. 2008), 7.28 to 7.42 (Ortolani 2003), PCO_2 = 34 to 45mmol/L (Ortolani 2003).

Table 2. Venous blood concentration of BE (mmol/L) in the ewes included in the two experimental groups (G1 and G2) at different times (M) of collection

M	BE		P
	G1	G2	
MI	1.0 (-3.0 – 2.2)^{a}	1.0 (-2.0 – 3.0)^{a}	0.467
MG30	-2.0 (-3.0 – -1.0)^{ab}	-3.0 (-5.0 – 1.0)^{ab}	0.300
MG90	-2.0 (-2.0 – -4.0)^{abc}	-2.0 (-3.0 – 0.0)^{ab}	0.495
MG120	-1.0 (-1.0 – -5.0)^{ab}	-4.0 (-5.0 – -1.0)^{b}	0.174
MG130	-4.0 (-4.0 – -5.0)^{bc}	-4.0 (-5.0 – -2.0)^{b}	0.908
MG140	-3.0 (-3.0 – -5.0)^{abc}	-3.5 (-5.0 – 0.0)^{b}	0.834
MP	-5.0 (-5.0 – -7.0)^{cB}	-4.0 (-5.0 – -2.0)^{bA}	0.046
MPP1	-3.0 (-3.0 – -6.0)^{bc}	-2.5 (-6.0 – 0.0)^{ab}	0.480
MPP2	-2.0 (-2.0 – -5.0)^{abc}	-2.0 (-5.0 – 1.0)^{ab}	0.939
P	<0.001	<0.001	

Data are shown as medians, first and third percentiles; ^{a,b,c} Medians followed by the same lower case letter in the columns did not differ statistically by Dunn's test (P>0.05); ^{A,B} Medians followed by different upper case letters in the rows differed statistically by the Mann-Whitney test (P<0.05); BE = -4 to 2mmol/L (Ortolani 2003).

presented lower base excess compared to the simple gestation group. Only the MP median in G1 was below the normal range for the species, according to Ortolani (2003). In both groups, the highest values were observed in MI, when the ewes were still empty, and the lowest results were found in MP. This finding represented a consumption of alkaline reserve during delivery, when ketone bodies were probably produced and, therefore, with compensated metabolic acidosis due to the acid character of the circulating ketone bodies, given the lower food intake due to the increase in the volume of the pregnant uterus (Santos et al. 2011). However, moments MG120, MG130, and MG140 were equal to MP in G2. This may be justified by the fact that twin-gestated ewes are already subject to a higher energy demand from the last month of gestation, due to the greater development of two lambs, and thus already develop ketonemia and, consequently, present a base deficit (Santos et al. 2011).

The HCO_3^- means (Table 3) of G1 were higher than those of G2 in MG120; in MP, the opposite occurred, which also coincided with the BE values. In both groups and parameters, the highest means were observed in MI, whereas the lowest were observed in MP. Group 1 presented a greater variation between the moments, while in G2 only MI differed from the others. Although there were differences across moments, all means were within the normal range for the species, according to Ortolani (2003), which shows changes in metabolism during pregnancy that can be considered physiological.

The lactate means (Table 3) were different between the groups in the MI and MG130 moments, where the ewes in G1 presented higher values than those in G2. In MG30, i.e. the first moment of analysis of the gestational period, the lowest values were found in both groups. It was also the only moment with results within the normal range for the species. In MP, the mean lactate values were higher than in the other moments analyzed. As in other species such as human beings, horses, and cattle this elevation during parturition in ewes is physiological and due to the generalized stimulation of the sympathetic nervous system with adrenaline and cortisol release during and after birth, promoting the increase of lactate and globular volume, glucose, and free fatty acids (Comline & Silver 1972). Similarly to this study, Silva et al. (2013) reported higher lactate levels in Suffolk crossbred ewes at the time of eutocic parturition (3.0±0.5mmol/L).

The ewes of both groups presented hyperlactatemia during MI when experiencing stress during administration of FTAI and throughout their whole pregnancy and postpartum, except for MG30. Santos et al. (2011) described high lactate levels in natural cases of PT in ewes and attributed it to ketonemia leading to metabolic acidosis, in addition to associating the glycolytic process (anaerobic glucose), which produces lactate from glucose, contributing to hyperlactatemia. In the absence of clinical manifestation of PT in this study, high lactate levels were probably related to a high energy demand, which promoted the more exacerbated production of this component, besides physiological elevation during delivery.

The groups differed in MG140 with regard to the Na^+ dosage (Table 4), where G2 was greater than G1. All means were within the normal range for the species. Overall, the means were

Table 3. Venous blood concentrations of HCO_3^- and lactate (mmol/L) in the ewes included in the two experimental groups (G1 and G2) at different times (M) of collection

M	HCO_3^-			Lactate		
	G1	G2	P	G1	G2	P
MI	24.4 ± 3.5^{a}	25.6 ± 2.6^{a}	0.196	3.1 ± 2.9^{bcA}	1.7 ± 1.1^{cdB}	0.033
MG30	22.4 ± 1.8^{ab}	21.9 ± 2.2^{b}	0.348	1.0 ± 0.7^{d}	0.8 ± 0.5^{d}	0.208
MG90	22.7 ± 2.1^{ab}	22.7 ± 2.0^{b}	0.980	2.3 ± 1.9^{bcd}	1.8 ± 1.5^{bcd}	0.393
MG120	22.7 ± 2.3^{abA}	21.2 ± 2.7^{bB}	0.035	1.9 ± 1.7^{cd}	1.5 ± 1.0^{cd}	0.396
MG130	21.2 ± 2.0^{bc}	21.2 ± 2.5^{b}	0.940	2.1 ± 1.4^{bcdA}	1.4 ± 0.6^{cdB}	0.045
MG140	21.9 ± 2.9^{b}	21.8 ± 2.8^{b}	0.862	2.2 ± 2.8^{bcd}	1.4 ± 0.9^{cd}	0.185
MP	19.4 ± 3.2^{cB}	21.6 ± 3.0^{bA}	0.016	5.3 ± 4.2^{a}	3.3 ± 2.5^{a}	0.060
MPP1	21.2 ± 3.2^{bc}	22.1 ± 2.9^{b}	0.345	3.9 ± 2.8^{ab}	3.0 ± 2.0^{ab}	0.214
MPP2	22.2 ± 3.2^{b}	22.2 ± 3.7^{b}	0.980	2.4 ± 1.6^{bcd}	2.5 ± 1.7^{abc}	0.841
P	<0.001	<0.001		<0.001	<0.001	

Data are shown as mean ± standard deviation; ^{a,b,c,d} Means followed by the same lower case letter in the columns did not differ statistically by the Tukey test (P>0.05); ^{A,B} Means followed by different upper case letters in the row differed statistically by the t-test (P<0.05); HCO_3^- = 20 to 25mmol/L (Kaneko et al. 2008), 19 to 25 mmol/L (Ortolani 2003), Lactate = 1 to 1.33mmol/L (Kaneko et al. 2008).

Table 4. Venous blood concentrations of Na^+ and K^+ (mmol/L) in the ewes included in the two experimental groups (G1 and G2) at different times of collection

M	Na^+			K^+		
	G1	G2	P	G1	G2	P
MI	143.6 ± 2.8^{e}	144.7 ± 1.5^{d}	0.088	5.0 ± 1.9^{a}	4.5 ± 0.9^{a}	0.269
MG30	146.1 ± 2.0^{d}	146.1 ± 1.8^{d}	0.967	4.0 ± 0.4^{bc}	3.8 ± 0.3^{bc}	0.106
MG90	145.1 ± 1.8^{de}	145.3 ± 1.5^{d}	0.597	4.4 ± 1.0^{ab}	4.1 ± 0.3^{b}	0.175
MG120	147.7 ± 1.5^{c}	147.9 ± 1.5^{c}	0.667	3.9 ± 0.4^{bc}	3.8 ± 0.3^{bc}	0.281
MG130	150.5 ± 2.1^{a}	150.5 ± 2.3^{a}	0.918	4.0 ± 0.5^{bcA}	3.7 ± 0.3^{cB}	0.003
MG140	147.7 ± 1.1^{cB}	148.7 ± 2.0^{cA}	0.039	3.9 ± 0.4^{bcA}	3.7 ± 0.3^{cB}	0.012
MP	149.2 ± 2.2^{abc}	150.4 ± 2.6^{ab}	0.077	3.8 ± 0.3^{bcA}	3.6 ± 0.4^{cB}	0.035
MPP1	149.6 ± 1.7^{ab}	149.0 ± 2.1^{bc}	0.219	3.6 ± 0.4^{c}	3.8 ± 0.4b^{c}	0.106
MPP2	148.8 ± 1.7^{bc}	149.0 ± 1.3^{abc}	0.699	3.6 ± 0.3^{c}	3.7 ± 0.4^{c}	0.687
P	<0.001	<0.001		<0.001	<0.001	

Data are shown as mean ± standard deviation; ^{a,b,c,d,e} Means followed by the same lower case letter in the columns did not differ statistically by the Tukey test (P>0.05); ^{A,B} Means followed by different upper case letters in the rows differed statistically by the t-test (P<0.05); Na^+ = 139 to 152mmol/L, K^+ = 3.9 to 5.4mmol/L (Kaneko et al. 2008).

equal in both groups from MI to MG90, increased in MG130 (higher values), decreased in MG140, and presented higher values again in MP. As in this study, Azab & Abdel-Maksoud (1999) noted lower Na^+ levels in goats one week before calving. This fact may be justified by lower peripartum food intake and great loss of body fluids at delivery. Additionally, the aqueous fraction of colostrum is mainly composed of Na^+, K^+, and Cl^- ions, and its production occurs seven days before delivery (Azab & Abdel-Maksoud 1999).

The means of K^+ (Table 4) at the MG130, MG140, and MP moments were different between the groups, where G1 was greater than G2. The potassium means declined from the time the ewes were empty (MI) to the first moment of pregnancy analyzed (MG30) and continued to fall until the postpartum moments. The same situation was observed by Azab & Abdel-Maksoud (1999), who analyzed the electrolyte balance of goats four weeks before and after delivery. Decreased food intake due to increased uterine volume may lead to hypokalemia. Regarding the normality pattern for sheep, the ewes were hypokalemic from MP to MPP2 in G1, and in MG30 and from MG120 to MPP2 in G2.

There was a statistical difference between groups for the measurement of Cl^- (Table 5) only in MG120, in which G2 was greater than G1. All means of both groups showed that the ewes presented hyperchloremia, according to the normality standards proposed by Kaneko et al. (2008). The highest values were found in MG130, whereas the lowest values were found in MG90 for both groups. In concentrations close to the upper limit in this study, hyperchloremia may not necessarily be related to pregnancy, since the mean values of Cl^- in the empty ewes were also slightly above reference; therefore, it cannot be considered clinically significant. As in this study, Cosenza et al. (2013) described the mean value of 116.46±1.88mmol/L chloride in healthy, but non-pregnant or lactating ewes. The chloride levels did not mean a pathological process, but could be linked to the mineral salt intake of the ewes during pregnancy (Souza et al. 2016), which occurred *ad libitum* from the salt trough and was added to the feed. Souza et al. (2016) described reference values for Dorper ewe electrolytes in Bahia considering gender and age, showing that the lower limit of chloride values of animals aged 12 to 24 months (73,57-142,37mg/dL) was higher than

those of young animals from one to six months, due to the higher consumption of mineral salt in this age group, although without statistical difference.

With regard to iCa^{+2} (Table 5), no difference was found between groups at any of the moments analyzed. A similar behavior was found in both groups in the different moments, with the highest values observed in MG90, the lowest in MI, and similar values in the others. However, all means were below normal for the species, although the ewes showed no clinical signs of hypocalcemia. Overall, the ewes presented increased calcium levels from MI to MG30, as well as from MG30 to MG90, and decreased values from MG90 to MG120, which remained the same until MPP2. This decrease in calcium levels may be related to the higher demand of the last month of pregnancy due to fetal mineralization and the production of the maternal colon, which requires a considerable amount of circulating levels of this element (Souto et al. 2013).

The mean values of AG (Table 6) of the groups only differed in MG120, being greater in G1 compared to G2. Additionally, in both groups, the MI and MG30 moments were equal. The results increased from MG90 until the moment of delivery (MP), remaining the same until 48h postpartum. During this period from MG90 to MPP2 the AG mean of the pregnant ewes in both groups showed metabolic acidosis, however compensated, since the blood pH remained unchanged.

The SID values (Table 6) were lower at the first moment of pregnancy analysis (MG30), whereas the highest values were found 48h postpartum (MPP2) for the ewes in both groups. A priori, this fact can be explained by the adaptation of the maternal organism to the gestation observed at 30 days. In the MPP2 moment, the metabolic normalization in the acid-base imbalance observed in the peripartum was observed and confirmed, albeit physiologically, by the bicarbonate and base excess levels (Table 2). A slight decrease in pre-partum SID values from MG130 to MG140 was observed. This represents a decrease below the reference values for the species and the healthy ewe data described by other authors. Ferreira et al. (2014) studied the influence of ammonium chloride supplementation in confined lambs and described that animals in the control group (not supplemented) presented mean SID values between 41.78±1.56 and 44.46±1.69mEq/L. On the other hand, Conseza et al. (2015), in a study on acute ruminal

Table 5. Venous blood concentrations of Cl^- and iCa^{+2} (mmol/L) in the ewes included in the two experimental groups (G1 and G2) at different harvest times

M	Cl^-			iCa^{+2}		
	G1	G2	P	G1	G2	P
MI	109.2 ± 3.2^{cd}	108.8 ± 2.7^{cd}	0.612	0.72 ± 0.10^{c}	0.76 ± 0.11^{c}	0.202
MG30	111.4 ± 2.2^{abc}	111.7 ± 2.6^{ab}	0.652	0.91 ± 0.10^{b}	0.91 ± 0.10^{b}	0.928
MG90	107.3 ± 2.4^{d}	108.1 ± 2.5^{d}	0.268	1.04 ± 0.13^{a}	1.05 ± 0.16^{a}	0.869
MG120	110.5 ± 2.6^{bcB}	112.6 ± 3.0^{abA}	0.009	0.90 ± 0.13^{b}	0.86 ± 0.11^{bc}	0.291
MG130	113.0 ± 2.6^{a}	113.4 ± 2.4^{a}	0.529	0.91 ± 0.15^{b}	0.85 ± 0.14^{bc}	0.117
MG140	111.2 ± 2.7^{abc}	112.8 ± 3.4^{b}	0.067	0.87 ± 0.09^{b}	0.88 ± 0.18^{b}	0.849
MP	112.5 ± 2.9^{ab}	112.3 ± 3.7^{ab}	0.792	0.91 ± 0.12^{b}	0.88 ± 0.14^{b}	0.424
MPP1	112.1 ± 2.9^{ab}	111.2 ± 3.1^{ab}	0.263	0.85 ± 0.15^{b}	0.89 ± 0.11^{b}	0.350
MPP2	110.4 ± 4.5^{c}	110.5 ± 2.7^{bcd}	0.904	0.90 ± 0.13^{b}	0.93 ± 0.11^{ab}	0.346
P	<0.001	<0.001		<0.001	<0.001	

Data are shown as mean ± standard deviation; ^{a,b,c,d} Means followed by the same lower case letter in the columns did not differ statistically by the Tukey test (P>0.05); ^{A,B} Means followed by different upper case letters in the rows differed statistically by the t-test (P<0.05); Cl^- = 95 to 103mmol/L, iCa^{+2} = 1.4 to 1.6mmol/L (Kaneko et al. 2008).

Table 6. Venous blood concentrations of AG (mEq/L) and SID (mmol/L) in the ewes included in the two experimental groups (G1 and G2) at different times (M) of collection

M	AG			SID		
	G1	G2	P	G1	G2	P
MI	18.3 ± 3.5^{e}	18.0 ± 2.0^{e}	0.789	39.4 ± 3.4^{bc}	40.4 ± 3.1^{abcd}	0.254
MG30	18.9 ± 1.3^{e}	19.1 ± 1.6^{de}	0.640	38.7 ± 2.2^{c}	38.2 ± 2.2^{d}	0.467
MG90	21.6 ± 3.7^{bcd}	21.3 ± 2.1^{bc}	0.712	42.2 ± 2.6^{a}	41.4 ± 2.8^{abc}	0.278
MG120	20.9 ± 2.3^{dA}	19.3 ± 1.8^{deB}	0.009	41.1 ± 2.7^{abA}	39.0 ± 2.9^{cdB}	0.011
MG130	22.9 ± 1.9^{bcd}	22.3 ± 2.4^{abc}	0.373	41.5 ± 2.8^{b}	40.8 ± 3.4^{abc}	0.392
MG140	21.1 ± 1.8^{cd}	20.5 ± 1.4^{cd}	0.267	40.5 ± 2.9^{abc}	39.6 ± 3.1^{bcd}	0.301
MP	24.1 ± 2.8^{a}	23.3 ± 2.9^{a}	0.317	40.5 ± 1.8^{abc}	41.8 ± 2.7^{ab}	0.056
MPP1	23.0 ± 2.1^{abc}	22.6 ± 1.9^{ab}	0.571	41.1 ± 2.8^{ab}	41.6 ± 1.9^{abc}	0.488
MPP2	23.0 ± 3.1^{ab}	23.3 ± 1.5^{a}	0.966	42.1 ± 4.1^{a}	42.2 ± 2.5^{a}	0.932
P	<0.001	<0.001		<0.001	<0.001	

Data are shown as mean ± standard deviation; ^{a,b,c,d,e} Means followed by the same lower case letter in the columns did not differ statistically by the Tukey test (P>0.05); ^{A,B} Means followed by different upper case letters in the rows differed statistically by the t-test (P<0.05); AG = 10 to 20 mmol/L, SID = 47.9 to 54.4mmol/L (Kaneko et al. 2008).

lactic acidosis, described a mean SID value of 34.5±4.04mEq/L in empty and non-lactating ewes, which proved metabolic acidosis. Contrarily, this study found 38.2±2.2mEq/L as the lowest mean value, where the absence of clinical signs was compatible with the acid-base imbalance.

The blood gas analysis in this experiment showed that pregnancy caused higher alkaline reserve expenditure during delivery (MP), elucidated by BE values (Table 2), with decreased SID (Table 6). However, no metabolic acidosis was observed. Since there was no acidemia (Table 2), the events occurred physiologically, and the ewes were healthy and did not present pregnancy toxemia.

CONCLUSIONS

The ewes of this study were healthy until the last moment analyzed (48h postpartum), although they showed higher consumption of alkaline reserve in the peripartum, which was more evident in animals with single gestation. Nevertheless, this occurrence can be considered physiological and a consequence of the increased energy demand during pregnancy, which is more exacerbated in the peripartum.

The electrolytic changes observed across the moments were also considered physiological.

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