Scientific Works. Series C. Veterinary Medicine. Vol. LXIX (1), 2023
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

STUDY OF AN EPISODE OF SUBCLINICAL KETOSIS IN A SHEEP FARM IN SOUTHERN ROMANIA

Adrian MIHAI, Roxana Mariana IGNĂTESCU (ȚÎMPĂU), Nicoleta Andreea MINCĂ, Carmen IONIȚĂ, Raluca Mihaela TURBATU, Lucian IONIȚĂ

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: roxana_mariana_12@yahoo.ro

Abstract

Subclinical ketosis in sheep is considered a disorder of carbohydrate metabolism, which causes the mobilization of lipids, disturbance of fatty acid metabolism and the formation of ketone bodies. The main objective of this work is to identify local and general factors that contribute to the occurrence of subclinical ketosis in a sheep farm in Dolj County (Southern Romania) and to highlight by laboratory analysis the disturbances of energy metabolism, as well as other conditions closely related to them. A good nutritional management of the ewes before and after lambing is essential knowing that their nutritional requirements increase in this period and the peak will be reached in the first two-three weeks of lactation. Careful monitoring of the ewes in this period: trained personal to identify early signs of disease, diagnostic tests and quick intervention is also needed to prevent these episodes and other complications, such as pregnancy toxemia, which can lead to severe nervous manifestations (secondary to hypoglycemia) and significant economic losses.

Key words*: subclinical ketosis, sheep, ketonemia, anemia, ketonoruria.*

INTRODUCTION

Subclinical ketosis (SK) is very common in ruminants, but in sheep herds is often underdiagnosed because of the absence of clinical signs. Identification of this condition is possible through its main dominant: hyperketonemia (Duffield et al., 2009). The pathophysiological mechanism is based on the increase of the ewe's nutritional needs, while the ability to store feed decreases because of the abdominal expansion of the pregnant uterus, which will result in a negative energy balance. SK is characterized also by imbalance of carbohydrate and fat metabolism (Bergman, 1971).

In general, preventive measures (proper nutrition and energy suppliers) are taken to avoid this condition, but routine testing for SK should be done at least in the last four weeks of pregnancy, knowing the increased risk of complications that may appear during this period. The gold standard for SK diagnosis is analysis of blood β-Hydroxybutyric acid (BHBA), which according to Bostedt et al., 1990 is the most stable ketone, being significant for approximately 85% of the total ketones in sheep with pregnancy toxemia, but it requires specialized testing and increased prices. Identification of ketone bodies in the urine is also used, but it is not a very relevant test, knowing that ketonuria may occur in other conditions too. Determination of elevated BHBA is possible now on the farm by using a ketone meter. This method is very affordable and with great diagnostic value according to various authors. (Jones et al., 2018; Pichler et al., 2014). SK should be differentiated using liver enzymes activity tests from other liver disease that induce incorrect glucose metabolism (Braun, 2010).

Subclinical ketosis may rapidly degenerate into clinical ketosis, in case of inappropriate environmental conditions, inadequate nutrition, transport stress. In practice, nutrition is one of the factors of sensitization of sheep and associated with other stressors, may be the trigger for the disease (Marutsova et al., 2018).

Ketosis is associated with pregnancy toxemia in sheep, being a metabolic disease that often occurs in the last 3 weeks of gestation and is considered more aggressive in twin or triplet-bearing ewes (Fthenakis et al., 2012).

Symptomatology includes low food intake or selective anorexia, ketone breathing odor, weight loss, or, in decompensated cases (pregnancy toxemia): muscle tremors, opisthotonos 1-3 weeks before lambing. In a few days, blindness, ataxia, prolonged sternal decubitus, comatose state or death may occur. (Van Saun, 2000; Schlumbohm et al., 2008)

MATERIALS AND METHODS

The study was carried out in a privately-owned farm of Tsurcana breed with 122 heads (12 males and 110 females), located in the South of Romania, approximately 20 km away from Danube River (Figure 1).

Figure 1. View from the sheep herd in Southern Romania

The analysed population included 84 ewes (in second or third gestation), 5 with twin pregnancies (diagnosed by ultrasound by the farm physician). Anamnesis showed that in the last years, 6 of these sheep aborted, without suffering from an infectious disease.

Table 1. The flock effective in the period of our study (December 2021-February 2022)

	110 EWES	Previous Abortion	Previous Twin lambings
Flock N=122	32 in 3rd gestation	3	4
	3 twin lambings		
	52 in 2nd gestation	2	2
	2 twin lambings		
	26 in 1st gestation	-	-
	12 RAMS		

All animals were up to date with preventive actions (vaccinations, internal-external deworming). They were monitored during their lambing period (December 2021 - February 2022) (Table 1) in the last three weeks of gestation for early signs of Ketosis (low food intake or selective anorexia, weight loss) and for high levels of blood BHBA, by using The Nova Vet Meter (UK). Diagnosis of SK was possible in 12 ewes with BHBA values: 0.8-1.6 mmol/l (group A). Also, a control group of healthy ewes (n=5) was established according to the absence of clinical signs and to the BHBA values (under 0.8 mmol/l: group B)
Blood samples were collected from both groups via jugular vein puncture, using 21 G needles, heparin and K3EDTA vacutainers. Hematological and biochemical tests were performed in order to assess the metabolic status of the SK group compared to the control group, using the hematology analyser Abacus Junior Vet 5 (SUA) and the biochemistry analyser Arkray Spotchem EZ SP4430 (Japan). Results were compared to the available relevant literature data, using the keywords: subclinical ketosis, sheep, ketonemia, anemia, ketonuria. The following sections will emphasize the obtained results.

RESULTS AND DISCUSSIONS

A very important role in this survey was taken by the clinical examination of the animals, their environmental conditions and their welfare. Their rations were in concordance with their physiological needs (late pregnancy), but we considered that the protein component was of low quality. Ewes were reared in facilities in compliance with the welfare standard for the species. At the group examination we concluded that the flock was in a good maintenance condition. The clinical exam of the individuals was performed and a special attention was paid to palpation of each sheep in order to establish the Body Condition Score (BCS), using the five-point system (1.0-5.0) at 0.5-point intervals between 2.0 and 4.0, the appearance of the skin, the colour of the mucous membranes. BCS of the group A was 3 and BCS of the group B was 2.5, except the twin bearing ewes which had the BCS 3, which means that the BCS is mild decreased in the SK

group, probably associated with the lower food intake and its quality of the fodder. Also the ability to consume the entire quantity of food was appreciated as good in both groups, according to the low litter size observed in the exploitation.

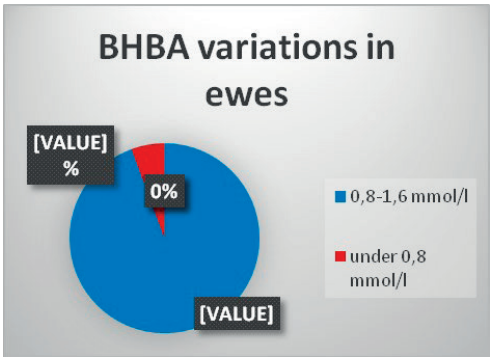

Figure 2. Values of BHBA in our study

The prevalence of SK in late pregnancy was 14.28%, which is similar to what Gupta et al. reported in their study from 2008 (14.86%), performed in pregnant ewes. We think that this percentage is quite significant and it can be directly associated to the occurrence of clinical ketosis and its economic implications, taking into account that all ewes with twin pregnancy were included. On the other hand, there are also studies with higher incidence of SK: during the last four weeks of gestation, 46.0% of ewes exhibited persistent BHBA >0.8 mmol/L, as Jones et al. (2018) report in their paper.

We believe that in appropriate environmental conditions, but with unbalanced rations, each breeder should pay more attention to his flock and more investigations should be carried out to assess the metabolic state of the ewes. Imbalances should be corrected in order to prevent other pathologies such as: fetal death, mammitis. In this sense, we implemented a minimal set of analyses for a more efficient monitoring of sheep during the peripartum period at a low cost. This panel included a hemoleucogram and some suggestive biochemistry parameters: glucose (GLU), total protein (TP), urea (BUN), γ-glutamyltransferase (GGT), calcium (Ca). Hematological parameters included: Red Blood Cells (RBC), White Blood Cells (WBC), Hemoglobin concentration (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Platelet Volume (MPV). The variation of the main indices of the red blood cells will be presented in Table 2 for group A and in Table 3 for group B. Also, the average of the values was calculated and compared to the reference interval (RI) to emphasize the differences between the 2 groups.

Table 2. Variation of the main erythrocyte indices, in pregnant sheep from group A

No	RBC mil/mm^3	Hct %	Hgb g/dl	MCV μ^3	HEM	MCHC g/dl Er.
RI	**12**	**36**	**11**	**33**	**10**	**32**
1	12.5	36.7	11.4	35	10.5	33
2	12.4	37.8	11.2	33	10.1	32.3
3	13.7	38.5	12.0	34.3	11.4	33.8
4	13.4	37.5	11.7	36.4	11	33.5
5	12.8	39.0	12.8	33.0	11.6	37.6
Average	**12.96**	**37.9**	**11.82**	**34**	**10.92**	**35.3**

Clinically healthy sheep had normal values in HLG, while those with SK had mild microcytic hypochromic anemia. This type of anemia has been reported due to some deficiency (Iron). In this exploitation, this pattern associated with mild decreased BCS sustain the assumption that the sheep with SK consume insufficient feed. On the other hand, this type of anemia can also occur as a result of the increase in the need of the fetus, a fact supported by the lower values of red blood cells in ewes with twin pregnancies (red colour in the Table 3).

Table 3. Variation of the main erythrocyte indices, in pregnant sheep from group B (Sk group)

No	RBC mil/mm^3	Hct %	Hgb g/dl	VEM μ^3	HEM	CHEM g/dl Er.
RI	**12**	**36**	**11**	**33**	**10**	**32**
1	11.1	34.2	10.4	34	9.8	30.1
2	10.9	33.8	10.2	33	10.1	31.3
3	10.7	33.5	10.0	31.3	9.3	29.8
4	12.3	32.5	9.7	26.4	7.8	29.8
5	9.0	34.0	8.8	31.7	9.7	25.8
6	9.3	33.6	9.5	31.1	10.2	28.2
7	9.7	36.1	9.0	31.4	9.2	29.5
8	9.3	39.0	10.8	41.0	11.6	27.6
9	10.0	33.6	9.6	33.6	9.6	28.5
10	10.7	38.8	10.2	36.2	9.5	26.2
11	10.0	34.5	9.7	32.5	10.7	31.0
12	10.5	33.9	9.5	32.2	9.0	28.0
Average	**9.30**	**34.85**	**8.8**	**33.5**	**9.70**	**27.6**

During periods of under-nutrition, tissue reserves are mobilized resulting in increases in plasma non-esterified fatty acids (NEFA) and β-hydroxybutyrate that may alter insulin responsiveness. (Brown et al., 2010). Because NEFA are not available in the area of the exploitation under study, energy metabolism was assessed using: total protein, glucose and BHBA. To differentiate the SK from hepatic disease, GGT was evaluated, knowing that in sheep it is specific for hepatobiliary damage, while AST, ALT are poorly specific to the liver. (Braun et al., 2010).

Table 4. Variation of the main biochemistry parameters, in pregnant sheep from group A

No	GLU mg/dl	TP g/DL	BHBA mmol/l	GGT UI/L	Ca mg/Dl
RI	**30-60**	**6.2-8.3**	**< 0.8**	**13-40**	**8.4-10**
1	54	7.8	0.5	22	9.9
2	53	8.1	0.6	28	10.1
3	62	7.1	0.6	32	8,7
4	58	6.9	0.4	32	9.0
5	55	6.4	0.4	26	8.5
Average	**56.4**	**7.38**	**0.5**	**28**	**9.24**

The values of GGT have been normal in the control group, while almost normal (borderline elevated) in SK group, which reveals that preventive actions (deworming) were done rigorously and there is no evidence of other concurrent liver damage.

Generally, serum concentrations of BHBA have been used to determine hyperketonemic status and subclinical ketosis. This rise in serum BHBA is a compensatory mechanism and a reflectionary response to carbohydrate deficiency and inhibition of Kreb's cycle, according to Anoushepour et al., 2014.

Plasma calcium concentration, as indicative of mineral metabolism has been tested too. About Calcium it is known that it is influenced by food supply, and is reported to be lower in twin than in single pregnant ewes, but no difference was observed between twin and triplet-pregnancies (Corner et al., 2008). In our study Calcium levels were within reference ranges in both groups.

Upon a closer analysis of the data obtained among group B, it is observed that sheep with twin pregnancy have lower blood glucose and total protein values than those with normal pregnancy, a fact that once again emphasizes the need to adjust the rations according to the needs of each individual. Also, the GGT values were higher in the twin bearing ewes (red colour in the Table 5) than in the rest of the ewes.

Table 5. Variation of the main biochemistry parameters, in pregnant sheep from group B

No	GLU (mg/dl)	TP (g/dl)	BHBA mmol/l	GGT UI/L	Ca mg/dl
RI	**30-60**	**6.2-8.3**	**< 0.8**	**13-40**	**8.4-10.3**
1	31	7.7	1.3	34	10.3
2	35	7.3	1.1	33	9.9
3	34	7.5	1.2	28	13.4
4	40	7.3	0.9	31	10.1
5	26	6.4	1.3	44	9.7
6	28	6.8	1.6	43	9.0
7	44	7.9	1.1	32	10.4
8	26	6.9	1.5	48	9.5
9	34	8.2	1.4	34	9.7
10	54	8.1	1.2	36	9.2
11	30	6.4	1.5	40	10.2
12	29	6.4	1.4	52	9.7
Average	**34.25**	**7.24**	**1.3**	**37.91**	**9**

Comparing the results of both groups, we can state that the Sk group has low levels of glucose, total protein and GGT at the upper limit, a fact that can lead to the clinical form of the disease at any time (Figure 3).

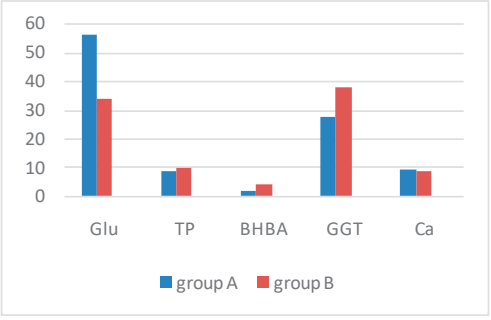

Figure 3. Variation of the main biochemistry parameters between the 2 groups

Once the clinical signs of ketosis are installed, there is only one more step (inadequate management) to pregnancy toxemia, which leads to significant productive and reproductive

losses. Knowing that sheep also have a tendency to develop anemia, that their BCS tends to decrease, we recommend re-evaluating the rations and improving the quality of the forage, as well as its nutritional values.

CONCLUSIONS

A good management of sheep flocks during lambing period should include: good quality feed, optimized according to individual needs, periodic measurement of BHBA levels, monitoring of the herd through blood tests. Ketonemia has been recognized as an essential characteristic of SK, the main predisposing cause was inadequate long-term feeding, sustained also by the decreased BCS and mild anemia found in group B.

We consider that a minimum database of blood tests, as those presented previously may be useful in evaluating the health status of the ewes, helping us take the necessary measures before the development of any clinical conditions. Also, in the case of subclinical ketosis, identifying and correcting imbalances as early as possible will mean a healthier herd, with fewer problems and higher productions.

REFERENCES

Anoushepour, A., Mottaghian, P., & Sakha, M. (2014). The comparison of some biochemical parameters in hyperketonemic and normal ewes. European Journal of Experimental Biology, 4.

Bergman, E.N., 1971. Hyperketonemia-ketogenesis and ketone body metabolism. J. Dairy Sci. 54, 936–48.

Bostedt, H., & Hamadeh, M. E. (1990). The significance of pregnancy-induced ketonuria in sheep and goats. Tierarztliche Praxis, 18(2), 125–129.

Braun, J. P., Trumel, C., & Bézille, P. (2010). Clinical biochemistry in sheep: A selected review. Small Ruminant Research, 92(1-3), 10–18.

Corner, R.A., Kenyon, P.R., Stafford, K., West, D.M., López-Villalobos, N., Morris, S.T., & Oliver, M.H. (2008). Effect of nutrition from mid to late pregnancy on the performance of twin- and triplet-bearing ewes and their lambs. *Animal Production Science, 48*, 666-671.

Duffield, T.F., Lissemore, K.D., McBride, B.W., Leslie, K.E. (2009). Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92, 571–580.

Fthenakis, G. C., Arsenos, G., Brozos, C., Fragkou, I. A., Giadinis, N. D., Giannenas, I., Mavrogianni, V. S., Papadopoulos, E. and I. Valasi. (2012). Health management of ewes during pregnancy. Anim. Reprod. Sci., 130, 198–212.

Gupta, V. K., Sharma, S. D., Vihan, V. S. and A. Kumar. (2008). Prevalence and changes in haemogram in subclinical ketosis in sheep reared under organized farming system. Ind. J. Anim. Sci., 78, 453–456

Jones, A. K., Gately, R. E., Kellogg, T. D., Zinn, S. A., Govoni, K. E., & Reed, S. A. (2018). Evaluation of the Nova Vet Meter for sheep-side monitoring of β-hydroxybutyric acid (BHBA) and description of ewe BHBA during late gestation in three flocks from the Northeastern U.S. Research in veterinary science, 118, 491–497.

Marutsova, V. & Marutsov, P. (2018). Subclinical and Clinical Ketosis in Sheep-Relationships between Body Condition Scores and blood B-Hydroxybutyrate and Non-Esterified Fatty Acids concentrations. Tradition and Modernity in Veterinary Medicine. 3. 30-36.

Pichler, M., Damberger, a, Schwendenwein, I., Gasteiner, J., Drillich, M., Iwersen, M., 2014. Thresholds of whole-blood β-hydroxybutyrate and glucose concentrations measured with an electronic hand-held device to identify ovine hyperketonemia. J. Dairy Sci. 97, 1–12.

Schlumbohm, C. and J. Harmeyer. (2008). Twin-pregnancy increases susceptibility of ewes to hypoglycaemic stress and pregnancy toxaemia. Res.Vet. Sci., 84, 286–299.

Van Saun, Robert. (2000). Pregnancy toxemia in a flock of sheep. Journal of the American Veterinary Medical Association. 217. 1536-9.10.2460/javma.2000.217.1536.