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# Different prepartum feeding systems on ewe metabolic profile and lamb growth

Diferentes sistemas alimentares no pré-parto sob perfil metabólico das ovelhas e crescimento dos cordeiros

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# Highlights .

Sheep metabolic conditions limit lamb growth. Twin calving determines different concentrations of metabolites in sheep. Feed level of ewes influences the weight of lambs.

# Abstract \_

Identifying the metabolic profile of farm animals constitutes an important tool for the clinical diagnosis of metabolic diseases that can affect animal production performance. This study investigates the prepartum metabolic profile of ewes reared in two feeding systems native pasture and cultivated pasture of black oat and ryegrass and their effects on lamb development. Forty adult Texel ewes and their lambs were used and evaluated for metabolites representative of energy, protein, and mineral metabolism in the prepartum period. Ewes kept on cultivated pasture exhibited higher glucose levels than those kept on native pasture (59.67 vs. 31.98 mg/dL, respectively), whereas those kept on native pasture had higher serum Ca and P levels (7.62 and 4.58 mg/dL, respectively) than the ewes on cultivated pasture (6.21 and 3.73 mg/dL, respectively). Albumin was higher in the ewes with single pregnancy (2.92 vs. 2.76 g/dL), while urea levels were affected by the interaction between feeding system and type of pregnancy. Prepartum levels of glucose, phosphorus, and calcium in the blood of the ewes were correlated with lamb weight at 30 days. The feeding system

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influenced the metabolic profile of the ewes in the prepartum period, with lamb weight at 30 days of age being mainly associated with the mother's glucose level at the end of the gestation period. **Key words:** Fetal programming. Glucose. Metabolites. Sheep. Urea.

# Resumo \_\_

A identificação do perfil metabólico em animais de produção apresenta-se como uma importante ferramenta no diagnóstico clínico de doenças do metabolismo que podem afetar no desempenho produtivo dos animais. Avaliou-se o perfil metabólico no pré-parto de ovelhas criadas em dois sistemas alimentares, a pasto (pastagem nativa) e pastagem cultivada de aveia preta e azevém e seus efeitos sobre o desenvolvimento dos cordeiros. Foram utilizadas 40 ovelhas adultas, da raça Texel, e seus respectivos cordeiros, nas quais foram mensurados no pré-parto os metabólitos representativos do metabolismo energético, proteico e mineral. Animais mantidos em pastagem cultivada apresentaram maiores valores de glicose em relação aos mantidos em pastagem nativa (59,67 vs 31,98 mg/dL, respectivamente), e os animais mantidos em pastagem nativa apresentaram maiores níveis séricos de Ca e P (7,62 e 4,58 mg/dL, respectivamente) do que os em pastagem cultivada (6,21e 3,73 mg/dL, respectivamente). A albumina foi maior nas ovelhas com gestação simples (2,92 vs 2,76 g/dL) enquanto os níveis de ureia sofreram efeito da interação sistema alimentar vs tipo de gestação. Os teores de glicose, fósforo e cálcio no sangue das ovelhas no pré-parto se mostraram correlacionados com o peso dos cordeiros aos 30 dias. O sistema alimentar influenciou o perfil metabólico das ovelhas no pré-parto sendo o peso dos cordeiros aos 30 dias de idade principalmente associado com o nível de glicose da mãe no final do período gestacional. Palavras-chave: Glicose. Metabólitos. Programação fetal. Ovinos. Ureia.

# Introduction \_\_\_\_\_

In the Brazilian South and Northeast, the regions that concentrate the largest part of sheep farming in the country, most production systems use natural pasture as the feed base. However, forage quality is poor, particularly in the winter or in the dry season, when animals cannot meet their nutritional demand during growth, gestation, or lactation (Vaz et al., 2016). In the south region, to overcome the shortage of native forage, temperate climate grasses of higher yield and nutritional quality are cultivated (Savian et al., 2020).

Gestation is a critical physiological period for the ewe due to the increased nutritional requirements for the development of the fetus and the udder (EI-Sherif & Assad, 2001; Oliveira, Maduro, Lima, & Oliveira, 2014). In ewes, nutritional requirements increase during the last six weeks prepartum, when 70% of fetal development occurs (El-Sherif & Assad, 2001). Feed restriction to ewes during pregnancy is responsible for decreased fetal growth, lamb birth weight, and milk production (Ortunho & Marçal, 2014). For the lamb, nutritional restrictions during its gestation prevent the adequate growth of muscle and fat fibers, causing less development, which can be potentiated by the mother's age, type of birth, and genetics.

In ruminants, the transition period (or peripartum) occurs between three weeks pre- and three weeks postpartum. It is considered crucial for determining the health and production levels of herds (Oliveira et al.,



2014), which may be lower due to metabolic disorders (Libardi et al., 2018; Silva et al., 2020).

Identifying the metabolic profile of production animals works as an auxiliary method in the assessment of herds and as a tool in the diagnosis of metabolic diseases (Diaz González, 2018). Monitoring the protein, energy, and mineral patterns is important to adapt the sheep diet, since there are often imbalances between nutrient input and output (Siqueira et al., 2020).

The objective of this study was to evaluate the prepartum metabolic profile of ewes subjected to two feeding systems on pasture and their effects on lamb weight at 30 days of age.

# Material and Methods \_\_

# Study location

The study was carried out on a private property in the municipality of Pedro Osório - RS, located in the south region of Brazil (31°20'47" S 52°44'14" W), during the months of July and August. The climate of the region is humid subtropical.

# Animals, diets, treatments, and management

Forty Texel ewes with an average age of three years and an average prepartum body condition score of four were divided into the following feeding systems: native pasture (18 animals) or cultivated pasture (22 animals). The natural pasture consisted of a mixture of grasses and legumes as well as species of other botanical families, including *Andropogon lateralis, Axonopus fissifolius, Paspalum notatum, Trifolium* sp., and *Desmodium incanum.* Poor pasture management increases the number of weeds, such as *Soliva pterosperma* and *Vernonia nudiflora.* Lately, there has been an increase in the population of *Eragrostis plana* Nees. The cultivated pasture consisted of an intercrop of black oat (*Avena strigosa*) and annual ryegrass (*Lolium multiflorum*). After lambing, ewes and lambs were managed in a single lot and kept on cultivated pasture.

The lambs originated from artificial insemination, which was carried out in February, with ewes synchronized using the same sire to generate all lambs.

At birth, the lambs were identified as to sex and type of birth (single and twin) and then allocated with their mothers to the cultivated pasture. At 30 days after birth, the lambs had their development evaluated by weighing on an appropriate scale.

# Blood collection and metabolite analysis methods

Fifteen days before the expected date of lambing, blood samples were collected from the jugular vein of the ewes using a vacutainer system (Stober & Grunder, 1993), with the animals restrained in individual cages, observing welfare criteria. Two tubes were collected: one containing sodium fluoride and 10% EDTA to obtain the plasma and another without anticoagulant and with coagulation activator gel to obtain the serum. The samples were centrifuged at 3,500 rpm for 15 min, stored in Eppendorf<sup>®</sup> tubes, and frozen at -20 °C until analysis.

Seven components of energy, protein, and mineral metabolism were measured, in addition to analyses of glucose, urea,



albumin, total protein, calcium, phosphorus, and magnesium by photocolorimetry. A visible spectrophotometer (FEMTO 435®) and commercial kits (Labtest Clinical Diagnosis System®) were used for all colorimetric analyses.

Glucose levels were measured by the method of glucose oxidase from plasma with antiglycolytic agents. Urea, albumin, and total protein levels were measured using the urease, bromocresol green, and Biuret methods, respectively, from the serum samples. Calcium, phosphorus, and magnesium levels were determined by the o-cresolphthalein complexone, ammonium molybdate, and xylyl blue methods, respectively, also using serum samples.

The soil and pastures where the animals were managed were also sampled for mineral evaluation. Soil and pasture analyses were carried out in a laboratory accredited by the Official Soil Analysis Laboratory Network (ROLAS), using routine methodology (Table 1).

#### Table 1

Mineral composition of forages and soil in the different feeding systems.

	Forage			Soil					
Feeding system	Ca g/kg	Mg g/kg	P g/kg	K g/kg	Ca cmol <sub>c</sub> /dm³	Mg cmol <sub>c</sub> /dm³	P mg/dm <sup>3</sup>	K mg/dm <sup>3</sup>	pH water
Cultivated pasture	6.38	2.61	2.05	36.81	5.1	2.5	18.2	108	6.1
Native pasture	4.33	2.29	1.34	5.11	2.2	0.9	7.9	133	5.1

# Experimental design and statistical analysis

The experiment was laid out in a completely randomized design with a  $2 \times 2$  factorial arrangement (two prepartum feeding levels for ewes and single and twin births). The data were subjected to analysis of variance (P< 0.05) to check the effects of feeding system (native pasture and cultivated pasture), type of pregnancy (single and twin), and their interactions, using the weight of lambs at 30 days as a covariate. Correlation analyses were also carried out between ewe metabolic profile and lamb development.

# **Results and Discussion** \_

Therewasnointeraction effect between feeding systems and birth types (single or twin). Thus, the results will be presented separately. The prepartum feeding systems influenced the ewes' blood metabolites (P<0.05), with higher glucose and urea levels found in the animals kept on cultivated pasture. This higher glucose in animals reared on cultivated pasture is associated with the availability and quality of forage offered, since plant species from temperate climates have high crude protein and energy contents (Savian et al., 2020).

The normal physiological range for glucose values is between 50-80 mg/dL (Diaz González & Silva, 2019). Ewes kept on natural



pasture had glucose levels lower than those recommended for their development. Ribeiro et al. (2003) evaluated the metabolic profile of Corriedale ewes on native pasture in the different seasons of the year in Rio Grande do Sul and found a lower plasma glucose content (49.29 mg/dL) in the summer, with no differences between the other seasons (54.61, 53.03, and 53.61 mg/dL for spring, fall, and winter, respectively). The plasma glucose value recorded during the summer period, which was below recommended standards, is a consequence of the likely negative energy balance in this season due to the drought that occurred during the experiment.

The higher glucose values of animals kept on cultivated pasture coincided with lower levels of phosphorus, as this mineral participates in energy metabolism. Accordingly, higher intakes of energy substrates translate into a larger amount of phosphorus spent in the intracellular space, reducing its concentration in the plasma (Ribeiro et al., 2003; Kaneko, Harvey, & Bruss, 2008).

Indicators of protein metabolism in the ewes' metabolic concentration did not differ between the feeding systems (Table 2), lying within the reference ranges of 6.6-9.0 and 2.4-4.2 g/dL described by Oliveira et al. (2014) for total proteins and albumin, respectively. Decreases in total plasma proteins are related to a dietary deficiency of protein (Diaz González & Silva, 2019). This was not detected in any of the feeding systems in the present study, although total protein levels decrease at the end of pregnancy due to the demand for fetal growth and udder development (Sigueira et al., 2020). The lack of differences in albumin values indicates that despite qualitative restrictions of prepartum feeding, these were probably not long enough to induce changes in the levels of this metabolite, due to the mobilization of body reserves. The phenomena of hypoproteinemia or hypoalbuminemia do not occur immediately (Antunovic et al., 2011), but rather take several days of restriction for physiological changes to take place, which are reflected in the concentration of blood metabolites (Blagitz et al., 2012). In the final third of gestation, with increased fetal growth, a greater supply of nutrients is necessary, given the physiological need imposed. This nutrient supply can cause a decrease in total protein and albumin concentrations (Ribeiro et al., 2004) or maintain their levels during the three phases of gestation, depending on the body status and the feed provided to the ewes (Brozos, Mavrogianni, & Fthenakis, 2011).

#### Table 2

#### Means and standard errors of prepartum blood metabolites of ewes in different feeding systems.

Matabalita	Feeding			
Metabolite	Cultivated pasture	Native pasture	F test	
Glucose (mg/dL)	59.67 ± 1.94	31.98 ± 2.15	0.0001	
Total proteins (g/dL)	7.59 ± 0.13	7.97 ± 0.14	0.0785	
Albumin (g/dL)	2.79 ± 0.04	2.88 ± 0.04	0.1498	
Urea (mg/dL)	41.12 ± 1.71	34.89 ± 1.89	0.0310	
Calcium (mg/dL)	6.21 ± 0.14	7.62 ± 0.16	0.0001	
Phosphorus (mg/dL)	3.73 ± 0.26	4.58 ± 0.26	0.0008	
Magnesium (mg/dL)	2.54 ± 0.15	2.39 ± 0.17	0.5554	



For the urea metabolite, the ewes kept on cultivated pasture showed a 17.86% higher content than those managed on natural pasture, due to the better protein nutritional intake of the former. Urea is synthesized in the liver in amounts proportional to the concentration of ammonia produced in the rumen, being related to dietary protein levels and the energy/protein ratio of the diet (Siqueira et al., 2020). During the winter months, natural pastures have around 6% crude protein (Vaz et al., 2016), whereas, because they are in vegetative development, cultivated pastures such as oat and ryegrass may have high energy contents and up to 20% crude protein (Savian et al., 2020).

Ewes kept on natural pasture had higher calcium and phosphorus levels than those on cultivated pasture (Table 2). The higher phosphorus levels in the ewes managed on native pasture may be related to their blood glucose levels (Ribeiro et al., 2004; Kaneko et al., 2008; Brozos et al., 2011), and the presence of other minerals such as potassium in the diet that may compete with phosphorus absorption (Diaz González, 2018).

Phosphorus deficiency during pregnancy/lactation can lead to poor lamb growth (Radwinska & Zarczynska, 2014). Minervino et al. (2014) reported that situations with phosphorus levels below 4 mg/dL are consistent with mineral deficiency, resulting in slow growth, low fertility, and decreased appetite. Ribeiro et al. (2004) worked with ewes in the final third of gestation and observed decreases in the phosphorus concentration caused by low availability of the mineral in the animals' diet, thus recommending supplementation in this period.

As for calcium, the animals kept on natural pasture showed higher values than those reared on cultivated pasture. In both treatments, the ewes were in hypocalcemia, with serum calcium levels below the recommended physiological values (Kaneko et al., 2008), as there is an increase in demand for fetal growth and milk synthesis in the evaluated phase (Ribeiro et al., 2004; Brozos et al., 2011; Blagitz et al., 2012). The higher calcium content of animals kept on natural pasture is related to the levels of total protein in those that were kept on cultivated pasture, which influences the metabolism of this mineral. The apparent decrease in calcium may be because this mineral is transported by proteins in the blood plasma (Ribeiro et al., 2003), or even due to the imbalance between the minerals present in the diet (Diaz González, 2018).

The type of pregnancy influenced (P<0.05) only the albumin levels in the prepartum period, which were lower in the twin-pregnant ewes (Table 3). Lower albumin levels are a result of the greater protein requirement of ewes carrying twins, which was not met via the feed. According to Oliveira et al. (2016), albumin is the most sensitive indicator to determine the protein nutritional status of sheep.



#### Table 3

Means and standard errors of blood metabolites in the prepartum period of ewes with single and twin pregnancies.

Motabolito	Pregna			
Wietabolite	Single	Twin	F test	
Glucose (mg/dL)	47.49 ± 2.92	45.06 ± 2.38	0.5345	
Total proteins (g/dL)	7.95 ± 0.15	7.61 ± 0.12	0.0886	
Albumin (g/dL)	2.92 ± 0.04	2.76 ± 0.03	0.0080	
Urea (mg/dL)	37.0 ± 2.08	38.85 ± 1.69	0.5061	
Phosphorus (mg/dL)	4.02 ± 0.19	4.13 ± 0.15	0.6679	
Magnesium (mg/dL)	2.68 ± 0.18	2.25 ± 0.15	0.0773	
Calcium (mg/dL)	6.82 ± 0.17	7.01 ± 0.14	0.4214	

There was an interaction effect (P<0.05) between the feeding systems and the type of pregnancy only for the blood urea titration analyzed in the prepartum period. Among the animals kept on native pasture, urea levels were higher in the single-pregnant ewes, whereas in the group kept on cultivated pasture, higher urea levels were found in the ewes with twin pregnancy. This difference was not expected at the time of prepartum between the different feeding systems.

The correlation between the ewes' prepartum metabolites and lamb weight at 30 days (W30) was significant only for the glucose, phosphorus, and calcium levels (Figure 1). The high correlation of glucose with W30 is explained by the influence of this metabolite on milk production and mammary gland development, which allows lambs to have a greater nutritional supply and, consequently, greater weight gain from birth to 30 days of age. Glucose is important in the regulation of mammary secretion, as the

volume of milk is dependent on the water flow caused by the osmotic pressure exerted by the lactose granules secreted inside the alveolus (Machado et al., 2011). For the synthesis of one liter of milk, approximately 100 g of glucose must be extracted from the bloodstream, as it is not the same synthesized in the mammary gland (Oliveira et al., 2014). In addition, about 35% of the pregnant ewes' circulating glucose is directed to meet the energy demand of the fetal-placental unit (Ortunho & Marçal, 2014; Brito et al., 2008).

Calcium and phosphorus were negatively correlated with W30. These variables had no influence on lamb weight, as the demand for these nutrients is higher for the pregnant ewe, which will transform them for the fetal growth of the lamb. Despite the uptake of calcium and phosphorus by the mammary gland for the formation of casein for milk secretion, these were not significant to improve lamb performance.



**Figure 1.** Correlation between blood metabolites of Texel ewes prepartum and lamb weight at 30 days (W30). \*0.05; \*\*\*0.001. TP: total plasma proteins; P: phosphorus; Mg: magnesium; Ca: calcium.

# Conclusion \_\_\_\_\_

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The prepartum nutritional level and the number of lambs gestated influence the metabolic profile of ewes. Lamb weight at 30 days of age is influenced by the ewe's serum blood glucose level at the end of the gestation period.

# Conflict of interest \_\_\_\_

The authors declare that there is no conflict of interest.

# Statement of animal rights \_\_\_\_\_

The study was approved by the Ethics Committee on Animal Experimentation at UFPel (approval no. 7486/2011).

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