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# Oral sugar and vasopressin: Possible alternative in the management of ovine pregnancy toxemia

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ABSTRACT

Ovine pregnancy toxemia, a disease that affects adult ewes in the final stages of pregnancy and which is caused by a negative energy imbalance, requires the usage of dextrose substances for treatment. It has been proven that it is possible to induce reticular groove closure by means of an intravenous injection of lysine-vasopressin (LVP) at doses of 0.08 IU/kg B.W., which enables oral administration of glucose solutions that can be used in pregnancy toxemia therapy. The aim of this research is to compare the effectiveness of conventional treatment with i.v. glucose solution versus coadministration of LVP and a commercial sugar-water solution. This was administered orally to eight ewes to which we experimentally had provoked pregnancy toxemia by fasting. The use of commercial sugar, replacing other glucose solutions, could facilitate and cheapen the therapy for this disease significantly. The results obtained indicate that the joint administration of LVP and an oral solution of commercial sugar produce a noteworthy increase in glycaemia, which has a longer effect over time despite being less marked than with i.v. administration of glucose solution. Therefore, it could be used in the therapy of this disease. Besides, it is also able to normalize other parameters used to evaluate energy metabolism in ewes during the days of the trial.

## 1. Introduction

Ovine pregnancy toxemia (PT) is a disease that affects pregnant ewes in the last third of pregnancy and is generally associated with multiple gestations, although it can occur in single gestations when nutritional conditions and/or climatic conditions are poor (Andrews, 1997; Cal-Pereyra et al., 2012; Crilly et al., 2021; Duehlmeier et al., 2011; Macrae, 2020; Sargison, 2007; Simões et al., 2020; Van Saun, 2000). It is due to the inability of the ewe to meet the increased metabolic demand of the growing fetuses during the final period of gestation (Cal-Pereyra et al., 2012; Mohammadi et al., 2016; Sargison, 2007; Silva et al., 2022; Simões et al., 2020; Vasava et al., 2016) and according to Affan et al. (2022) can present acutely or chronically.

Traditionally, this disease appears in extensive sheep farming, but it is more and more frequent to find it in intensive production systems, especially when assisted reproduction techniques are applied, obtaining a large number of twin births and/or in overfed ewes and with significant accumulation of fat (Cal-Pereyra et al., 2012; Crilly et al., 2021; Ratanapob et al., 2018; Rook, 2000; Van Saun, 2000). There are many studies dedicated to explaining the etiology of the disease, the etiopathogenic mechanisms, the symptoms, the clinicopathological characteristics and the therapeutic options (Affan et al., 2022; Aly and Elshahawy, 2016; Andrews, 1997; Cal-Pereyra et al., 2015a, 2015b, 2012; Crilly et al., 2021; Duehlmeier et al., 2011; González-Montaña

*Abbreviations:* PT, pregnancy toxemia, LTI-ULE, Laboratorio de Técnicas Instrumentales de la Universidad de León; LVP, lysine-vasopressin; i.v, intravenously; B. W, body weight; IU, International Units; β-HBA, β-hydroxybutyrate acid; NEFA, non-esterified fatty acids; AST, aspartate aminotransferase; ALT, alanine aminotransferase, GGT, γ-glutamyltransferase.

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et al., 2014; Karagiannis et al., 2014; Macrae, 2020; Rook, 2000; Sargison, 2007; Van Saun, 2000; Vasava et al., 2016).

When blood glucose is lower than 2.22 mmol/l, pregnant ewes are forced to use their fat and muscle protein reserves for energy, giving rise to the formation of ketone bodies (Crilly et al., 2021; Sargison, 2007; Simões et al., 2020). For this reason, hypoglycemia and ketonemia are frequent findings in toxemic ewes (Aly and Elshahawy, 2016; Andrews, 1997; Cal-Pereyra et al., 2015a, 2012; Constable et al., 2017; Crilly et al., 2021; Harmeyer and Schlumbohm, 2006; Jyothi et al., 2014; Roubies et al., 2003; Russel, 1985; Scott, 1995; Simões et al., 2020, 2020), although other laboratory assessments have been used such as metabolic acidosis (Crilly et al., 2021; Simões et al., 2020; Van Saun, 2000), decreased fructosamine serum (Iqbal et al., 2022; Rook, 2000; Silva et al., 2022), increased AST and other enzymes (Cal et al., 2009, 2009; Jyothi et al., 2014; Ortolani and Benesi, 1989; Silva et al., 2022; Yarim and Ciftci, 2009), increased plasmatic cortisol (Aly and Elshahawy, 2016; Cal-Pereyra et al., 2015a; Crilly et al., 2021; Silva et al., 2022), increased non-esterified fatty acids (NEFA) (Raoofi et al., 2013; Silva et al., 2022) and hypocalcemia (Raoofi et al., 2013; Roubies et al., 2003; Silva et al., 2022; Van Saun, 2000).

The clinical signs generally appear in the 2–3 weeks prior to delivery and although at first are mild and may go unnoticed (Affan et al., 2022; Rook, 2000; Sargison, 2007). These signs quickly evolve towards a neurological and digestive condition, which culminates after a comatose phase, with the death of the animal (Cal-Pereyra et al., 2012; Constable et al., 2017; Duehlmeier et al., 2011; Sargison, 2007; Scott, 1995; Simões et al., 2020; Van Saun, 2000). Up to 40% of the ewes in a flock can be affected and with a mortality of 80% of the affected animals (Crilly et al., 2021; Rook, 2000), and even between 80% and 90% of the untreated ewes die within a week from the beginning of the symptoms (Cal-Pereyra et al., 2012). The recovery of clinically affected ewes, even with treatment, is around 30% (Crilly et al., 2021; Macrae, 2020) and the mortality of lambs born to ewes with a negative energy balance is greater than lambs born to ewes without this problem (Ratanapob et al., 2018). Thus, the rate of lambs live births, fetal birth weight, and lamb viability are severely affected, leading to higher rates of perinatal mortality (Crilly et al., 2021; Sargison, 2007).

Due to the high mortality of ewes and the fact that the treatment is expensive and laborious, and in many cases without ensuring a positive result, it is very important to carry out an early diagnosis of the disease. It is necessary to establish the appropriate measures early, always before there is severe metabolic acidosis, renal failure, irreversible neurological lesions have been established and the animal is not yet in decubitus (Andrews, 1997; Cal-Pereyra et al., 2012; Rook, 2000). In addition, in most cases, this disease should be seen more as a herd problem than an individual problem (Cal-Pereyra et al., 2012). Diagnosis is often based on history and clinical signs, and confirmation is done by laboratory analysis, detecting hyperketonemia, usually hypoglycemia, and approximately 25% of affected ewes will also be hypocalcemic and have elevated enzymes (AST, GGT, GLDH, etc.) (Macrae, 2020; Vasava et al., 2016).

All therapeutic trials are aimed at normalizing blood glucose. Therefore, it is a priority to facilitate the formation of glucose and its use at the tissue level, as well as promoting the use of ketone bodies until their normal concentration in blood is restored (Braun et al., 2010; Cal-Pereyra et al., 2012; Constable et al., 2017; Crilly et al., 2021; Jyothi et al., 2014; Rook, 2000).

Glucose administration is the basis of therapy in ovine pregnancy toxemia. Thus, the most common has been to resort to intravenous isotonic glucose solution, accompanied by oral glycoprecursors such as glycerol, sodium propionate or propylene glycol (Cal-Pereyra et al., 2015b; Constable et al., 2017; El-Hamamsy et al., 1990; Sargison, 2007). Therefore, the exclusive use of parenteral glucose is a palliative rather than a curative treatment, and in addition, its repeated administration can cause polypnea, muscle tremors, weakness and collapse, and even swelling and infection in the inoculation area are frequent (Constable et al., 2017).

These glycoprecursors must be administered orally for their metabolization in the rumen, although, on the other hand, it has been verified that the stimulation of the reticular groove allows the oral administration of glucose solutions that reach the abomasum and therefore can be used, at least experimentally, in the therapy of ovine toxemia of pregnancy. Taking this last appreciation into account, we want to go one step further in therapy at the field level. Therefore, this research aims to verify if the joint use of lysine-vasopressin and a commercial solution of sugar water by mouth can be used in the treatment of ovine pregnancy toxemia. We intend that the intravenous administration of lysine-vasopressin induces the closure of the reticular groove in adult sheep, in negative energy balance, allowing the sugar solution to reach the abomasum directly, from where it can be immediately absorbed. The use of commercial sugar, instead of other glucose solutions, could significantly facilitate and reduce the cost of therapy for this disease.

## 2. Material and methods

### 2.1. Animals and handling. Animal protection regulations

We used 8 healthy Assaf ewes, aged between 4 and 6 years, not pregnant, not lactating, with a mean weight of 59.1 + 3.5 kg (Mean + SD), a body condition of 3.5–4.0 according to the Russell scale (Russel, 1985) and without clinical signs of disease. The animals remained stabled in the Experimental Farm of the Faculty of Veterinary Medicine of the University of León.

All experimental procedures were carried out in compliance with the provisions of the Directive that regulates the use of animals for scientific purposes in the European Union (European Commission, 2010/63/UE) (European-Union, 2010) and the Royal Decree that regulates experimentation, teaching and animal protection in Spain (Royal Decree 53/2013) (BOE, 2013), and prior approval of the local Animal Welfare Committee [Subcomité para la Experimentación y Bienestar animal (OEBA- ULE)] and the Dirección General de Producción Agropecuaria en Infraestructuras Agrarias de la Junta de Castilla y León, case number OEBA-ULE-013–2016.

### 2.2. Feed and water

Ewes were overfed with concentrate and forage during the last month, until causing a slight fatness. The ration administered was composed of concentrate (0.50 kg/day of barley and 0.15 kg/day of soybean meal, 45%) and with free access to good quality hay (around 1.5 kg/day), which provides a final ration of 2.03 kg, 92% dry matter (DM), 14.0% crude protein (CP) and 2.65Mcal/kg DM. Subsequently, they were subjected to a fasting period, which ranged from 3 to 5 days (72–120 h), to achieve a negative energy balance, verified with the appearance of ketonuria diagnosed using semiquantitative test strips. The water supply was maintained at all times, although the bedding material was removed from the area where the ewes were stabled to avoid the ingestion of straw or other components. Food fasting was maintained throughout the experimental protocol. Once the test was finished, the ewes were fed again as at the beginning.

### 2.3. Experimental protocol

One group, called Treatment group, consisted of 4 ewes to which lysine-vasopressin (LVP) ([Lys8]-Vasopressin Sigma, 250 IU/mg) was applied intravenously (i.v.) at a dose of 0.08 IU/kg B.W. followed by the immediate administration of a sugary solution, orally. This solution was obtained by adding 100 g of commercial sugar (Azúcar, Azucarera AB Sugar Company) dissolved in 1000 ml of water, through a probe introduced into the initial section of the esophagus. The sugar used is the one normally used for human consumption, which is chemically sucrose ($C_{12}H_{22}O_{11}$), also called common sugar or table sugar, and it is a

disaccharide made up of glucose + fructose, obtained in our case from sugar beet (*Beta vulgaris L.*), with 99.9% sugar purity and 4 kcal per gram. The treatment was repeated every 12 h for four days (Table 1).

The Control group (ewes n = 4) received a standard treatment based on 50 ml of 50% commercial glucose serum (Glucose 50%, B. Braun, glucose 50%, glucose 500 mg/ml, equivalent to 550 mg/ml of anhydrous glucose, with energy value 2000 kcal/l), intravenously, every 12 h, similar to the therapy recommended by Andrews (1997, 1982) and Sargison (2007). This group also received LVP at the previously mentioned dose to eliminate possible variables attributable to its application. This therapeutic protocol, as in the previous group, was repeated every 12 h for four days (Table 1).

### 2.4. Sampling, storage, and processing of samples

Blood samples were taken from the jugular vein, by venipuncture with 10 ml syringes and 18 G needles, always before the administration of each treatment and 15 min after administration. To check the evolution of treatment, once the test was finished, a sample was taken 12 h after the last application. In the first experimental cycle, the animals were also sampled at 30 and 60 min, and after 2, 4, 8, and 12 h. This last sampling coincided with the next administration of LVP, carried out 12 h after the first application (Table 1, Fig. 1). Urine samples were collected at the start of the protocol and before each therapeutic trial, following the transient apnea method (Benech et al., 2015).

The blood samples, except for a fraction for the assessment of non-esterified fatty acids (NEFA) that must be performed on whole blood, were centrifuged at 4000 rpm (2200 xg) for 10 min and the plasma was frozen at − 20 °C until analysis at the Laboratorio de T é cnicas Instrumentales (LTI) de la Universidad de León.

### 2.5. Laboratory analysis

Analyzes were performed using a Cobas Integra 400 autoanalyzer (Roche Diagnostics, S.L). Glucose, creatinine, urea, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT) were analyzed using Roche Diagnostics reagents, while ß-hydroxybutyrate (βOHB) and non-esterified fatty acids in blood (NEFA) were measured by Ranbut® (D-3-Hydroxybutyrate, Randox Laboratories Ltd, UK) and NEFA® (Non-Esterified Fatty Acids, Randox Laboratories Ltd, UK) reagents, respectively.

Urine samples were analyzed *in situ* using semiquantitative test strips (Multistix 10 Visual, Bayer, USA) evaluating the pH, glucose, density, and total ketones.

**Table 1**
Experimental design. Treatment and sampling times.

| | Treated group (n = 6) | Standard treatment group (n = 4) |
|---|---|---|
| **Premedication (every 12 h, 4 days, 8 applications)** | LVP 0.08 UI/kg PV, i.v. | LVP 0.08 UI/kg PV, iv. |
| **Treatment (every 12 h, 4 days, 8 applications)** | 100 g of sugar (sacarose) dissolved in 1 L of water, *per os* | 50% glucose solution, 50 ml, i.v. |
| **Sampling in 12 h** | 0 h, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h (=0 h) | 0 h, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h (=0 h) |
| **Sampling in 4 days** | 0 h, + 15 min;<br>12 h, + 15 min;<br>24 h, + 15 min;<br>36 h, + 15 min;<br>48 h, + 15 min;<br>60 h, + 15 min;<br>72 h, + 15 min;<br>84 h, + 15 min;<br>96 h | 0 h, + 15 min;<br>12 h, + 15 min;<br>24 h, + 15 min;<br>36 h, + 15 min;<br>48 h, + 15 min;<br>60 h, + 15 min;<br>72 h, + 15 min;<br>84 h, + 15 min;<br>96 h |

### 2.6. Data study

Data were analyzed using the statistical software SPSS 20.0 (IBM, Armonk, NY). We studied both the evolution of samples during the first 12 h and throughout the four days of the experiment. For this, we carried out a descriptive analysis and a repeated-measures ANOVA to analyse the effects of the treatments over time (intragroup effect), whereas a Student's t-test was used for intra-group analysis (between treatments). Results are expressed as mean±SEM, and P-value was considered statistically significant when $P<0.05$.

Data are shown using tables where the data obtained in the descriptive analysis have been integrated, using letters and/or numbers to indicate the existence of statistically significant differences. In some cases, and when the data obtained have advised it, graphs have been used to better visualize the results.

## 3. Results

Four ewes, and only the first time they received LVP i.v., were more restless than normal, with slight tremors and vocalizing for a few minutes. Also, two of them urinated small amounts of urine several times and showed flatulence for a few minutes. These signs were not appreciated in the following administration of vasopressin.

After applying glucose i.v. for 15 min a significant increase in blood glucose was observed (from 2.71 to 20.76 mmol/l), while it only doubled with the administration of oral sugar (from 2.84 to 5.57 mmol/l). In both cases the maximum value was reached 15 min after therapy, to subsequently decrease progressively. At 8 h, higher glycemia values were observed in animals treated orally than those treated i.v. (3.77 vs. 3.45 mmol/l) and at 12 h post-therapy the values, in both treatments, were higher than the initial ones (Table 2, Fig. 2). Therefore, treatment with the sugary solution causes blood glucose to remain longer, without increasing excessively, and always showing higher values than the initial ones.

When studying the evolution of glycemia throughout the trial, it could be seen that the pattern was repeated in the successive applications of glucose therapy, observing a rapid and marked rise in i.v. therapy and a moderate rise in oral therapy. In both experimental groups, glycemia always remained above the initial values until the moment of the next administration, also observed higher glycemia, from 48 h until the end of the experiment, in those animals treated with the sugar solution than in animals treated intravenously (Table 3, Fig. 3).

In all the ewes, and from the first sampling, we detected the presence of ketonuria using test strips, in variable amounts, although, in most of them, the values were low and medium. Ketonuria was not detected in any ewes after starting the treatments. When studying the values of ßOHB we found that the evolution is similar in both experimental groups, with a significant decrease after 15 min, showing minimum values after one hour and being slightly higher in oral sugar therapy. Thus, with this treatment, the ßOHB in the blood required more time to decrease, but after 4 h it already presented values similar to the reference treatment and they were maintained until the end, being even lower than those found after the administration of i.v. glucose (Table 2, Fig. 2). In both therapies, the blood concentration of ßOHB was always lower than that found at the beginning of the trial. If we observe the values of ßOHB found throughout the experiment, we find that after all the applications the values of this ketone always decrease after 15 min, always remaining at much lower values than the initial ones (Table 3, Fig. 3).

The NEFAs, at all times, are lower in the oral sugar treatment than in the glucose solution treatment i.v., their values decrease 15 min after treatment and always remain below the initial values. We observed that the evolution of NEFA is similar to that of ßOHB, and is contrary to that of glycemia, decreasing from the beginning and increasing when glycemia decreases (Table 2, Fig. 2). The significant drop in NEFA observed after the first treatment is repeated after each therapeutic application

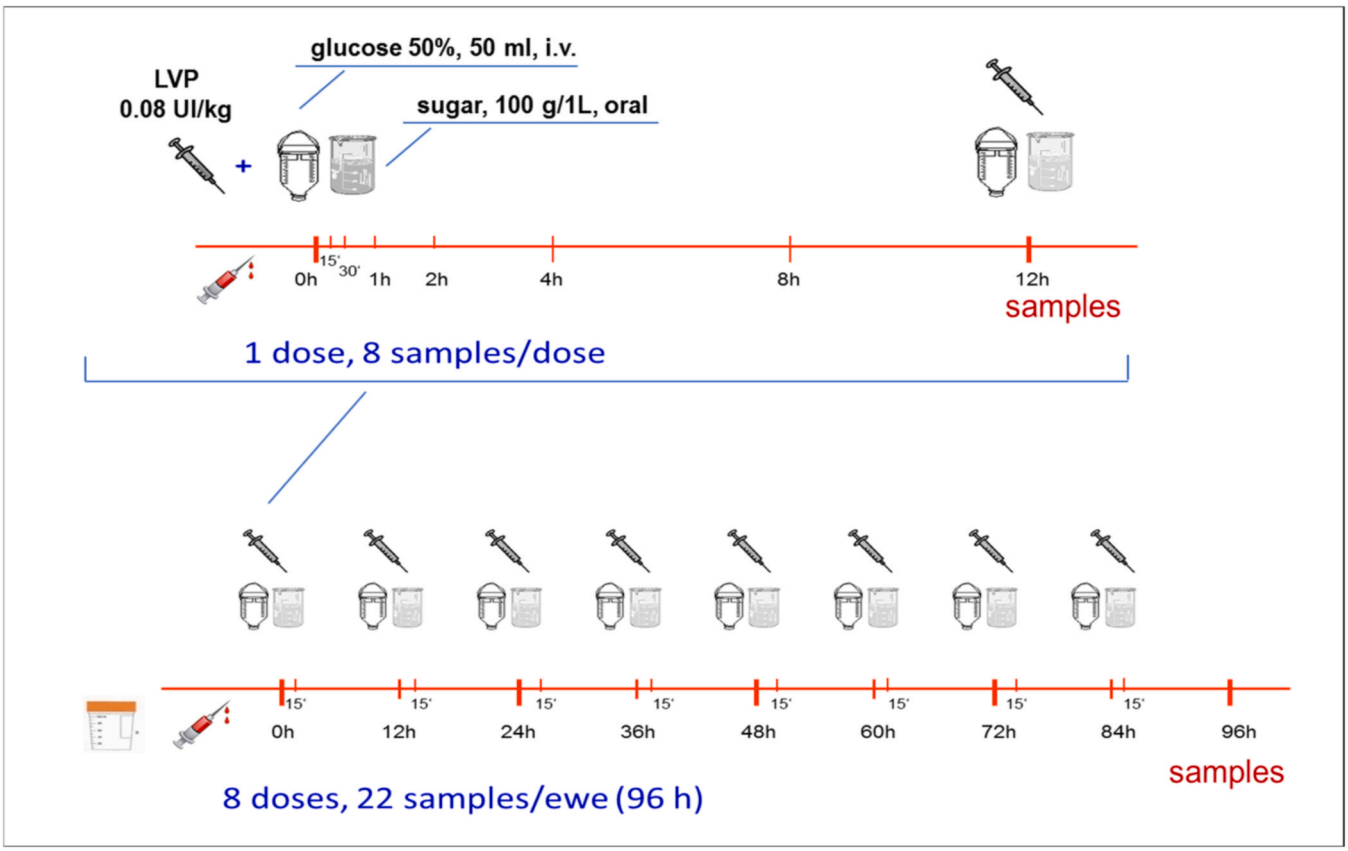


**Fig. 1.** Experimental design. Treatment and sampling times.

**Table 2**
Blood levels of glucose, βOHB, NEFA, and calcium (in mmol/l) in two treatments and in different sampling times, corresponding to 12 h after the administration of the treatment.

| Sampling | Glucose | | | | ß-OHB | | | | NEFA | | | | Calcium | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LVP + Sugar (n = 4) X̄ ± SD | | LVP + Glc i.v. (n = 4) X̄ ± SD | | LVP + Sugar (n = 4) X̄ ± SD | | LVP + Glc i.v. (n = 4) X̄ ± SD | | LVP + Sugar (n = 4) X̄ ± SD | | LVP + Glc i.v. (n = 4) X̄ ± SD | | LVP + Sugar (n = 4) X̄ ± SD | | LVP + Glc i.v. (n = 4) X̄ ± SD | |
| 0 h | 2.84 ± 0.34 | a | 2.71 ± 0.25 | a,e | 0.79 ± 0.36 | a, c | 0.75 ± 0.12 | a, d | 0.69 ± 0.13 | a | 0.79 ± 0.14 | a, c | 2.46 ± 0.15 | | 2.41 ± 0.11 | a,b |
| 15 min | 5.57 ± 1.09 | b, * | 20.76 ± 1.93 | b, * | 0.61 ± 0.22 | | 0.39 ± 0.07 | a, c | 0.54 ± 0.14 | b,c,e | 0.61 ± 0.08 | b, c | 2.42 ± 0.10 | * | 2.33 ± 0.19 | a,b, * |
| 30 min | 5.52 ± 1.11 | b, * | 16.57 ± 2.45 | c, * | 0.39 ± 0.17 | | 0.25 ± 0.05 | b, c | 0.40 ± 0.14 | b,d, e | 0.50 ± 0.17 | | 2.36 ± 0.12 | | 2.41 ± 0.10 | |
| 1 h | 4.88 ± 1.11 | b, * | 12.40 ± 3.05 | d, * | 0.32 ± 0.07 | a, b | 0.24 ± 0.09 | c, | 0.36 ± 0.11 | c,d,e | 0.44 ± 0.09 | b, c | 2.34 ± 0.16 | | 2.30 ± 0.16 | |
| 2 h | 4.28 ± 0.43 | c | 7.76 ± 3.87 | a | 0.36 ± 0.14 | b | 0.32 ± 0.11 | | 0.45 ± 0.25 | a,b, c,d | 0.43 ± 0.12 | b | 2.35 ± 0.15 | * | 2.30 ± 0.11 | a,c, * |
| 4 h | 3.68 ± 0.31 | d | 4.26 ± 1.77 | e | 0.35 ± 0.16 | b | 0.37 ± 0.11 | | 0.36 ± 0.15 | d | 0.46 ± 0.11 | b, c | 2.36 ± 0.14 | * | 2.32 ± 0.10 | a, * |
| 8 h | 3.77 ± 0.23 | e | 3.45 ± 0.49 | a,e | 0.50 ± 0.12 | c | 0.51 ± 0.05 | d | 0.37 ± 0.10 | b,c, d,e | 0.66 ± 0.27 | | 2.36 ± 0.19 | | 2.40 ± 0.08 | b,c |
| 12 h | 3.29 ± 0.18 | f | 3.53 ± 0.28 | a,e | 0.48 ± 0.09 | c | 0.45 ± 0.07 | d | 0.55 ± 0.25 | c | 0.67 ± 0.23 | c | 2.37 ± 0.11 | | 2.51 ± 0.12 | c |

LVP + Sugar: lysine-vasopressin + oral sugar; LVP + Glc i.v.: lysine-vasopressin + commercial glucose solution i.v. NEFA - non-esterified fatty acids; βOHB - β-hydroxybutyrate. The values are expressed as mean± standard deviation. Different letters per column indicate statistical differences between sampling times. Asterisk (*) indicates statistical differences between treatments. Statistical differences: $P < 0.05$.

and in general the values are lower with oral therapy than with the application of i.v. glucose solution, except after 84 h (Table 3, Fig. 3).

Enzymes do not show significant variation in the first 12 h and are maintained throughout the trial. Both ALT and GGT are higher in the ewes in the sugar-treated group, probably because these ewes already have higher values from the start. It is important to note the increase in ALT in ewes with oral therapy as the trial progresses, whereas with i.v. therapy they remain constant.

Urea and creatinine are similar in both groups. We only have to point out that with the administration of oral sugar, they increase slightly up to 30 min and then remain constant, except at 12 h when they decrease slightly, coinciding with a negative energy balance. In contrast, in ewes treated with i.v. solution urea and creatinine remain constant, except in the last sampling. When observing the evolution of these parameters in the entire trial, we see that the administration of glucose (both i.v. and oral) does not cause significant changes.

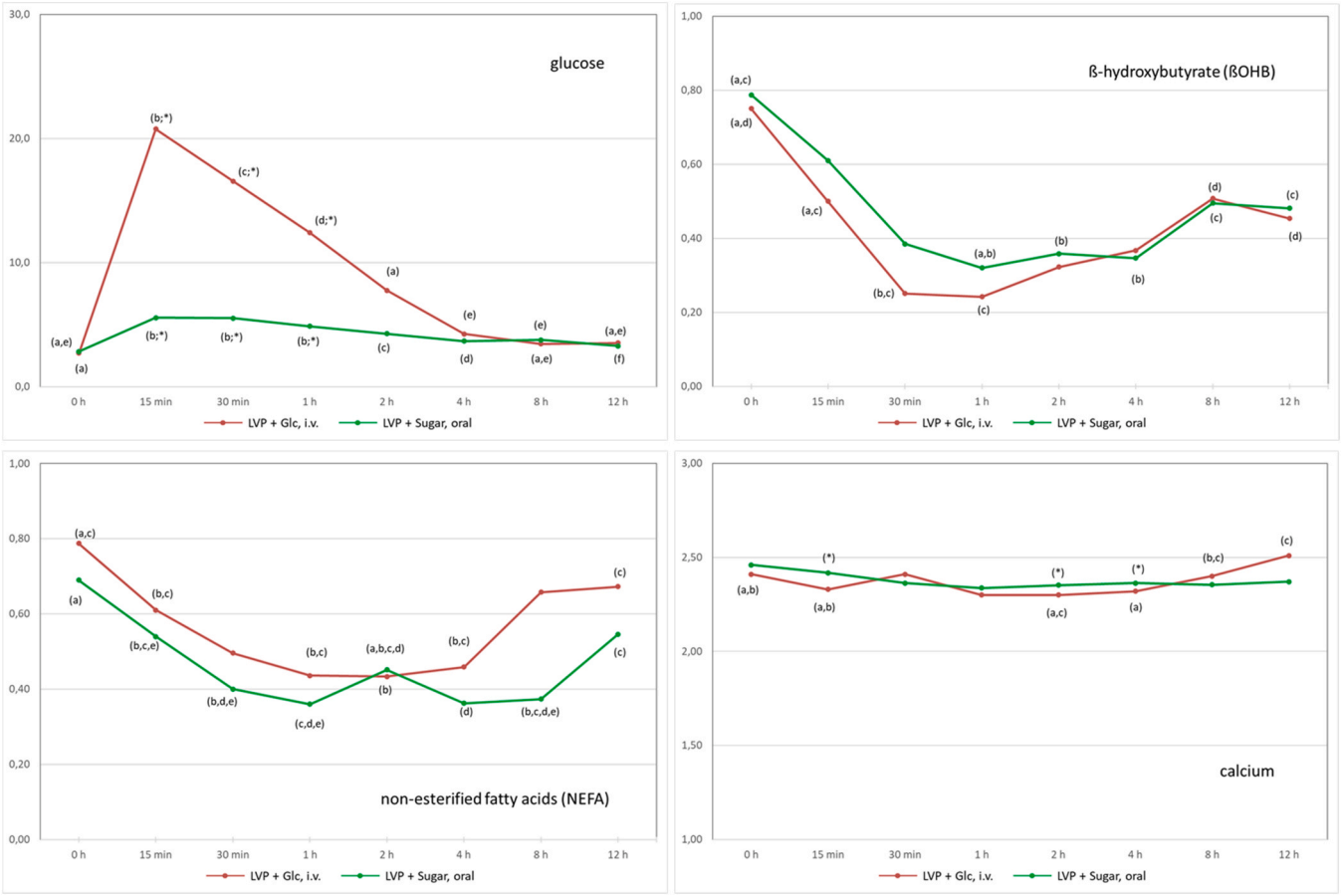


**Fig. 2.** Evolution of blood glucose, βOHB, NEFA, and calcium (in mmol/l) at different sampling times, corresponding to 12 h after the administration of treatment. LVP + Sugar: lysine-vasopressin + oral sugar; LVP + Glc i.v.: lysine-vasopressin + commercial glucose solution i.v. Different letters indicate statistical differences between sampling times. Asterisk (*) indicates statistical differences between treatments ($P < 0.05$).

Calcemia, in ewes treated with oral sugar, remains constant throughout the experience, although after decreasing slightly in the first-hour post-administration, while i.v. produces some variations in calcium, with a slight decrease in the first 15 min to increase until the end of the first treatment, and always with values above the initial value (Table 2, Fig. 2). Throughout the entire trial, the different treatment cycles cause a slight decrease in calcium which, although not significant, is lower when oral sugar therapy has been applied. The final calcemia is less than that found at the beginning of the experience (Table 3, Fig. 3).

## 4. Discussion

In previous experiences, adverse reactions such as bleating, more restless animals, tachypnea, tremors, flatulence, and ewes repeatedly adopting urination postures had already been described (González-Montaña et al., 2014). These effects have been justified by the applied vasopressin, by acting on vascular and gastrointestinal smooth muscle, and by alterations in vagal and sympathetic tone (Jackson, 2003). Some similar symptoms were cited in ovine to which NaCl solutions were applied to the carotid artery trying to stimulate the reticular groove, since by producing an increase in plasmatic osmolarity, the release of endogenous vasopressin was caused (Mikhail et al., 1988).

This behavior of ewes becomes more serious when higher doses of LVP (González-Montaña et al., 2014) have been tested, and nausea, cutaneous vasoconstriction, urgency to defecate, diarrhea, colic, cardiac complications such as arrhythmias, decreased cardiac output, and myocardial ischemia have been referred (Jackson, 2003). On the contrary, using higher doses several investigators have not reported the adverse effects that we have found (El-Hamamsy et al., 1990; Mikhail et al., 1988).

In Russel's opinion (1985), glycemia, NEFAs and ketones (especially β-hydroxybutyrate) are the metabolites that must be studied to determine the energy situation of an animal, and therefore of a herd, since they are less affected by handling and sampling. However, they have some limitations such as the low specificity of NEFA and β-HBA, the diurnal variation of glucose and the cost of measurement (Iqbal et al., 2022). Glycemia, by itself, should not be used as a metabolic indicator due to the fluctuations it presents (Rook, 2000), being able to find normal and even hyperglycemia in females with clinical signs of pregnancy toxemia (Cal-Pereyra et al., 2012; Crilly et al., 2021; Raoofi et al., 2013; Sargison, 2007; Vijayanand et al., 2022). According to Sargison glycemia does not correlate with the severity of clinical signs or prognosis (Sargison, 2007), therefore normoglycemia does not exclude OPT and often occurs after fetal death, which is why it is associated with a poorer prognosis (Kabakci et al., 2003; Sargison, 2007; Souto et al., 2013).

In our case, by causing a fasting situation in the ewes in the trial, we have verified a significant decrease in glycemia and an increase in NEFA and βOHB. The initial glycemia recorded in the test ewes ranged between 2.36 and 3.18 mmol/l, which is slightly lower than that considered "physiological" for this species (Kaneko et al., 2008; Lacetera et al., 2001; Sargison, 2007; Silva et al., 2022; Van Saun, 2000), and coincides with that found in ewes subjected to hypo-feeding. In the opinion of Cal-Pereyra et al. (2015a) blood glucose levels of 1.59 ± 0.24 mmol/l (28.62 ± 4.33 mg/dl), along with β-HBA (2.26 mmol/l) and cortisol (5.47 mg/ ml), allow the diagnosis of subclinical pregnancy toxemia, although values between 1.0 mmol/l and 2.7 mmol/l have been found in ewes with clinical signs of this disease (Kabakci et al., 2003; Lacetera

**Table 3**
Blood levels of glucose, βOHB, NEFA, and calcium (in mmol/l) in two treatments and at different sampling times throughout the trial.

| Sampling | Glucose | | | | ß-OHB | | | | NEFA | | | | Calcium | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LVP + Sugar (n = 4) | | LVP + Glc i.v. (n = 4) | | LVP + Sugar (n = 4) | | LVP + Glc i.v. (n = 4) | | LVP + Sugar (n = 4) | | LVP + Glc i.v. (n = 4) | | LVP + Sugar (n = 4) | | LVP + Glc i.v. (n = 4) | |
| | X̄ ± SD | | X̄ ± SD | | X̄ ± SD | | X̄ ± SD | | X̄ ± SD | | X̄ ± SD | | X̄ ± SD | | X̄ ± SD | |
| 0 h | 2.84 ± 0.34 | a | 2.71 ± 0.25 | a,i | 0.79 ± 0.36 | | 0.75 ± 0.12 | | 0.69 ± 0.13 | a,c, e,f | 0.79 ± 0.14 | a,c | 2.46 ± 0.15 | | 2.41 ± 0.11 | a,b, d, |
| + 15 min | 5.57 ± 2.20 | b, * | 20.76 ± 2.14 | b,f, * | 0.61 ± 0.31 | | 0.39 ± 0.13 | | 0.54 ± 0.10 | b,c, f | 0.61 ± 0.14 | | 2.42 ± 0.14 | | 2.33 ± 0.17 | |
| 12 h | 3.40 ± 0.30 | a | 3.39 ± 0.41 | a,c,i | 0.42 ± 0.23 | | 0.47 ± 0.02 | | 0.57 ± 0.30 | | 0.68 ± 0.24 | a,c,d, e,f | 2.40 ± 0.09 | | 2.48 ± 0.12 | d, |
| + 15 min | 5.75 ± 0.50 | b, * | 21.03 ± 1.76 | b,f, * | 0.27 ± 0.14 | | 0.31 ± 0.09 | | 0.45 ± 0.18 | b,c, d,f | 0.48 ± 0.19 | b,c,d | 2.41 ± 0.08 | | 2.38 ± 0.22 | |
| 24 h | 3.18 ± 0.64 | a | 3.68 ± 0.29 | c,d,i | 0.54 ± 0.12 | | 0.44 ± 0.15 | | 0.53 ± 0.21 | | 0.66 ± 0.25 | a,d,f | 2.35 ± 0.17 | | 2.54 ± 0.12 | |
| + 15 min | 5.11 ± 1.64 | b, * | 20.49 ± 2.89 | b,c, * | 0.37 ± 0.19 | | 0.29 ± 0.16 | | 0.47 ± 0.18 | | 0.50 ± 0.20 | c,d,e, f | 2.43 ± 0.20 | a | 2.37 ± 0.22 | |
| 36 h | 3.05 ± 1.49 | a | 4.12 ± 0.48 | d, | 0.44 ± 0.02 | a,c | 0.46 ± 0.19 | | 0.52 ± 0.08 | | 0.61 ± 0.43 | | 2.36 ± 0.10 | | 2.47 ± 0.12 | |
| + 15 min | 3.65 ± 0.39 | b, * | 20.67 ± 1.01 | b,c,f, g, * | 0.43 ± 0.10 | | 0.32 ± 0.08 | | 0.43 ± 0.10 | c,d, e,f | 0.45 ± 0.13 | b,c,e, f | 2.29 ± 0.09 | | 2.42 ± 0.10 | |
| 48 h | 5.19 ± 0.69 | a | 3.87 ± 0.42 | d,h, | 0.39 ± 0.14 | | 0.44 ± 0.11 | | 0.47 ± 0.06 | | 0.60 ± 0.21 | b,c, d,e,f | 2.25 ± 0.14 | | 2.36 ± 0.12 | b,c, d |
| + 15 min | 3.98 ± 0.73 | b, * | 21.47 ± 1.85 | e,f, * | 0.26 ± 0.09 | a, b | 0.33 ± 0.11 | | 0.44 ± 0.07 | | 0.47 ± 0.14 | b,c, d,e,f | 2.23 ± 0.08 | | 2.27 ± 0.15 | |
| 60 h | 5.62 ± 0.85 | a | 4.25 ± 0.74 | a,d, | 0.35 ± 0.03 | | 0.30 ± 0.11 | | 0.47 ± 0.05 | | 0.63 ± 0.15 | d,e,f | 2.23 ± 0.13 | | 2.46 ± 0.10 | |
| + 15 min | 3.57 ± 0.01 | b, * | 22.99 ± 3.38 | f,g, * | 0.27 ± 0.01 | b,c | 0.30 ± 0.06 | | 0.44 ± 0.07 | | 0.49 ± 0.14 | a,b,c, d,e | 2.14 ± 0.18 | b | 2.26 ± 0.11 | c,d, |
| 72 h | 5.56 ± 0.94 | a | 3.83 ± 0.62 | a,c,d, i, | 0.46 ± 0.08 | c, * | 0.27 ± 0.05 | * | 0.46 ± 0.21 | | 0.67 ± 0.18 | a,c,d, e,f | 2.17 ± 0.15 | | 2.35 ± 0.13 | |
| + 15 min | 4.56 ± 0.72 | b, * | 22.44 ± 1.85 | g, * | 0.33 ± 0.08 | | 0.20 ± 0.08 | | 0.38 ± 0.11 | d,f | 0.46 ± 0.09 | | 2.22 ± 0.43 | | 2.26 ± 0.12 | |
| 84 h | 7.26 ± 0.92 | a | 3.65 ± 0.29 | a,h, | 0.34 ± 0.19 | | 0.39 ± 0.11 | | 0.54 ± 0.13 | e,f | 0.42 ± 0.18 | e,f | 2.12 ± 0.22 | | 2.37 ± 0.14 | a,b, c |
| + 15 min | 5.15 ± 0.92 | b, * | 19.87 ± 0.33 | b,c,f, g, * | 0.26 ± 0.07 | | 0.28 ± 0.15 | | 0.42 ± 0.05 | | 0.43 ± 0.13 | f | 2.17 ± 0.26 | | 2.30 ± 0.11 | c,d |
| 96 h | 3.10 ± 0.26 | c, * | 3.24 ± 0.26 | I,j, * | 0.33 ± 0.07 | | 0.30 ± 0.15 | | 0.38 ± 0.07 | f | 0.49 ± 0.17 | a,b,c, d,f | 2.11 ± 0.25 | | 2.22 ± 0.14 | d, |

LVP + Sugar: lysine-vasopressin + oral sugar; LVP + Glc i.v.: lysine-vasopressin + commercial glucose solution i.v. NEFA - non-esterified fatty acids; βOHB - β-hydroxybutyrate. The values are expressed as mean±standard deviation. Different letters per column indicate statistical differences between sampling times. Asterisk (*) indicates statistical differences between treatments. Statistical differences: $P < 0.05$.

et al., 2001). Sargison reports that plasma glucose concentrations for confirmed cases of ovine gestational toxemia range from 0.5 to 7.0 mmol/l (Sargison, 2007). Therefore, we could say that our ewes, since they do not show obvious symptoms, are in subclinical toxemia.

All ewes in the experiment had been subjected to a slight overfeeding in the weeks prior to the test, followed by a fast of 72–120 h to experimentally induce a "pregnancy toxemia" (Cal et al., 2009; Cal-Pereyra et al., 2015a), which justifies that these animals had blood glucose in the lower part of the reference values, and also with variable amounts of ketones in urine, detected by test strips. The increased energy requirement in ewes, as a result of increased fetal demands, together with restricted intake, either due to fasting or resulting from the uterine expansion in ewes with multiple fetuses, contribute to a negative energy balance during this period, which usually manifests in a drop in blood glucose (Aly and Elshahawy, 2016; Cal-Pereyra et al., 2012; Raoofi et al., 2013; Ratanapob et al., 2018; Vasava et al., 2016). Prolonged hypoglycemia also leads to hyperactivity of the adrenal glands, increasing cortisol secretion. The higher cortisol level is due not only to hypoglycemia but also to reduced liver metabolism, as well as continuous stress levels, circumstances present in the ewes in our experiment (Aly and Elshahawy, 2016). Iqbal (2022) recommends measuring fructosamine, which is the product of a non-enzymatic and irreversible reaction between glucose and protein, whose concentration depends on the average blood glucose concentration during the previous two weeks and on the half-life of proteins in the blood. Therefore, fructosamine can be used as a potential indicator to diagnose pregnancy toxemia, even when β-HBA levels are still within reference values (Iqbal et al., 2022).

Monitoring the energy status of pregnant ewes by measuring the serum concentration of NEFA and β-HBA is an alternative and useful technique (Iqbal et al., 2022). The revised bibliography indicates that samplings in fasting ewes, as well as in toxemic ewes, show an increase in both βOHB (Iqbal et al., 2022; Lacetera et al., 2001; Russel, 1985; Silva et al., 2022) and NEFA (Aly and Elshahawy, 2016; Iqbal et al., 2022; Lacetera et al., 2001; Silva et al., 2022). This increase in ketone bodies is caused by fasting (with a reduction of oxalacetate) which, together with the increase in glucagon and epinephrine secretion, will stimulate lipolysis and glycogenesis, causing high lipomobilization (Sargison, 2007; Silva et al., 2022; Vasava et al., 2016). This hyperketonemia is aggravated by the lower utilization of ketones in peripheral tissues, since they cannot be burned or eliminated (Cal-Pereyra et al., 2015b; Riis, 1983). However, this author, Riis (1983), states that they will be used when the plasma concentration of β-hydroxybutyrate is above 2 mmol/l.

The βOHB values measured range between 0.34 and 1.15 mmol/l in fasting ewes, and several show values above 0.70 mmol/l, while the NEFA range between 0.45 and 1. 0.0 mmol/l and with several ewes above 0.60 mmol/l. βOHB values are slightly higher than the reference values (Kaneko et al., 2008; Lacetera et al., 2001; Silva et al., 2022), similar to those considered “normal” and which in healthy animals should be less than 0.8 mmol/l (Andrews, 1997; Mohammadi et al., 2016; Olfati et al., 2013) or of 1 mmol/l (Macrae, 2017; Russel, 1985), while β-HB concentrations of 0.8–1.6 mmol/l could suggest moderate malnutrition (Andrews, 1997; Olfati et al., 2013). βOHB concentrations above 1.6 mmol/l in individual ewes represent severe energy malnutrition, with gestational toxemia likely to develop as pregnancy progresses and fetal energy requirements increase, unless changes are

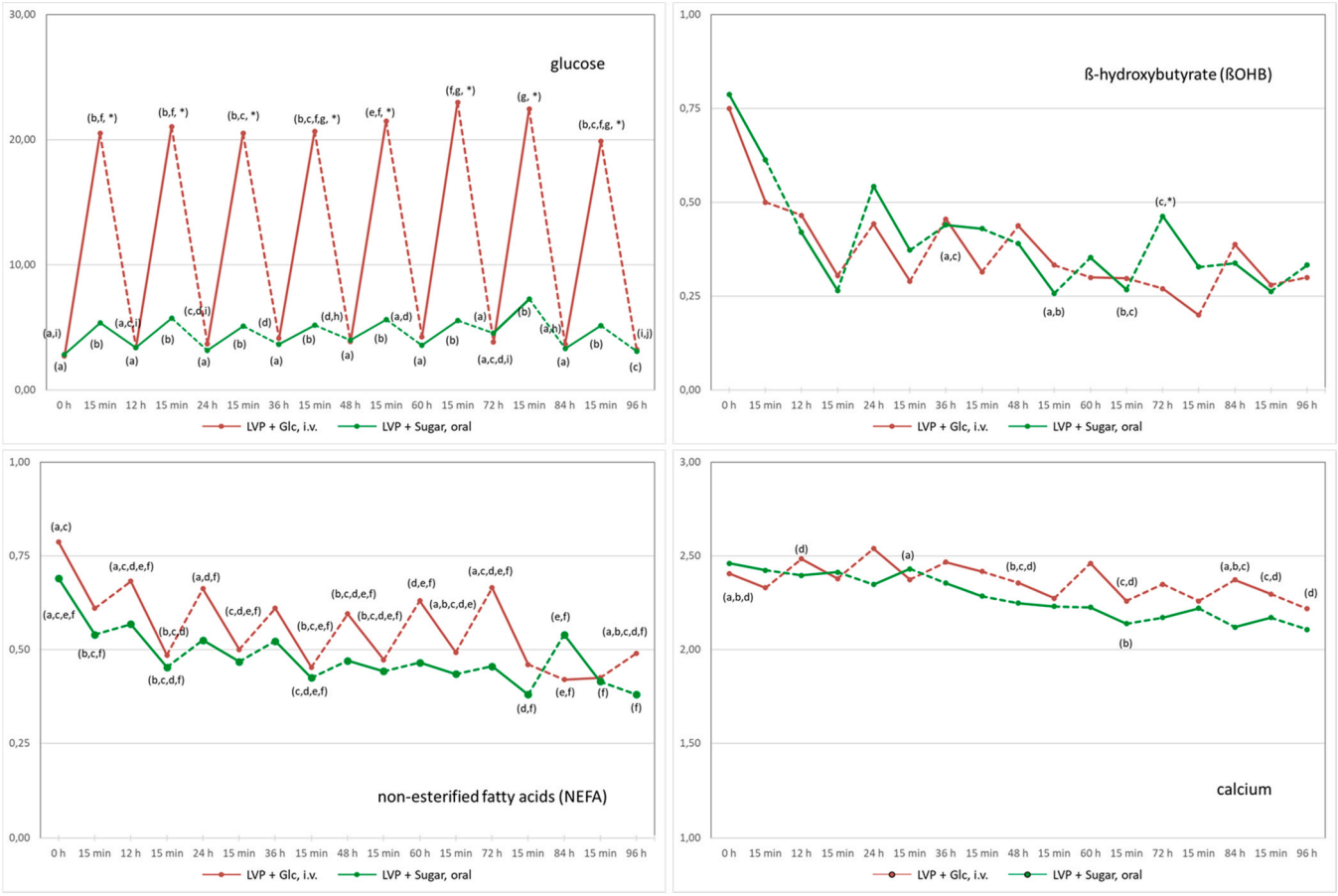


**Fig. 3.** Evolution of blood glucose, βOHB, NEFA, and calcium (in mmol/l) in two treatments and at different sampling times throughout the trial. LVP + Sugar: lysine-vasopressin + oral sugar; LVP + Glc i.v.: lysine-vasopressin + commercial glucose solution i.v. Different letters indicates statistical differences between sampling times. Asterisk (*) indicates statistical differences between treatments ($P < 0.05$).

implemented in feed (Macrae, 2017). Malnutrition in pregnant mothers produces ketone bodies, which reduce ruminal motility, causing reduced intake and altering rumen contractions that further deteriorate body condition (Khan et al., 2021). Hyperketonemia worsens hypoglycemia by inhibiting hepatic glucose production, glucose uptake and utilization by peripheral tissues, worsening negative energy balance, and increasing lipolysis (Harmeyer and Schlumbohm, 2006; Iqbal et al., 2022). Therefore, the reduction of food consumption by ewes during the end of gestation, or in our case by fasting, is not only capable of inducing the accumulation of fat in the liver but also an increase in liver fibrosis in ewes, causing a significant alteration of liver architecture and therefore liver dysfunction (Yang et al., 2019). Also, the concentration of βOHB increases with the size of the litter. The concentration of this ketone body in ewes with twins was significantly higher than those in ewes with only one child by 19.2%, while ewes-bearing triplets and ewes-bearing quadruplets or quintuplets showed βOHB values by 30.4% and 85.2% higher compared to pregnant uniparous ewes, respectively (Ratanapob et al., 2018).

In pregnancy toxemia of goats, blood ß-OHB values above 1.6 mmol/l have been found (Vijayanand et al., 2022), while with values of 3.0 mmol/l, they could suffer severe toxemia during pregnancy both goats (Khan et al., 2021) as ewes (Macrae, 2017; Sargison, 2007), although commonly they exceed 5.0 mmol/l (90.09 mg/dl) (Andrews, 1997; Kabakci et al., 2003; Kasimanickam, 2016; Lima et al., 2016; Ratanapob et al., 2018; Santos et al., 2011; Sargison, 2007), and even up to 19.0 mmol/l (Amirul et al., 2016). Cal-Pereyra et al. (2015a) established a value of 2.26 ± 1.03 mmol/l as the threshold for diagnosing subclinical pregnancy toxemia, although serum concentrations higher than 3 mmol/l have been associated with a poor prognosis in ewes (Simões et al., 2020). Ewes affected by hyperketonemia have also been cited as having a shorter gestation and being at increased risk of dystocia (50% *vs* 8.3%) and metritis (25% *vs* 0%) compared to unaffected ewes (Crilly et al., 2021).

Ketonuria, detected in some ewes, appears when the blood concentration of β-hydroxybutyrate reaches 0.6–0.7 mmol/l (Rook, 2000), and even this ketonuria can be detected before ketonemia, being very useful in the early diagnosis of pregnancy toxemia, and especially in subclinical disease (Maine, 2000; Rook, 2000). Under field conditions, Vijayanand et al. (2022) recommend using the presence of ketone bodies in urine and a blood β-hydroxybutyrate concentration above 0.8 mmol/l for the diagnosis of toxemic goats.

NEFA concentration reflects the magnitude of fat mobilization from reserve stores in response to negative energy balance (Aly and Elshahawy, 2016; Mohammadi et al., 2016) and during the peripartum period should be < 0.45 mmol/l (Aly and Elshahawy, 2016; Kaneko et al., 2008; Mohammadi et al., 2016). Both βOHB as NEFA concentrations in ewes with reduced energy intake (Yang et al., 2019) or in ewes affected by gestational toxemia are higher than those in the control group (Iqbal et al., 2022), with blood NEFA values that can triple those of the control group (415.75 vs. 1236.8 and 1186.5 μmol/l) (Yang et al., 2019). According to Iqbal, the cut-off point of NEFA is 0.390 mmol/l and 0.657 mmol/l, for subclinical PT and clinical PT respectively (Iqbal et al., 2022), and also when the sheep exceed these concentrations they have a probability of 4.66 and 5.14 times greater risk of developing subclinical and clinical disease, respectively, than control ewes (Iqbal et al., 2022).

Despite being glucose the primary metabolic fuel and being required for the normal function of crucial organs, when an animal is unable to consume enough food to meet its maintenance requirements it enters a prolonged hypoglycemic state causing utilization of its body fat and protein stores, resulting in increased plasma levels of NEFA and urea due to fat and protein catabolism (Aly and Elshahawy, 2016; Iqbal et al., 2022). Therefore, the increase in NEFA could be attributed to hypoglycemia, which results in an excessive mobilization of adipose tissue, and therefore an elevated concentration of blood NEFA (Iqbal et al., 2022). De Sousa et al. reported that during late gestation peripheral tissue becomes less sensitive to insulin (de Souza et al., 2019), resulting in decreased glucose uptake and increased lipolysis (Iqbal et al., 2022).

A large amount of NEFA reaches the liver where it is oxidized to carbon dioxide and water. However, when a large amount of NEFA is produced, they undergo incomplete oxidation to ketone bodies (Iqbal et al., 2022; Yang et al., 2019).

The increased concentration of β-HBA is the result of a compensatory mechanism for energy deficiency and inhibition of the Krebs cycle (Kaneko et al., 2008). During energy deficiency, gluconeogenesis is highly active in the liver and much of the mitochondrial oxaloacetate is consumed in the process, making it unavailable for citrate formation from its binding to acetyl-CoA. As a result, large amounts of acetyl-CoA become available for ketogenesis, leading to hyperketonemia (Aly and Elshahawy, 2016; Iqbal et al., 2022; Kaneko et al., 2008).

The analyzed enzymes showed values similar to those cited in the bibliography at all times (Kaneko et al., 2008; Ortolani and Benesi, 1989; Sargison, 2007) and hardly show variations throughout the experience, indicating that there is no destruction of liver or muscle cells. Traditionally, an increase in the enzymes indicative of liver failure (AST, ALT, and GGT) is cited in ewes affected by pregnancy toxemia (Aly and Elshahawy, 2016; de Souza et al., 2020, 2019; Jyothi et al., 2014; Khan et al., 2021; Ortolani and Benesi, 1989; Silva et al., 2022; Yarim and Ciftci, 2009). However, Scholz did not find variations in AST, GGT, or GLDH when administering LVP, glucose solutions, and sodium propionate in cows affected by primary ketosis (Scholz, 1988).

Increased AST, ALT, GGT, and LDH activity in toxemic pregnant ewes is mainly due to damage to liver cells caused by hepatic lipidosis, leading to increased membrane permeability and release of cellular enzymes into the blood (de Souza et al., 2020, 2019). GGT is a more specific and sensitive indicator of liver damage than AST, which makes it a good diagnostic marker. Thus, the observed increase in GGT activity in ewes with gestational toxemia indicates liver dysfunction due to fatty infiltration, also evidenced by elevated levels of AST, NEFA, and β -HBA (de Souza et al., 2020, 2019; Iqbal et al., 2022; Kabakci et al., 2003; Khan et al., 2021; Vasava et al., 2016).

Low calcium levels have been found in ketotic animals (Affan et al., 2022; Aly and Elshahawy, 2016; Rook, 2000), to the point that approximately 20% of ewes with toxemia also suffer from hypocalcemia (Crilly et al., 2021; Rook, 2000; Sargison, 2007), although its significance is unclear (Roubies et al., 2003). Hypocalcemia and hypokalemia, which are often associated with gestational toxemia, can be attributed to anorexia and metabolic acidosis, respectively (Aly and Elshahawy, 2016; Rook, 2000; Van Saun, 2000).

It has been pointed out that hypocalcemia can also be induced by increased lipolysis (Aly and Elshahawy, 2016; Rook, 2000), by high circulating cortisol levels (Aly and Elshahawy, 2016), associated with poor hydroxylation of vitamin D in ewes with severe liver fatty damage (Aly and Elshahawy, 2016; Sargison, 2007) or even with the great need for calcium for the development of the fetal skeleton (Vasava et al., 2016). In our opinion, the slight decrease in calcium, observed immediately after the application of the therapy, could also be caused by the consumption of calcium at the muscular level, due to the stress caused by restraining and immobilizing the animal, and even by the effect of the LVP on the smooth muscle fiber. The decrease in calcium is more evident in animals treated with i.v. glucose but has got a quick recovery. However, it persists for at least 30 min post-administration in ewes treated with oral sugar. According to Schlumbohm and Harmeyer (2003), if hyperketonemia and hypocalcemia occur in the last trimester of pregnancy in ewes, the appearance of gestational toxemia is facilitated. Hypocalcemia does not promote pregnancy toxemia *per se* but facilitates its development when combined with hyperketonemia. For this reason, some authors recommend including calcium solutions in treatment protocols given the similarity of the clinical signs of gestational toxemia and hypocalcemia (Crilly et al., 2021; Sargison, 2007).

The evolution of metabolic indicators, and especially glycemia, in this trial in which a solution with sugar dissolved in water is administered *per os* with prior stimulation of the reticular groove by LVP, is practically similar to that observed in a previous experience (Martin-Alonso et al., 2019). In it, an oral glucose solution was administered with a significant elevation from 15 min post-application, remaining at high levels for at least 2 h and with values clearly higher than the initial ones until the sampling carried out 8 h post-treatment. If we take into account that the therapy is repeated every 12 h, we can affirm that glycemia, at all times, presents acceptable values, which, without any doubt, must be attributed to the closure of the reticular groove (Martin-Alonso et al., 2019). Stimulation of the reticular groove, as lysine-vasopressin acts on its musculature, allows the sugar solution to pass through the rumen, reaching directly to the abomasum, where, as described when other glucose solutions are administered orally, the sugar can be rapidly absorbed (El-Hamamsy et al., 1990; González-Montaña et al., 2014; McAllan and Lewis, 1985; Scholz, 1995).

The administration of LVP, by itself, is already capable of causing a slight increase in blood glucose, attributed to the release of ACTH and its glycogenolytic effect (Jackson, 2003; Mikhail et al., 1988; van Weeren-Keverling Buisman et al., 1990), as well as due to the stress produced when handling sheep (van Weeren-Keverling Buisman et al., 1990). According to the experiences of Mikhail et al. (1988), who administer glucose solutions orally to goats, the administration of the sugary solution orally is not enough to stimulate the closure of the esophageal groove. However, it is similar to the significant increase in blood glucose observed after the abomasal administration of an oral glucose solution after stimulation of the reticular groove with either 1.5 ml of a saturated NaCl solution, or with 10.5 ml of a 1.5% NaCl solution or with 0.5 IU/kg B.W. vasopressin (Mikhail et al., 1988). Therefore, the significant increase in blood glucose can only be accounted for by the closure of the reticular groove caused by LVP and the passage of the sugar solution directly into the abomasum.

In lambs or calves when oral rehydration solutions are administered the reticular groove closes, so these solutions reach the abomasum directly, producing their active absorption in the small intestine, causing a rapid co-transport of water, glucose, and sodium. The rationale for oral glucose-electrolyte therapy in ovine pregnancy toxemia is based on this potential for rapid co-transport of glucose from the intestine (Sargison, 2007). However, when concentrated oral rehydration solutions are administered to adult ewes, they are deposited mainly in the rumen-reticulum, where glucose is rapidly metabolized by the microflora. Therefore, it is doubtful that significant amounts of glucose will be rapidly absorbed after treatment with concentrated oral rehydration solutions (Sargison, 2007).

Annison was able to slightly increase glycemia (up to 5 mg/dl) in some treated animals after administering glucose orally (0.5 g/kg in 50 ml of water), which could be attributed to a slight absorption from the rumen (Annison, 1960). Though, Mc Allan and Lewis found that 80% or even more glucose will be rapidly absorbed if it reaches the abomasum (McAllan and Lewis, 1985). Mikhail et al. (1988) confirmed that rapid glucose absorption from the abomasum produces an increase in blood glucose 30 min after treatment, and that is capable of maintaining high levels up to 4 h later, to return to initial levels after 8 h. The evolution that we have verified is similar, since although we did not administer glucose, but commercial sugar, we observed a marked elevation of glycemia in all the ewes, appreciable already at 15 min and which remains at acceptable values, even when the ewes are in fasting,

during the 12 h of each experimental cycle.

Glycemia is even higher than that observed in the ewes of the control group, treated with i.v. glucose, where the extremely high initial glycemia drops to values lower than those of the problem group (treated with sugar solutions) 8 h after therapy. El-Hamamsy et al. (1990) in ewes with experimental toxemia obtained increases in blood glucose from the first treatment when administering LVP to the ewes at doses of i.v. 1.0 IU/kg B.W. and 50 g of glucose orally, twice a day for three days, remaining at significant levels even after the end of the therapy and the ewes recovering before parturition.

In the control group, standard therapy, with i.v. glucose solution, has been carried out taking into account the recommendations of multiple researchers (Constable et al., 2017; Simões et al., 2020; Van Saun, 2000), although some advocate the use of hypertonic solutions (between 40% and 50%) (Andrews, 1997, 1982; Sargison, 2007; Simões et al., 2020).

These solutions cause a very important rise of glycemia, producing transient hyperglycemia, which drops rapidly due to its urinary elimination (Prasad and Kaul, 1981) and according to Fox, glucose excreted in the urine can be up to 80% of the i.v. glucose administered (Fox, 1971).

For this reason, the application of gluco-precursors is recommended to maintain glycemia at stable values. Thus, treatment with i.v. glucose has been recommended. (100 ml at 25%, in the morning) and 100 ml of oral glycerol in the afternoon, finding a non-significant increase in plasma glucose, which subsequently decreased to hypoglycemic values, similar to baseline on the last day of treatment (El-Hamamsy et al., 1990). Andrews also recommends applying propylene glycol, after i.v. glucose, to maintain blood glucose and take into account its renal excretion (Andrews, 1982).

In addition, intravenous glucose treatment should be associated with parenteral administration of insulin (Simões et al., 2020) or 10% dexamethasone (Kabakci et al., 2003), since both insulin and corticosteroids are associated with the metabolism of body fat so that a low insulin level reduces the use of glucose in the body, but accelerates lipolysis and increases hepatic ketogenesis (Affan et al., 2022; Iqbal et al., 2022). In Sousa et al. opinion during late gestation peripheral tissue becomes less sensitive to insulin, resulting in decreased glucose uptake and increased lipolysis (de Souza et al., 2020). Therefore, these substances promote the rapid utilization of glucose, being very effective in advanced processes of the disease and/or to stimulate gluconeogenesis.

Levels of total ketones, and therefore of βOHB, vary inversely proportional to blood glucose, except if the hyperketonemia is severe (Kaneko et al., 2008), which is why we have verified that the increase in blood glucose, in both experimental groups, causes a much less marked decrease in βOHB and also in NEFA. This behavior also coincides with that described by El-Hamamsy et al. (1990), with a significant decrease (from 0.652 to 0.292 mmol/l) of ketone bodies 24 h after the first treatment combining LVP and oral glucose in toxemic ewes. We have verified this decrease already 15 min after therapy, with values below those shown by the ewes after fasting and that remained lower at least 24 h after the last dose of oral sugar. In contrast, El-Hamamsy et al. found no decrease in ketonemia in ewes affected by toxemia when treating them with glucose serum (i.v.), combined with glycerol orally (El-Hamamsy et al., 1990).

In the experimental protocol, we have chosen to use a probe introduced in the initial section of the esophagus to prevent receptors located in the mouth and pharynx from being stimulated by the tested solutions (van Weeren-Keverling Buisman et al., 1990). In clinical practice, and to facilitate the application of the sugary solution, it could be administered through a bottle applied directly to the ovine's mouth, and in our opinion, the results would be similar.

## 5. Conclusions

Stimulation of the reticular groove allows the oral administration of glucose solutions that can be used in the therapy of pregnancy toxemia. The combination of intravenous lysine-vasopressin (0.08 IU/kg B.W.) with subsequent oral administration of sugar (100 g) dissolved in one liter of water causes an increase in blood glucose that remains within acceptable values for 12 h, at which time a new application of the treatment is required. This is due to the stimulation of the reticular groove, which allows the sugar to reach the abomasum, where it can be absorbed immediately. The use of commercial sugar, substituting other glucose solutions, makes it possible to significantly reduce the cost of therapy for this disease and could be considered in new therapeutic protocols related to ovine pregnancy toxemia.

## Ethics approval and consent to participate

All experimental procedures were carried out in compliance with the provisions of the EU Directive of the European Parliament which regulates the use of animals for scientific purposes and the Royal Decree regulation in Spain of the experimentation and protection of animals used for scientific purposes.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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## References

Affan, A.A., Salleh, A., Zamri-Saad, M., Jayanegara, A., Hassim, H.A., 2022. Clinical signs and blood variables of pregnancy toxemia goats during late gestation and postpartum. Trop. Anim. Sci. J. 45, 84–90. https://doi.org/10.5398/tasj.2022.45.1.84.

Aly, M., Elshahawy, I., 2016. Clinico-biochemical diagnosis of pregnancy toxemia in ewes with special reference to novel biomarkers. Alex. J. Vet. Sci. 48, 96–102. https://doi.org/10.5455/ajvs.215993.

Amirul, F.M.A., Mokrish, A., Lai, K.S., Zamri-Saad, M., Zuki, A.B., Hassim, H.A., 2016. Clinical, biochemical and histological changes during developement of pregnancy ketosis in goats. J. Vet. Malays. 28, 1–6.

Andrews, A., 1997. Pregnancy toxaemia in the ewe. Pract 19, 306–312.

Andrews, A.H., 1982. Effects of glucose and propylene glycol on pregnancy toxaemia in ewes. Vet. Rec. 110, 84–85.

Annison, E.F., 1960. Plasma non-esterified fatty acids in sheep. Aust. J. Agric. Res 11, 58–64.

Benech, A., Cal-Pereyra, L., Da Silva, S., Acosta-Dibarrat, J., González-Montaña, J.R., 2015. Transient apnoea in sheep: an alternative method for serial urine sample collection. Vet. Arh. 85, 293–307.

BOE, 2013, Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia., Boletín Oficial del Estado.

Braun, J.P., Trumel, C., Bezille, P., 2010. Clinical biochemistry in sheep: A selected review. Small Rumin. Res. 92, 10–18. https://doi.org/10.1016/j.smallrumres.2010.04.002.

Cal, L., Borteiro, C., Benech, A., Rodas, E., Abreu, M.N., Cruz, J.C., González-Montaña, J. R., 2009. Alterações histológicas do fígado e correlações metabólicas em ovelhas com toxemia da prenhez. (Histological changes of the liver and metabolic correlates in ewes with pregnancy toxemia). Arq. Bras. Med. Vet. E Zootec. 61, 306–312. https://doi.org/10.1590/S0102-09352009000200004.

Cal-Pereyra, L., Acosta-Dibarrat, J., Benech, A., Da Silva, S., Martin, A., González-Montaña, J.R., 2012. Toxemia de la gestación en ovejas. Revisión. Ewe pregnancy toxemia. Rev. Rev. Mex. Cienc. Pecu. 3, 247–264. ⟨http://scielo.unam.mx/pdf/rmcp/v3n2/v3n2a7.pdf⟩.

Cal-Pereyra, L., Benech, A., González-Montaña, J.R., Acosta-Dibarrat, J., Da Silva, S., Martin, A., 2015a. Changes in the metabolic profile of pregnant ewes to an acute feed restriction in late gestation. N. Z. Vet. J. 63, 141–146. https://doi.org/10.1080/00480169.2014.971083.

Cal-Pereyra, L., González-Montaña, J.R., Benech, A., Acosta-Dibarrat, J., Martin, M.J., Perini, S., Abreu, M.C., Da Silva, S., Rodriguez, P., 2015b. Evaluation of three therapeutic alternatives for the early treatment of ovine pregnancy toxaemia. Ir. Vet. J. 68. https://doi.org/10.1186/s13620-015-0053-2.

Constable, P., Hinchcliff, K.W., Done, S., Gruenberg, W., 2017. Veterinary medicine. A Textbook of the diseases of cattle, horses, sheep, pigs and goats, 11th ed..,. Elsevier,, St. Louis, USA.
Crilly, J.P., Phythian, C., Evans, M., 2021. Advances in managing pregnancy toxaemia in sheep. Pract 43, 79–94. https://doi.org/10.1002/inpr.17.
de Souza, L.M., de Mendonça, C.L., de ASSIS, R.N., Oliveira Filho, E.F., Gonçalves, D.N. A., Souto, R.J.C., Soares, P.C., Afonso, J.A.B., 2019. Cardiac biomarkers troponin I and CK-MB in ewes affected by pregnancy toxemia. Small Rumin. Res. 177, 97–102. https://doi.org/10.1016/j.smallrumres.2019.06.020.
de Souza, L.M., Mendonça, C.L., Assis, R.N., Oliveira Filho, E.F., Soares, G.S.L., Souto, R. J.C., Soares, P.C., Afonso, J.A.B., 2020. Changes in cardiac biomarkers in goats naturally affected by pregnancy toxemia. Res. Vet. Sci. 130, 73–78. https://doi.org/10.1016/j.rvsc.2020.02.016.
Duehlmeier, R., Fluegge, I., Schwert, B., Parvizi, N., Ganter, M., 2011. Metabolic adaptations to pregnancy and lactation in German Blackheaded Mutton and Finn sheep ewes with different susceptibilities to pregnancy toxaemia. Small Rumin. Res. 96, 178–184. https://doi.org/10.1016/j.smallrumres.2010.12.002.
El-Hamamsy, H.T., El-Neweehy, T.K., Abdou, O.M., Kubesy, A.A., 1990. Clinical significance of oesophageal groove vasopressin induced-closure. II. A new concept in the oral glucose treatment of pregnancy toxaemia in ewes. Vet. Med. J. Giza 38, 373–384.
European-Union, 2010, Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes.
Fox, F.H., 1971. Clinical diagnosis and treatment of ketosis. J. Dairy Sci. 54, 974–982.
González-Montaña, J.R., Martin, M.J., Benech, A., Alonso, M.E., Alonso, A.J., Cal-Pereyra, L.G., 2014. Handling the gastric groove closure in adult sheep using lysine-vasopressin. Small Rumin. Res. 121, 418–424. https://doi.org/10.1016/j.smallrumres.2014.07.025.
Harmeyer, J., Schlumbohm, C., 2006. Pregnancy impairs ketone body disposal in late gestating ewes: Implications for onset of pregnancy toxaemia. Res. Vet. Sci. 81, 254–264. https://doi.org/10.1016/j.rvsc.2005.10.010.
Iqbal, R., Beigh, S.A., Mir, A.Q., Shaheen, M., Hussain, S.A., Nisar, M., Dar, A.A., 2022. Evaluation of metabolic and oxidative profile in ovine pregnancy toxemia and to determine their association with diagnosis and prognosis of disease. Trop. Anim. Health Prod. 54, 338. https://doi.org/10.1007/s11250-022-03339-9.
Jackson, E.K., 2003. Vasopresina y otros fármacos que afectan la conservación renal del agua. In: Goodman, L.S., Gilman, A.G. (Eds.). Mc-Graw-Hill-Education, Mexico DF, pp. 767–784.
Jyothi, K., Sudhakara Reddya, B., Pridhvidhar Reddy, Y.V., Rao, K.P., Sivajothi, S., Ganesan, A., 2014. Pregnancy toxemia associated with dystocia in a Nellore Brown Ewe. Adv. Appl. Sci. Res. 5, 325–327.
Kabakci, N., Yarim, G., Yarim, M., Duru, O., Yagci, B.B., Kisa, U., 2003. Pathological, clinical and biochemical investigation of naturally occuring pregnancy toxemia of sheep. Acta Vet. -Beogr. 53, 161–169. https://doi.org/10.2298/AVB0303161K.
Kaneko, J.J., Harvey, W.J., Bruss, M.L., 2008. Clinical Biochemistry of Domestic Animals, 6th ed..,. Elsevier- Academic Press,, San Diego, USA. https://doi.org/10.1016/B978-0-12-370491-7.X0001-3.
Karagiannis, I., Panousis, N., Kiossis, E., Tsakmakidis, I., Lafi, S., Arsenos, G., Boscos, C., Brozos, C., 2014. Associations of pre-lambing body condition score and serum β-hydroxybutyric acid and non-esterified fatty acids concentrations with periparturient health of Chios dairy ewes. Small Rumin. Res. 120, 164–173. https://doi.org/10.1016/j.smallrumres.2014.05.001.
Kasimanickam, R.K., 2016. Subclinical pregnancy toxemia-induced gene expression changes in ovine placenta and uterus. Front. Vet. Sci. 3, 69. https://doi.org/10.3389/fvets.2016.00069.
Khan, Y.R., Durrani, A.Z., Muhammad, I., Ahmad, A.L.I., Khan, R.L., Hussain, K., Rabbani, A.H., 2021. Determination of hemato-biochemical biomarkers, associated risk factors and therapeutic protocols for pregnancy toxemia in Beetal goats. Kafkas Üniversitesi Vet. Fakültesi Derg. 27 (525–532), 2021. https://doi.org/10.9775/kvfd.2021.25931.
Lacetera, N., Bernabucci, U., Ronchi, B., Nardone, A., 2001. Effects of subclinical pregnancy toxemia on immune responses in sheep. Am. J. Vet. Res 62, 1020–1024. https://doi.org/10.2460/ajvr.2001.62.1020.
Lima, M.S., Silveira, J.M., Carolino, N., Lamas, L.P., Pascoal, R.A., Hjerpe, C.A., 2016. Usefulness of clinical observations and blood chemistry values for predicting clinical outcomes in dairy goats with pregnancy toxaemia. Ir. Vet. J. 69, 1–9. https://doi.org/10.1186/s13620-016-0075-4.
Macrae, A., 2020. The recumbent ewe. Livestock 25, 136–140. https://doi.org/10.12968/live.2020.25.3.136.
Macrae, A., 2017. Managing and feeding the pregnant ewe. Livestock 22, 92–96. https://doi.org/10.12968/live.2017.22.2.92.
Maine, D., 2000. Role of nutrition in the prevention of toxemia, 1242–1242 Am. J. Clin. Nutr. 72. https://doi.org/10.1093/ajcn/72.1.298S.
Martin-Alonso, M.J., Escalera-Valente, F., Cal-Pereyra, L.G., Benech, A., Alonso, M.P., González-Montaña, J.R., 2019. Energetic metabolism in fasting sheep: regularization of metabolic profile by treatment with oral glucose, with prior handling of gastric groove. Rev. Bras. Zootec. 48. https://doi.org/10.1590/rbz4820180290.
McAllan, A.B., Lewis, P.E., 1985. The removal of glucose, maltose and different starches from the small intestines of steers. Arch. Anim. Nutr. 35, 495–505.
Mikhail, M., Brugère, H., Le Bars, H., Colvin Jr., H.W., 1988. Stimulated esophageal groove closure in adult goats. Am. J. Vet. Res 49, 1713–1715.
Mohammadi, V., Anassori, E., Jafari, S., 2016. Measure of energy related biochemical metabolites changes during peri-partum period in Makouei breed sheep. In: Veterinary Research Forum. Faculty of Veterinary Medicine. Urmia University, Urmia, Iran, p. 35.
Olfati, A., Moghaddam, G., Bakhtiari, M., 2013. Diagnosis, treatment and prevention of pregnancy toxemia in ewes. Int. J. Adv. Biol. Biomed. Res. 1, 1452–1456.
Ortolani, E.L., Benesi, F.J., 1989. Ocorrência de toxemia da prenhez em cabras (Capra hircus, L) e ovelhas (Ovis aries, L) criadas no Estado de São Paulo. Bras. Rev. Fac. Med. Veter-.-. E Zootec. Univ. São Paulo 26, 229–234.
Prasad, M.C., Kaul, P.L., 1981. Glucose tolerance test in goats. Zent. Für Veter-.-. Reihe A 28, 732–736.
Raoofi, A., Jafarian, M., Safi, S., Vatankhah, M., 2013. Fluctuations in energy-related metabolites during the peri-parturition period in Lori-Bakhtiari ewes. Small Rumin. Res. 109, 64–68. https://doi.org/10.1016/j.smallrumres.2012.06.012.
Ratanapob, N., VanLeeuwen, J., McKenna, S., Wichtel, M., Rodriguez-Lecompte, J.C., Menzies, P., Wichtel, J., 2018. The association of serum β-hydroxybutyrate concentration with fetal number and health indicators in late-gestation ewes in commercial meat flocks in Prince Edward Island. Prev. Vet. Med. 154, 18–22. https://doi.org/10.1016/j.prevetmed.2018.03.009.
Riis, P.M., 1983, World animal science: Dynamic biochemistry of animal production. Elsevier Science Publishers BV, Amsterdan.
Rook, J.S., 2000. Pregnancy toxemia of ewes, does, and beef cows. Vet. Clin. North Am. Food Anim. Pract. 16, 293–317. https://doi.org/10.1016/S0749-0720(15)30107-9.
Roubies, N., Polizopoulou, Z.S., Minas, A., Papasteriadis, A., 2003. A pre- and postpartum study of selected biochemical parameters in ewes for the early detection of pregnancy toxemia. J. Hell. Vet. Med. Soc. 54, 11–20. https://doi.org/10.12681/jhvms.15213.
Russel, A., 1985. Nutrition of the pregnant ewe. Pract 7, 23–28.
Santos, F.C.O., Mendonça, C.L., Silva Filho, A.P., Carvalho, C.C.D., Soares, P.C., Afonso, J.A.B., 2011. Indicadores bioquímicos e hormonais de casos naturais de toxemia da prenhez em ovelhas. Pesqui. Vet. Bras. 31, 974–980. https://doi.org/10.1590/S0100-736×2011001100006.
Sargison, N.D., 2007. Pregnancy toxaemia. In: Aitken, I.D. (Ed.), Diseases of Sheep. Blackwell Publishing Professional, Ames, Iowa, USA, pp. 359–363. https://doi.org/10.1002/978047075331.
Schlumbohm, C., Harmeyer, J., 2003. Hypocalcemia reduces endogenous glucose production in hyperketonemic sheep. J. Dairy Sci. 86, 1953–1962. https://doi.org/10.3168/jds.S0022-0302(03)73783-7.
Scholz, H., 1995. Sulla funcionalità della doccia esofagea. Atti Della Soc. Ital. Buiat. 27, 551–561.
Scholz, H., 1988. Utilization of the reticular groove contraction in adult cattle. A Ther. Altern. Pr. ? Bov. Pr. 23, 148–152.
Scott, P., 1995. Differential diagnosis of common metabolic disorders of sheep. Pract 17, 266–269. https://doi.org/10.1136/inpract.17.6.266.
Silva, S.R., Sacarrão-Birrento, L., Almeida, M., Ribeiro, D.M., Guedes, C., González Montaña, J.R., Pereira, A.F., Zaralis, K., Geraldo, A., Tzamaloukas, O., Cabrera, M. G., Castro, N., Argüello, A., Hernández-Castellano, L.E., Alonso-Diez, Á.J., Martín, M. J., Cal-Pereyra, L.G., Stilwell, G., de Almeida, A.M., 2022. Extensive sheep and goat production: The role of novel technologies towards sustainability and animal welfare. Animals 12, 885. https://doi.org/10.3390/ani12070885.
Simões, P.B.A., Bexiga, R., Lamas, L.P., Lima, M.S., 2020. Pregnancy Toxaemia in Small Ruminants. In: Advances in Animal Health, Medicine and Production. Springer, pp. 541–556.
Souto, R.J.C., Afonso, J.A.B., Mendonça, C.L., Carvalho Cleyton, C.D., Silva Filho, A.P., Cajueiro, J.F.P., Lima, E.H.F., Soares, P.C., 2013. Biochemical, electrolytic and hormonal findings in goats affected with pregnancy toxemia. Pesqui. Veter-.-. Bras. 33, 1174–1182. https://doi.org/10.1590/S0100-736×2013001000002.
Van Saun, R.J., 2000. Pregnancy toxemia in a flock of sheep. J. Am. Vet. Med. Assoc. 217, 1536–1539. https://doi.org/10.2460/javma.2000.217.1536.
van Weeren-Keverling Buisman, A., Kuiper, R., Wensing, T., Breukink, H.J., 1990. The effect of vasopressin on the closure of the reticular groove in the veal calf. J. Anim. Physiol. Anim. Nutr. 64, 240–249. https://doi.org/10.1111/j.1439-0396.1990.tb00229.x.
Vasava, P.R., Jani, R.G., Goswami, H.V., Rathwa, S.D., Tandel, F.B., 2016. Studies on clinical signs and biochemical alteration in pregnancy toxemic goats. Vet. World 9 (869). https://doi.org/10.14202/vetworld.2016.869-874.
Vijayanand, V., Balagangatharathilagar, M., Gnanaraj, P.T., Vairamuthu, S., 2022. Diagnostic indicators and therapeutic evaluation of pregnancy toxaemia in goats. Indian J. Anim. Res 56, 451–459. https://doi.org/10.18805/IJAR.B-4456.
Yang, H., Wang, Y., Ma, C., Sun, C., Liu, Y., Wu, K., Li, M., Borjigin, G., Gao, F., 2019. Effects of feed intake restriction during late pregnancy on the function, anti-oxidation capability and acute phase protein synthesis of ovine liver. Asian-Australas. J. Anim. Sci. 32, 217–223. https://doi.org/10.5713/ajas.18.0331.
Yarim, G.F., Ciftci, G., 2009. Serum protein pattern in ewe with pregnancy toxemia. Vet. Res. Commun. 33, 431–438. https://doi.org/10.1007/s11259-008-9189-9.