

Variation in blood glucose, cholesterol, urea and calcium levels according to the physiological stage in Ouled-Djellal ewes in Algerian arid zone

Technical note

Variación de los niveles de glucosa, colesterol, urea y calcio en sangre según el estado fisiológico en ovejas Ouled-Djellal en la zona árida de Argelia

Nota técnica

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ABSTRACT

Sheep farming in arid and semi-arid regions of Algeria is confronted with large fluctuations in the availability of fodder, which is all the more restrictive for pregnant ewes whose needs are at a maximum and constitute a major obstacle to the development of this sector. This study aims to evaluate the influence of the physiological stage on the energy, nitrogen and mineral status of Ouled-Djellal ewes living in arid zones (Biskra), by determining the plasma levels of glucose, cholesterol, urea and calcium. Blood samples were taken from 40 ewes divided into two groups according to their physiological stage (pregnant and empty). The obtained results showed that the physiological stage had a significant influence only on blood glucose, being higher in empty sheep than in pregnant sheep ($P < 0.05$), whereas for the other blood parameters: cholesterol, urea, and the plasma concentrations of calcium do not seem to be influenced by the physiological stage.

Key words: Sheep; Ouled-Djellal breed; blood metabolites; physiological stage; arid zone

RESUMEN

La cría de ganado ovino en las regiones áridas y semiáridas de Argelia se enfrenta a grandes fluctuaciones en la disponibilidad de forrajes, lo que resulta aún más restrictivo para las ovejas gestantes, cuyas necesidades son máximas y constituyen un obstáculo importante para el desarrollo de este sector. Nuestro estudio tiene como objetivo evaluar la influencia del estado fisiológico sobre el estado energético, nitrogenado y mineral de ovejas Ouled-Djellal que viven en zonas áridas (Biskra), mediante la determinación de los niveles plasmáticos de glucosa, colesterol, urea y calcio. Se tomaron muestras de sangre de 40 ovejas divididas en dos grupos según su estado fisiológico (preñada y vacía). Los resultados obtenidos mostraron que el estado fisiológico tuvo influencia significativa sólo en la glucemia, cuando es muy mayor en ovejas vacías que en ovejas preñadas ($P < 0,05$), mientras que para los demás parámetros sanguíneos: colesterol, urea y concentraciones plasmáticas de calcio no parecen estar influenciados por el estado fisiológico.

Palabras clave: Oveja; raza Ouled-Djellal; metabolitos sanguíneos, estado fisiológico, zona árida

INTRODUCTION

Sheep farming in Algeria ranks among the most traditional activities and holds a very significant position in the field of animal production. It constitutes the country's primary supplier of red meat. Sheep (*Ovis aries*) herd in Algeria is estimated at 18 million heads, of which 63% are of the breed Ouled-Djellal [1]. The majority of herds are concentrated in steppe regions, high plateaus, and a portion is exploited in the Saharan regions. The Ouled-Djellal breed represents between 54-63% of the ovine herd [2]. Sheep farming in Algeria is managed traditionally in the vast majority of private farms and some state-owned farms. It faces challenges of climatic, nutritional, and pathological hazards. The low production of herds is linked to poor management of reproduction and feeding, which are often extensive in nature [3].

Understanding the metabolic profile of ewes is important to clarify their nutritional status as well as to prevent metabolic disorders that lead to disruptions in production and reproduction [4]. Additionally, the serum levels of minerals and various biochemical indicators in sheep have been widely discussed [5, 6]. However, a limited number of studies have reported the influence of reproductive status in different sheep breeds as important elements for the interpretation of biochemical indicators in ewes [7, 8].

The main objective of this investigation is to analyze the influence of physiological stage on the variations or evolution of the following biochemical parameters: blood glucose, blood cholesterol, blood urea, and blood calcium levels. This analysis aims to evaluate the metabolic state and identify which of these parameters could be used as indicators for predicting the metabolic status of the ewe according to its physiological stage.

MATERIAL AND METHODS

Ethical approval and statement

Adequate measures were taken to minimize pain or discomfort in accordance with the International Animal Ethics Committee. The study was approved by the committee framed for the research by the university authority. During the study visits, the researchers introduced themselves and explained the objective and methodology of the study to all animal breeders.

Region of the study and animals

This study was conducted in Biskra governorate, it is a semi-arid region, located in the south-eastern part of Algeria, at 440 Km from the capital city Algiers at an altitude of about 110 m of the sea-level, a latitude of 34°51' North and a longitude of 5°43' East.

Farm presentation

The farm extends over an area of 27 hectares and is organized into two main components:

- Crop production: of which cereal farming, namely wheat (*Triticum aestivum*) and oats (*Avena sativa*), is the primary activity, followed by the cultivation of forage crops of barley (*Hordeum vulgare*) in second place.
- Animal production: this consists mainly of about 220 ewes managed in an extensive system, along with 4 dairy cows. Milk production is reserved solely for self-consumption.

Animals

The ewes included in this study are from the same farm described above. They are Ouled-Djellel breed ewes (*Ovis aries*), which is the predominant breed in this region. A total of 40 clinically healthy, multiparous ewes aged between 2 and 5 years were selected. The animals were divided into two groups based on their physiological stage:

- 20 gestating ewes in their last trimester;
- 20 non-gestating ewes in maintenance.

The farming method employed is extensive, with the sheep grazing on the pasturelands available in the region, locally referred to as 'Watn'. Supplementary feed is provided during favorable seasons, especially to pregnant ewes in the last trimester of gestation. This feed mainly consists of barley and straw (*Imperata cylindrica*) produced on the farm.

In this arid region, supplemental feeding is provided for almost three-quarters of the year during dry periods; in particular, concentrated energy foods (wheat, corn, beets in pulp form) provided in daily rations of approximately 0.25 to 1 kg per head. Mineral supplements (calcium, phosphorus) and vitamins can also be provided; they are made freely available to the animals, in the stable, in the form of a block of mineral salts that breeders call the lick stone. In the summer, the herd migrates to neighboring provinces such as Khenchela and Oum El-Bouaghi to graze on cereal stubbles.

Blood sampling

Blood samples were collected using the protocol described by Polizopoulou [9]. The samples were taken in the morning before feeding (fasted) after disinfecting the area. They were drawn from the jugular vein using a single-use needle into vacutainer tubes containing lithium heparin. After collection, the samples were divided into two batches and transported in a cooler (Haier Biomedical brand, HZY SERIES, China). The first batch was sent to the laboratory of the local public health facility in Zéribet El-Oued. There, they were immediately centrifuged with BioSan centrifuge (model LMC-3000 22005, Latvia) at 1008 G and then frozen at 25°C in Haier Biomedical* (China) freezer until analysis for blood glucose levels, blood cholesterol levels, and blood urea levels, carried out by a spectrophotometer SECOMAM model PRIM 500 (France). The second batch of samples was sent to the laboratory of Ben Badis polyclinic in Biskra specifically for the analysis of blood calcium levels.

Analytical methods

The commercial kits used were manufactured by Biomaghreb (Spain):

- **Glucose Assay:** by enzymatic method (GOD-PAP). Result is done using spectrophotometry at a wavelength of 505nm
- **Cholesterol Assay:** by colorimetric enzymatic test (CHOD-PAP). Reading is carried out at a wavelength of 505 nm.
- **Urea Assay:** with modified urease + Berthelot method. Reading is carried out at a wavelength of 590 nm.
- **Calcium Assay:** by colorimetric method. Reading is done spectrophotometrically at a wavelength of 570 nm.

Statistical analysis

For a better analytical treatment of the recorded results, statistical analysis was conducted using the MedCalc 2017 software (version

17.2 copyright © 1993-2017 MedCalc software bvba); it is a powerful and useful statistical tool, designed and developed for researchers in biology and medical field. The main test used is the Student's t-test for comparing the means of two independent samples (groups).

RESULTS AND DISCUSSION

The biochemical characterization of animals is of particular interest for evaluating the nutritional and/or metabolic status of the animal through the exploration of the results of the biochemical profile as well as the understanding of factors that can influence the plasma concentrations of some blood metabolites [10].

Blood glucose levels

Results indicated in TABLE I shows that blood glucose is significantly higher in non-gestating ewes than in gestating ewes ($P < 0.05$ by Student's t-test).

Physiological stage	M ± SD
Non-gestating ewes (n = 20)	0.61 ± 0.11*
Gestating ewes (n = 20)	0.43 ± 0.06

M: Mean, SD: Standard deviation, *: P-Value <0.05

The blood glucose levels obtained in this study are within the lower limit of the reference values for gestating ewes (0.50 – 0.80 g·L⁻¹) as reported by Dubreuil *et al.* [11], Frye *et al.* [12] and Varanis *et al.* [13], and within the upper limit for non-gestating (0.41 – 0.65 g·L⁻¹) ewes as reported by Dubreuil *et al.* [11]. They also fall within the range of reference values reported by Kaneko *et al.* [14] and Sarmin *et al.* [15]. Additionally, the recorded results were higher than those obtained by Nedeva *et al.* [16], Abdelsattar *et al.* [17], Nedeva *et al.* [18] and Xulu *et al.* [19].

The low blood glucose levels observed in ewes at the end of gestation could be explained by the abundant use of maternal glucose by the fetus(es). The development and rapid growth of the fetus(es) in the last trimester of gestation require energy input that the mother should satisfy [20]. The results obtained in the present study demonstrate that physiological stage has a significant influence on blood glucose levels, which is consistent with the findings reported by Varanis *et al.* [13] and Hamadech *et al.* [20], who concluded that blood glucose levels are lower in gestating ewes compared to those in lactation or non-gestating ewes.

Blood cholesterol levels

Regarding the parameter of blood cholesterol levels, statistical analysis did not reveal a significant difference ($P > 0.05$) between the two physiological stages (TABLE II).

In the current study, the cholesterol levels observed in both physiological stages are higher than those reported by Dubreuil *et al.* [11] (0.52 – 0.76 g·L⁻¹ for non-gestating ewes; and 0.50 – 1.39 g·L⁻¹ for gestating ewes); however, the reported results are lower than those recorded in studies conducted by Nedeva *et al.* [18] and Vicente *et al.* [21].

Physiological stage	M ± SD
Non-gestating ewes (n = 20)	1.54 ± 0.24
Gestating ewes (n = 20)	1.48 ± 0.24

M: Mean, SD: Standard deviation, P-Value >0.05

Blood cholesterol levels concentration in ruminants could be influenced by several factors: breed, age, sex, physiological stage, dietary composition and season [19, 22, 23]. It could also be an indicator of thyroid function because hypothyroidism is generally associated with hypercholesterolemia [21, 23, 24]. Furthermore, the obtained results are consistent with those demonstrated by Varanis *et al.* [13] who concluded that there is no significant difference in blood cholesterol levels concentrations between gestating ewes and non-gestating ewes.

Moreover, a significant decrease in total cholesterol at the end of gestation has been reported in other species: cows [25], goats [26] and mares [27]. This is likely related to the role of this metabolite in the synthesis of ovarian steroids, so total cholesterol concentrations are under the control of complex factors. Iriadam [28] described variations in blood cholesterol levels during estrus and gestation as precursors to steroid hormones.

Blood urea levels

In the TABLE III are presented the results of the influence of the physiological stage of ewes on the blood levels of urea. After comparing the averages using the analytical test Student T, it appears that there is no significant difference between the two batches of ewes (with $P > 0.05$).

Physiological stage	M ± SD
Non-gestating ewes (n = 20)	0.31 ± 0.08
Gestating ewes (n = 20)	0.28 ± 0.06

M: Mean, SD: Standard deviation, P-Value >0.05

The blood urea levels obtained in this study for both groups fall within the range described by several authors [11, 14, 15], it appears that there is no significant difference between the two experimental batches; indeed, uremia is subject to large fluctuations linked to the importance of protein intake in the ration, it constitutes a good indicator of nitrogen intake in sheep and goats. Contrary to the current results, Varanis *et al.* [13] and Sarmin *et al.* [29] found a significant difference in blood urea between non-gestating and gestating ewes, they observed higher urea levels in gestating ewes compared to non-gestating or lactating ewes.

Blood calcium levels

Results indicated in TABLE IV shows that blood calcium levels are slightly higher in non-gestating ewes compared to gestating ewes; however, this difference does not appear to have a significant influence on this parameter (with $P>0.05$).

TABLE IV
Variations in blood urea levels according to physiological stage (mg·L⁻¹)

Physiological stage	M ± SD
Non-gestating ewes (n = 20)	103.80 ± 8.46
Gestating ewes (n = 20)	96.82 ± 10.61

M: Mean, **SD:** Standard deviation, *P*-Value >0.05

Regarding the blood calcium levels, the comparative analysis of the results did not reveal a significant influence of the physiological stage on calcium levels ($P>0.05$) between the two groups studied; this finding was also demonstrated by Sarmin *et al.* [29] and Aksoy *et al.* [30]. Tanritanir *et al.* [31], did not report any significant difference in this element between ewes in the pre- and post-partum periods. On the other hand, Abdelrahman [32] reported that calcium requirements increase with the progression of gestation and noted that the reproductive stage has a very considerable influence on serum mineral concentrations, which is inconsistent with our results.

Physiological stage is a variable factor which is included in our study, and the different results observed, as well as those from other studies, may have different interpretations based on:

- » Experimental protocol: which varies among studies, particularly in terms of blood sampling for each physiological stage, that is to say, the day of sampling relative to the exact date of the physiological stage (non-pregnant, early pregnancy, mid-pregnancy, late pregnancy), and the sampling days are not identical for all studies.
- » Use of animals relative to physiological stage; some studies use a batch of ewes for each physiological stage, while others track the same group of animals through different physiological stages.
- » Feeding; different studies on Ouled-Djellal ewes use feed rations based on the forage availability in the region, whereas forage composition and nutritional value vary from one region to another Season; there is a seasonal effect (climate, temperature, photoperiod: long days, short days) on some biochemical parameters as reported by some studies, and additionally, the relief shows a significant effect of altitude on blood parameters.

CONCLUSIONS

The study of the influence of physiological stage on various blood biochemical parameters in Ouled-Djellal ewes living in the challenging conditions of arid zones has shown that the physiological state significantly affects blood glucose levels ($P<0.05$), while other parameters such as blood cholesterol, urea, and calcium concentrations do not seem to be affected by the physiological stage.

RECOMMENDATIONS

However, further research with larger sample sizes is needed to establish specific reference values for Ouled-Djellal ewes in arid zones and to assess the impact of challenging climatic conditions on the general metabolism and reproductive performance of the animals.

Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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