https://doi.org/10.52973/rcfcv-e34480 Revista Científica, FCV-LUZ / Vol. XXXIV, rcfcv-e34480

Risk factors and metabolic indicators associated with the percentage frequency of subclinical ketosis on Ouled Djellal ewes in eastern highlands of Algeria

Factores de riesgo e indicadores metabólicos asociados con la frecuencia de cetosis subclínica en ovejas Ouled Djellal en las tierras altas orientales de Argelia

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ABSTRACT

This study investigated the frequency, risk factors, and metabolic indicators for detecting subclinical ketosis (SCK) in Ouled Djellal ewes. Out of 54 enrolled ewes, those with BHB ≥ 0.86 mmol·L^{-1} without clinical signs formed the SCK group, while ewes with BHB < 0.86 mmol·L^{-1} were healthy controls, either in late pregnancy or early lactation. The SCK frequency was higher in early lactation (37%). Increased risk was associated with twin-bearing (OR=4.96, 95%CI=1.967-12.503, *P*=0.001) and thin ewes with BCS < 2.5 (OR=2.74, 95%CI=0.71-10.73, *P*=0.003). SCK ewes had significantly lower glucose, triglycerides, cholesterol, Ca, Mg, Na, and K levels, but higher AST, ALT, GGT, ALP, LDH, and CK levels. The best diagnostic indicators were Ca (AUC 94.4%, cut-off < 81 g·L^{-1}, SE 77.46%, SP 100%), AST (AUC 84.4%, cut-off > 94.19 U·L^{-1}, SE 74.65%, SP 83.78%), and K (AUC 79.3%, cut-off 4.1 mmol·L^{-1}, SE 71.83%, SP 75.68%). Monitoring BHB and BCS, especially in twin-bearing ewes during the transition period, is recommended for ketosis prevention. Further large-scale validation of these metabolic indicators as SCK predictors in Ouled Djellal ewes is warranted.

Key words: Metabolic indicators; Ouled Djellal ewes; prevalence; risk factors; subclinical ketosis

RESUMEN

Este estudio investigó la frecuencia, los factores de riesgo y los indicadores metabólicos para detectar cetosis subclínica (SCK) en ovejas Ouled Djellal. De 54 ovejas seleccionadas, aquellas con BHB ≥ 0,8 mmol·L^{-1} sin signos clínicos formaron el grupo SCK, mientras que, las ovejas con BHB < 0,8 mmol·L^{-1} fueron controles sanos, ya sea al final de la gestación o al principio de la lactancia. La frecuencia de SCK fue mayor al inicio de la lactancia (37 %). El mayor riesgo se asoció con la gestación gemelar (OR=4,96, IC del 95 %=1,967-12,503, *P*=0,001) y ovejas delgadas con BCS < 2,5 (OR=2,74, IC del 95 %=0,71-10,73, *P*=0,003). Las ovejas SCK tenían niveles significativamente más bajos de glucosa, triglicéridos, colesterol, Ca, Mg, Na y K, pero niveles más altos de AST, ALT, GGT, ALP, LDH y CK. Los mejores indicadores diagnósticos fueron Ca (AUC 94,4 %, punto de corte < 81 g·L^{-1}, SE 77,46 %, SP 100 %), AST (AUC 84,4 %, punto de corte > 94,19 U·L^{-1}, SE 74,65 %, SP 83,78 %) y K (AUC 79,3 %, punto de corte 4,1 mmol·L^{-1}, SE 71,83 %, SP 75,68 %). Se recomienda monitorear BHB y BCS, especialmente en ovejas con gestación gemelar durante el período de transición, para prevenir la cetosis. Se justifica una validación adicional a gran escala de estos indicadores metabólicos como predictores de SCK en ovejas Ouled Djellal.

Palabras clave: Indicadores metabólicos; ovejas Ouled Djellal; prevalencia; factores de riesgo; cetosis subclínica

Received: 04/07/2024 Accepted: 19/08/2024 Published: 30/09/2024

INTRODUCTION

Ketonemia is a common metabolic disorder affecting sheep (*Ovis aries*) and goats (*Capra hircus*), especially during late pregnancy and early lactation [1, 2, 3, 4]. It reflects disturbances in carbohydrate and fat metabolism resulting from severe negative energy balance (NEB) [5]. NEB promotes NEFAs (Non-esterified fatty acids) mobilization from adipose tissue and increased hepatic ketone body production, leading to pregnancy toxemia (PT) in late pregnancy or lactation ketosis in early lactation, in clinical or subclinical forms [1, 4, 6]. Subclinical ketosis (SCK) is characterized by hypoglycemia and hyperketonemia without clinical signs and can rapidly degenerate into clinical ketosis under unfavorable conditions [7, 8]. Among ketone bodies, β-hydroxybutyrate acid (BHB) is a sensitive marker for detecting maternal undernutrition and diagnosing ketosis in ewes [5, 9].

Commonly the risk of ketosis occurs most in older animals with multiple fetuses, which increases up to third parity and exacerbates with poor nutritional management [2, 3, 5, 10]. Other stress factors including concurrent diseases, poor sanitation, and severe cold affect energy balance and cause ketosis [3, 10, 11, 12].

Ketosis being the most frequent cause of deaths in pregnant ewes and causing economic losses by reducing productivity and infertility problems [12, 13]. It is also a risk factor for postpartum mastitis, dystocia, and retained fetal membranes [14, 15]. Ketosis can negatively affect lamb health, with up to 20% reduced lamb viability, and increased morbidity due to lower birth weight and inadequate colostrum production [12, 14, 15, 16].

Understanding the pathological changes associated with SCK is crucial for developing effective diagnostic, timely interventions and successful treatment [2, 17]. However, SCK is less studied in dairy ewes [16], and no studies have been conducted in Algeria in Ouled Djelall ewes. This study aimed to determine the prevalence of SCK and associated risk factors in Ouled Djelall ewes during the periparturient period, and to identify biomarkers for early SCK diagnosis.

MATERIALS AND METHODS

Animals

This study involved fifty-four clinically healthy Ouled Djellal ewes (10% of the total farm ewes), aged 3-6 years, with an average body condition score (BCS) of 2.89 ± 0.37 and weighing 59.25 ± 4.02 kg (Maquinaria Bar Hosteleria, MBHTMZ150, Spain) at the beginning of the research.

Study Area

The research was conducted at the Abassi Larbi pilot farm in Bordj Bou Arreridj Province, Eastern Algeria (35°N | 4°E, altitude 1065 m), characterized by a semi-arid climate. The 2020/2021 campaign had below-average rainfall (277.6 mm) and an annual average temperature of 15.9°C.

Feeding and breeding management

Estrus cycles were synchronized using the ram effect in late September, with pregnancy confirmed by transabdominal ultrasonography (DRAMINSKI iScan2, Poland) in 45 days later. Ewes grazed on natural pasture daily (11:00 to 17:00) and received wheat straw (1 kg·ewe^{-1}·day^{-1}) and 500 g of commercial concentrate composed of maize, wheat bran, barley, carob, soybean, molasses, salt, dicalcium phosphate, calcium carbonate, and CMV mineral and vitamin supplement. The corresponding nutritional value of the concentrate was 14.3% crude protein (DM), 3.3% crude fat (DM), 5.7% crude fiber (DM), 0.2% crude ash (DM), 0.85% calcium, and 0.6% phosphorus. They were provided fresh water twice daily and systematically vaccinated and dewormed.

Study design

Ewes were divided into two groups based on blood BHB concentration either during late pregnancy or early lactation: healthy (BHB < 0.86 mmol·L^{-1}) and SCK (BHB > 0.86 mmol·L^{-1}). All ewes had no clinical symptoms. Before blood sampling, BCS was assessed on a 1-5 scale [19].

Sample Collection

Blood samples were collected using vacutainer heparinized tubes from the jugular vein in the morning before feeding and centrifuged at 3000 rpm for 10 min (TDZ4-WS, Bioridge, Shanghai, China). Samples were taken during late pregnancy (18-3 days prepartum) and early lactation (3-18 days postpartum) and the plasma stored at -20°C until analysis.

Biochemical Analysis

The blood BHB concentrations were measured at ewe-side with rapid test strips using a handheld meter (FreeStyle Optium H for β-Ketone, Abbott Diabetes Care Ltd, Witney, Oxon, UK). This handheld meter was validated for use in sheep [20]. Whereas, plasma glucose, triglycerides (TGs), cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), and potassium (K) were all determined by an automatic biochemical analyzer (ADVIA® 1800 Chemistry System, Siemens Healthcare, Germany) at the biochemistry laboratory of the university hospital center of Constantine province.

Statistical Analysis

The data were examined utilizing the statistical program SPSS version 23.0. The variables were reported as the mean and standard error of the mean (SEM). A significance level of $P<0.05$ was used. Qualitative factors were categorized for logistic regression analyses. The pregnancy number was classified into two categories: 2 (biparous) and ≥ 3 (multiparous). The body condition score (BCS) of ewes was classified into three categories: thin (< 2.5), medium (2.5-3.5) and obese (> 3.5). The pregnancy size was classified into two classes: 1 (single) and 2 (twin). The period and sampling into late pregnancy and early lactation.

A chi-square test was used to assess potential risk factors linked with SCK outcomes for categorical variables. Variables having a significance level of $P<0.05$ were further analyzed using binary logistic regression. The SCK disease, represented as a binary variable, was treated as the dependent variable, while the other factors were treated as independent variables. The Hosmer and Lemeshow goodness of fit test was used to validate the final model.

An ANOVA two-factor test was used to assess the variability of metabolic blood parameters in sheep with and without SCK during late pregnancy and early lactation. Ultimately, the cut-off points or critical

thresholds of metabolic predictors for SCK diagnosis (dependent binary variable) were established by receiver operating characteristic (ROC) analysis. The diagnostic precision of the selected parameters was evaluated using the area under the curve (AUC). Only significant variables were chosen for prediction based on the hypothesis that the AUC is not equal to 0.5 (H_o: AUC = 0.5 vs H_o: AUC ≠ 0.5; P<0.05). The ideal thresholds of the significant parameters were determined by calculating Youden's index.

RESULTS AND DISCUSSION

Percentage frequency and risk factors associated with SCK in Ouled Djellal ewes

The percentage frequency of SCK was higher during early lactation (37%) than in late pregnancy (28.7%) and augmented with the increase in parity number, with the maximum cases was occurred in multiparous ewes (53.7%). Only the litter size and the BCS were significantly (P<0.001) associated with SCK. While the chi-square test did not detect any significant differences related to physiological state and parity on the occurrence of SCK (TABLE I).

Likewise, these findings also indicated that ewes bearing twins and under-conditioned ewes had a higher risk for developing SCK. The risk of SCK increases approximately 5 times in ewes bearing twins compared to ewes with simple litter size (OR=4.96, 95% CI=1.967-12.503, P=0.001), and approximately 3 times in thin ewes compared to over-conditionned ewes (OR=2.74, 95% CI=0.71-10.73, P=0.003) (TABLE II).

During the early lactation, Ouled Djellal ewes were also prone to ketosis, related to the deficiency of energy in ewes during this period. Similar rates have been reported in dairy ewes (Lacaune breed), with a higher percentage frequency during early lactation (33%) compared to late pregnancy (18%) [1]. However, ewes are more susceptible to ketosis in the last few weeks of pregnancy due to increased fetal energy demands and often inadequate nutrition [11, 21].

Several factors contribute to the development of SCK in ewes. This study identified litter size, and a BCS as significant risk factors in Ouled Djellal ewes. Under-conditioned ewes (BCS < 2.5), and those carrying multiple fetuses were at higher risk of SCK due to NEB and impaired gluconeogenesis [2, 8, 11, 15]. Higher blood BHB levels correlated with lower BCS, suppressed appetite, reduced ruminal motility, and further deterioration of body condition [1]. Microcytic hypochromic anemia, associated with SCK and low BCS, indicated insufficient feed intake in affected ewes [8]. BCS loss in postpartum increases the risk of ketosis, reduces milk yield and reproduction performance, and causes early embryonic death [1]. Hence, BCS is essential for managing feeding programs and detecting NEB and metabolic diseases in sheep herds.

TABLE I
Percentage frequency and risk factors associated with SCK in Ouled Djella ewes

Risk factors	Categorized variables	Healthy ewes		SCK ewes		Ch-square	df	*P* value
		N	percentage frequency	N	percentage frequency			
Physiological state	LP	23	21.3%	31	28.7%	3.33	1	0.068
	EL	14	13.0%	40	37.0%			
Parity	Biparous	10	9.3%	13	12.0%	1.103	1	0.294
	Multiparous	27	25.0%	58	53.7%			
Litter size	Single	25	23.1%	19	17.6%	16.77	1	0.000*
	Twins	12	11.1%	52	48.1%			
BCS	Thin	7	6.5%	40	37.0%	16.703	2	0.000*
	Medium	24	22.2%	19	17.6%			
	Obese	6	5.6%	12	11.1%			

The Chi-square statistic is significant at the 0.05 level. LP : late pregnancy; EL: early lactation

TABLE II
Association between litter size and BCS with the risk of SCK occurrence

Risk factors	Categorized variables	B	S.E	Wald	df	*P* value	OR	95%C.I for OD	
								Lower	Upper
Litter size	Twins	1.601	0.472	11.522	1	0.001	4.960	1.967	12.503
	Obese			11.406	2	0.003			
BCS	Thin	1.008	0.687	2.156	1	0.142	2.741	0.713	10.533
	Medium	-0.818	0.631	1.681	1	0.195	0.441	0.128	1.520
Constant		-0.194	0.586	0.109	1	0.741	0.824		

Association of biochemical parameters with the occurrence of SCK

BHB value increased significantly in both groups of SCK compared to the healthy ewes groups. A significant decrease in glucose and TGs concentrations only in subclinical pregnant toxemic ewes (SPT) were showed. Cholesterol followed the same behavior as glucose and TGs and the lowest values were reached during early lactation in SCK ewes (TABLE III).

The AUC for glucose was 70.7%, and the optimum cut-off point was 2.22 mmol·L^{-1} (SE: 40.85% and SP: 100%), and the AUC for cholesterol was 78.9%, and the optimum cut-off point was 1.55 mmol·L^{-1} (SE: 80.28% and SP: 64.86%) (TABLE IV).

The enzymatic activities of AST, ALP, and CK were significantly higher in both SCK groups than in the control groups. Whereas, the enzymatic activities of ALT, GGT, and LDH were significantly greater only in SPT ewes than in the healthy late pregnant ewes. Furthermore, the enzymatic activities of AST, ALT, GGT, ALP, CK, and LDH were highest in recently lambed ewes with SCK compared to SPT ewes (TABLE III).

The AUC for AST was 84.4%, and the optimum cut-off point was 94.19 UI·L^{-1} (SE: 74.65% and SP: 83.78%), and the AUC for ALP was 85%, and the optimum cut-off point was 123 UI·L^{-1} (SE: 77.46% and SP: 81.08%). The AUC for CK was 73.7%, and the optimum cut-off point was 99.43 UI·L^{-1} (SE: 53.52% and SP: 91.89%) (Table IV).

Lower levels of Ca, Mg, Na, and K were observed in ewes with SCK, either in late pregnancy or early lactation, compared to healthy ewes. However, P increased significantly in SPT ewes, followed by a significant decrease in recently lambed ewes with SCK (TABLE III).

The AUC for Ca was 94.4%, and the optimum cut-off point was 2,02 mmol·L^{-1} (SE: 77.46% and SP: 100%). For K, the AUC was 79.3%, and the optimum cut-off point was 4.1mmol·L^{-1} (SE: 71.83% and SP: 75.68%). (TABLE IV).

The study found that the mean blood BHB levels were 0.7±0.0 mmol·L^{-1} in healthy ewes and 1.5±0.0 mmol·L^{-1} in SCK groups, where the values ranging from 0.9–2.3 mmol·L^{-1}. Besides, hypoglycemia (<2.22 mmol·L^{-1}) was observed in ewes with SPT. These findings align with previous studies [22, 23]. Dietary restriction, lack of gluconeogenic precursors leading to NEB, and mobilization of body reserves, can explain the increased BHB levels during the transition period [7]. Thus, BHB < 0.8 mmol·L^{-1} indicates a good transition, while BHB > 0.85 mmol·L^{-1} is a sign of energy deficiency in ewes [24]. Appropriate nutritional management can maintain metabolic profiles within physiological ranges [16].

Hyperketonemia causes hypoglycemia by inhibiting gluconeogenesis, glucose uptake and utilization, reducing food intake, increasing lipolysis, and creating a vicious cycle [6, 25]. This cycle is further amplified in late pregnancy, as ewes may have a reduced capacity to utilize BHB as an energy source, contributing to the higher incidence of pregnancy toxemia (PT) during this period [25]. Insuline secretion, may have potential inhibitory role of ketogenesis and regulating role in the utilization of ketone bodies, and uptake of BHB as well as acetate [26]. Lipotoxicity of NEFAs on the function of β-pancratic cells compromises insulin production [9]. Hypocalcemia observed in SCK ewes may increase the depressive action of ketone bodies on gluconeogenesis [27]. This study found that 100% of SCK ewes also exhibited subclinical hypocalcemia, highlighting this close association. Measuring both Ca and BHB levels is crucial for accurate differentiation and diagnosis [13]. Early lactation creates NEB [16], confirmed by increased BHB in the study ewes. SCK occurs due to the energy demand for high milk yield exceeding dietary intake, leading to body reserve mobilization and increased NEFAs [26]. Insulin resistance during early lactation increases lipolysis and hepatic ketogenesis [1, 26]. Thus, hyperketonemia and hypoglycemia indicate early SCK and NEB, reflecting maternal energy deficiency in Ouled Djellal ewes [4, 9, 18, 23]. However, Ouled Djelall ewes were more stable with hyperketonemia, suffering from SCK rather than clinical ketosis (ck) despite BHB >1.6 mmol·L^{-1}. This raises interesting questions about the unique metabolic adaptation of this breed.

The study found lower levels of TGs and cholesterol in ewes with SCK. This is consistent partly with some previous studies that

TABLE III
Blood metabolic parameters comparison between ewes with and without SCK

Metabolic Indicators		Period	Group		*P*		
			CON	SCK	Group	Period	Group × Period
Energetic Indictors	BHB (mmol·L^{-1})	LP	0.7±0^{a}	1.5±0.1^{b}			
		EL	0.7±0^{a}	1.5±0.1^{b}			
	Glucose (mmol·L^{-1})	LP	2.77±0.11^{a}	2.33±0.05^{b}	0.0001	0.721	0.059
		EL	2.66±0.05	2.49±0.05			
	TGs (g·L^{-1})	LP	0.32±0.02^{aA}	0.23±0.01^{b}	0.001	0.002	0.088
		EL	0.23±0.01^{B}	0.21±0.01			
	Cholesterol (g·L^{-1})	LP	1.63±0.05^{a}	1.42±0.05^{bA}	0.0001	0.387	0.08
		EL	1.68±0.07^{a}	1.26±0.02^{bB}			
Enzymatic indicators	AST (U·L^{-1})	LP	82.04±1.75^{aA}	101,91±3.47^{bA}	0.0001	0.001	0.729
		EL	94.67±2.99^{Ab}	117.25±3.78^{bB}			
	ALT (U·L^{-1})	LP	12.77±0.34^{aA}	14.17±0.38^{bA}	0.003	0.005	0.91
		EL	14.1±0.57^{B}	15.61±0.45^{B}			
	GGT (U·L^{-1})	LP	41.75±0.73^{aA}	44.53±0.89^{bA}	0.013	0.0001	0.574
		EL	53.34±1.48^{B}	57.71±1.48^{B}			
	ALP (U·L^{-1})	LP	102.18±7.93^{a}	151.68±10.47^{bA}	0.0001	0.225	0.061
		EL	93.17±7.77^{a}	193.27±13.53^{bB}			
	CK (U·L^{-1})	LP	86.6±1.84^{a}	108.58±6.87^{bA}	0.001	0.074	0.079
		EL	87.05±3.8^{a}	159.39±16.75^{bB}			
	LDH (U·L^{-1})	LP	260.09±5.73^{aA}	291.38±8.14^{bA}	0.005	0.002	0.885
		EL	296.16±10.37^{B}	330.83±12.07^{B}			
Mineral indicators	Ca (mmol·L^{-1})	LP	2.17±0.02^{a}	1.83±0.04^{b}	0.0001	0.463	0.648
		EL	2.22±0.02^{a}	1.84±0.04^{b}			
	P (mmol·L^{-1})	LP	1.29±0.04^{aA}	1.59±0.04^{bA}	0.873	0.077	0.0001
		EL	1.67±0.06^{aB}	1.4±0.04^{bB}			
	Mg (mmol·L^{-1})	LP	0.75±0.008^{a}	0.69±0.01^{b}	0.0001	0.816	0.055
		EL	0.78±0.01^{a}	0.66±0.01^{b}			
	Na (mmol·L^{-1})	LP	144.6±0.4^{a}	141.00±0.8^{b}	0.0001	0.005	0.238
		EL	147,5±0,5^{a}	142,2±0,6^{b}			
	K (mmol·L^{-1})	LP	4.37±0.09^{a}	3.98±0.05^{b}	0.0001	0.423	0.429
		EL	4.48±0.11^{a}	3.98±0.06^{b}			

^{a,b}: Values within a row with different superscripts differ significantly at $P<0.05$, characterizing a group effect. ^{A,B}:Values within a column with different superscripts differ significantly at $P<0.05$, characterizing a period effect. LP: late pregnancy; EL: early lactation

TABLE IV
Optimum cut-off point of metabolic indicators for SCK

Metabolic indicators	cut-off	Sensitivity SE (%)	Specificity SP (%)	AUC	*P* (Area=0,5)
Glucose	≤ 2.22	40.85	100.00	70.7	< 0.0001
Cholesterol	≤ 1.52	80.28	64.86	78.9	< 0.0001
ASAT	> 94.19	74.65	83.78	84.4	< 0.0001
ALAT	> 13.60	63.38	64.86	69.1	0.0003
GGT	> 54.73	38.03	89.19	66.9	0.0015
ALP	> 123.00	77.46	81.08	85.0	< 0.0001
CK	> 99.43	53.52	91.89	73.7	< 0.0001
LDH	> 305.00	49.30	89.19	67.1	0.0009
Ca	≤ 2.10	77.46	100.00	94.4	< 0.0001
Mg	≤ 0.72	71.83	81.08	77.1	< 0.0001
Na	≤ 142.00	47.89	91.89	77.7	< 0.0001
K	≤ 4.1	71.83	75.68	79.3	< 0.0001

reported increased TGs and decreased cholesterol in ewes with PT [24, 28]. However, other studies found elevated cholesterol during SCK induction [7, 29], while some reported no influence of SCK on these variables [26]. During late pregnancy feed restriction, plasma TGs, VLDL–TGs and VLDL secretion decrease due to reduced liver VLDL synthesis capacity, causing TGs accumulation in ketotic ewes [21, 29]. In clinical PT, reduced cholesterol suggests compromised hepatic lipoprotein secretion inducing fat accumulation [11, 26]. As SCK reduces appetite [28] and cholesterol follows dry matter intake [30], NEB links to lower cholesterol [31]. Lower cholesterol and TGs levels in the present study are signs of hepatic lipidosis often accompanying ketosis. Thus, NEB may disrupt hepatic lipid metabolism, resulting in SCK. Lower cholesterol during severe NEB suggests it may predict energy balance in the transition period. This highlights the interconnectedness of energy metabolism and lipid homeostasis during metabolically challenging period like peripartum.

The study found increased activity of liver enzymes AST, ALT, and GGT in ewes with SCK, consistent with previous studies [21, 22, 32, 33]. However, some studies did not find changes in AST and ALT in PT [4, 23, 34]. The elevated liver enzymes may be attributed to hepatic damage or fatty infiltration leading to enzyme leakage, due to NEB [10]. High ketone bodies cause hepatic oxidative stress, apoptosis, and inflammation [21]. AST may be the best liver function marker in SCK and indicates early hepatic lipidosis [35]. The reduction in cholesterol with elevated liver enzymes suggests the liver's role in ketosis [28]. Previous studies reported that PT associated with an elevated level of ALP [10]. The reason of the highest ALP activity in SCK Ouled Djellal ewes could be a negative effect of NEB on hepatic tissue and consequently resulted in rise of ALP activity originating from the liver [31]. Plasma LDH and CK activities also increased in SCK ewes and highest in clinical ketosis [22, 32, 33], indicating possible muscle damage [37]. Elevated AST and CK suggest muscle protein mobilization due to NEB [34] or insufficient protein intake [11]. AST elevation may also be due to fatty infiltration in kidneys and heart, and CK elevation indicates muscle degeneration [36]. High CK in PT could relate to cardiac and skeletal muscle lesions due to lipotoxicity and oxidative stress from hyperketonemia [36, 37]. This is supported by the correlations between cardiac markers used to assist diagnoses of an acute myocardial infarction as CK-MB, troponin I and BHB [36, 37]. Increased AST, CK and LDH in the periparturient period may indicate reversible myocardial and skeletal muscle damage from subclinical hypocalcemia associated with SCK, as increased cardiac biomarkers suggest myocardial damage in parturient paresis [38]. Therefore, elevated AST and CK, along with hypocalcemia, may serve as critical diagnostic indicators of SCK in Ouled Djelall ewes, related to the pathogenesis involving cardiac, skeletal muscle lesions, NEB, and muscle protein catabolism. LDH increases may reflect liver, skeletal and cardiac muscle damage, indicated by increased AST and CK [33], though LDH is not an early SCK indicator in this study. Overall, elevations in AST, ALT, GGT, ALP, CK, and LDH can suggest that SCK in Ouled Djellal ewes may have broader metabolic impacts extending beyond the liver to involve muscle tissue, potentially including cardiac muscle. It's essential to interpret these findings in conjunction with other clinical signs, BHB levels, and the overall context of the animal's health status to arrive at an accurate diagnosis.

Hypocalcemia was observed in SCK ewes during late pregnancy (associated with hypomagnesemia and hyperphosphatemia) and early lactation (with hypomagnesemia and hypophosphatemia). This aligns with some previous studies [39] but contrasts others [22, 38]. The hypocalcemia may be attributed to increased fetal calcium demands [39], colostrum production [34], reduced feed intake [34, 35], or fatty liver impairing vitamin D hydroxylation [30]. Hypocalcemia and NEB are connected, with most natural PT cases associated with hypocalcemia [27]. Hypophosphatemia in early lactation may be due to hyperparathyroidism increasing urinary phosphorus loss to compensate for hypocalcemia, and increased phosphorus demands for milk production [34]. Hypomagnesemia is linked to enhanced lipolysis in PT [40]. Hypokalemia and hyponatremia were observed, likely due to reduced feed intake, dehydration, urinary losses from ketoacidosis, and impaired renal reabsorption [34]. Hypokalemia indicates metabolic acidosis and is a prognostic indicator for ketosis severity [22], also a good diagnostic indicator for SCK in Ouled Djellal ewes. Consequently, SCK caused electrolyte and mineral disturbances attributed to starvation, renal losses from ketoacidosis or renal damage, and impaired regulatory mechanisms [5, 34]. This study revealed significant electrolyte and mineral imbalances in Ouled Djellal ewes with SCK, further highlighting the role of kidney in the pathogenesis of ketosis.

CONCLUSION

Ketonemia characterized by elevated BHB levels, is an essential feature of SCK in Ouled Djelall ewes, reflecting NEB and inadequate long-term feeding. Twin pregnancy and low body condition score (BCS < 2.5) were major risk factors for SCK due to NEB. Significant changes of energetic, enzymatic and mineral metabolites reveal the involvement of the liver, kidney, and heart in ketosis pathogenesis, its impact on electrolyte and mineral balance, and suggest systemic and complexity of the disease.

Recommendations

Preventing ketonemia is essential to improving animal health, welfare, and productivity. Feeding ewes according to their energy and protein requirements during the periparturient period while monitoring BHB and BCS, especially in twin-bearing ewes, is recommended to prevent ketosis.

Ethical Statement

This study received approval from the Institutional Animal Care Committee of the National Administration of the Algerian Higher Education and Scientific Research (Approval no: 98–11, Law of August 22, 1998).

Conflict of Interest

The authors declared that there is no conflict of interest.

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