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Supplementation during pregnancy on the production and metabolic profile of Polwarth sheep

Suplementação gestacional na produção e perfil metabólico de ovelhas da raça ideal

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Highlights

Supplementation at the final gestation provides better conditions for calving.

Supplemented sheep have a higher fleece weight.

Supplementation at the end or during pregnancy determines greater lamb survival.

During pregnancy better nutrition leads to greater weight of ewes and lambs at birth.

Abstract _

The aim of this study was to evaluate the effect of supplementation during different stages of pregnancy on the development and productive performance of Polwarth sheep. Fifty-three adult ewes under synchronised pregnancy were submitted to a supplement of 1.5% body weight with commercial feed during the different stages of pregnancy on natural pasture: NS - no supplementation, SIG - supplemented during the initial third of pregnancy, SMG - supplemented during the middle third of pregnancy, SFG - supplemented during the final third of pregnancy, and STG - supplemented throughout pregnancy. The ewes were weighed at the time of laparoscopic insemination, at every third stage of the pregnancy, and two days before the expected lambing date, at which time the body condition score and metabolic markers were evaluated. The pre-lambing weight of the STG ewes was greater than that of the SFG and SMG ewes, which were, in turn, greater than the SIG and NS ewes. Body condition was superior in the STG and SFG ewes. Lamb survival was greater in the STG and SFG ewes, and lower in the SMG, SIG

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and NS ewes. Beta-hydroxybutyrate was lower in the SFG and STG ewes. Supplementation during the final third and throughout pregnancy increases the rates of survival and production in the lambs, with supplementation only during the final third of gestation being less costly for the production system. **Key words:** Albumin. Glucose. Lamb. Wool.

Resumo _

O objetivo do estudo foi avaliar o efeito da suplementação durante diferentes períodos da gestação no desenvolvimento e desempenho produtivo de ovelhas da raça Ideal. Foram utilizadas 53 ovelhas adultas prenhas por sincronização e submetidas a suplementação de 1,5% do peso corporal em diferentes fases da gestação em pastagem natural: SS - sem suplementação, SIG- suplementadas no terço inicial da gestação; SMG - suplementadas no terço médio da gestação; SFG - suplementadas no terço final da gestação e STG - suplementadas durante toda a gestação. As ovelhas foram pesadas na inseminação por laparoscopia, a cada terço da gestação e dois dias antes da data prevista para o parto, ocasião na qual foi avaliado o escore de condição corporal e os marcadores metabólicos. Após o nascimento os cordeiros foram mantidos com suas mães em pastagem cultivada de aveia preta (Avena strigosa) e azevém (Loluim multiflorum Lam.). No peso pré-parto ovelhas STG foram mais pesadas que as SFG e SMG e estas superiores as SIG e SS. A condição corporal foi superior para ovelhas STG e SFG. O peso de velo das ovelhas SS foi inferior ao das suplementadas, independente da fase da gestação ou na sua totalidade. A sobrevivência dos cordeiros foi maior para STG e SFG com inferioridade nas ovelhas SMG, SIG e SS. O betahidroxibutirato foi inferior nas ovelhas do SFG e STG (31 e 47 mmol/L, respectivamente). A suplementação realizada no terço final e durante toda a gestação proporcionam os melhores resultados, com aumento na taxa de sobrevivência dos cordeiros e otimizando a produção de lã das ovelhas.

Palavras-chave: Albumina. Cordeiro. Glicose. Lã.

Introduction _

The productive performance of a herd and the growth rate of its offspring are important aspects for the success of animal production (Young et al., 2014). Nutrition influences growth, both of the foetus and following the birth of the lamb (Thompson et al., 2011; Cranston et al., 2017); therefore, depending on the duration and timing of feeding restrictions on the pregnant ewe, foetal growth and the birth weight of the lamb can be affected (Behrendt et al., 2011; Brondani et al., 2016, 2022). The most critical periods are at fertilisation and during the

final stage of pregnancy, when the placenta reduces its ability to compensate for the supply of nutrients, and is not able to guarantee an adequate supply of nutrients to the ewes and foetuses (Rehfeldt et al., 2011; Du et al., 2013).

According to Gauvin et al. (2019), pregnancy in sheep is a critical period due to the increase in nutritional requirements and the mobilisation of nutrients for development of the udder and maintenance of the organism, where any feeding restrictions can affect maternal production, culminating in a reduction in the daily amount of milk (Kiani et al., 2008). From the haematological point of view, pregnancy is a stressful physiological event (Awad et al., 2021), and can reflect sub-clinically on the erythrogram results of the ewes, especially due to the high levels of endogenous cortisol during peripartum (Santarosa et al., 2022). In addition to monitoring the body condition of the herds, assessing nutritional levels by means of the metabolic profile in production animals acts as an auxiliary method of evaluating herds with different productive and reproductive indices, where an unbalanced diet during pregnancy can alter the metabolic profile of the sheep (Brondani et al., 2016, 2022). Metabolic disorders in sheep during peripartum result in significant economic loss to the producer, as they can lower milk production, reduce weight gain in the lamb, and even cause its death (Cardoso et al., 2011).

The aim of this study, therefore, was to evaluate the effect of supplementation during different stages of pregnancy on the metabolic profile, and its effects on the productive characteristics of Polwarth sheep.

Material and Methods _

The present study was carried out on private property located in the district of Capão do Cipó, Rio Grande do Sul, Brazil (28°93'45" S 54°57'80" W). All procedures were approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Santa Maria, under protocol no 8070070815.

Fifty-three adult Polwarth ewes (between 3 and 4 years of age) were used, shorn, with an average initial weight of 41.4 kg and a mean body score of 3 (scale of 1 to 5), evenly distributed for weight and body condition score over the different groups (with a different number of repetitions). The sheep were synchronised and inseminated by laparoscopy using fresh semen from a single male, with the pregnancy confirmed by ultrasound after 30 days, and gestating a single lamb. The ewes were divided into five groups and subjected to different feeding systems based on natural pasture, using supplementation during the different stages of pregnancy: NS - Ewes kept exclusively on natural pasture throughout pregnancy; SIG - Ewes kept on natural pasture and supplemented at 1.5% body weight during the first 50 days of pregnancy; SMG - Ewes kept on natural pasture and supplemented at 1.5% body weight from 50 to 100 days of pregnancy; SFG - Ewes kept on natural pasture and supplemented at 1.5% body weight from 101 days to lambing; STG - Ewes kept on natural pasture and supplemented at 1.5% body weight throughout the pregnancy.

The ewes remained in a single management group on an area of 10 hectares at an average stocking rate of 5.3 pregnant ewes/ha. The botanical composition of the native pastures in the region includes a predominance of Paspalum Notatum, Axonopus fissifolius and especially Andropogon lateralis. Among the legumes, some clovers stand out, but with heavy emphasis on family Desmoduim. The ewes were weighed at the beginning of each third stage of pregnancy and two days before the expected lambing date. Those that were consuming the supplement were separated and placed in pens equipped with a trough for regular consumption with the least possible stress. At both the start and end of the supplemented periods, the amount of supplement was increased or reduced,



respectively, with the purpose of adapting the animals to the intake or withdrawing the supplement. The supplement used for each treatment was a commercial feed with the following levels of guarantee/kg: moisture (max.) - 130 g; fibrous matter (max.) - 130 g; mineral matter (max.) - 140 g; crude protein (min.) - 140 g; ether extract (min.) -20 g; calcium (min.) - 10 g; calcium (max.) - 14 g; phosphorus (min.) - 4,000mg. The supplement irrespective of the period, was offered in individual troughs between 13:00 and 13:30, which was enough time for the stipulated amount to be consumed.

Two days before the expected lambing date, the ewes were weighed, and the body condition score was evaluated. At the time of the evaluation, a blood sample was taken to assess the metabolic markers glucose, betahydroxybutyrate, total proteins and albumin. Blood was collected in two tubes from the jugular vein using a vacutainer system; one tube containing sodium fluoride and 10% EDTA to obtain the plasma, and the other with no anticoagulant, but with coagulation activator gel to obtain the serum. The samples were centrifuged at 3,500 rpm for 15 minutes and stored in Eppendorf[®] tubes, after which they were frozen at -20°C for further analysis. When collecting the blood, the animals were immobilised in individual containment chutes, complying with animal welfare criteria (Stober & Grunder, 1993).

The metabolites glucose, betahydroxybutyrate, albumin and total proteins were quantified by photocolorimetry. A visible light spectrophotometer (FEMTO 435[®]) was used in the colorimetric analysis, together with the 1091, BT1004500, 19 and 1085 commercial kits (Labtest[®] Clinical Diagnosis System). The glucose oxidase method was employed to evaluate glucose levels, using plasma with an antiglycolytic agent (González & Silva, 2006). The levels of albumin and total proteins were measured in the serum samples using the bromocresol green and biuret methods (González & Silva, 2006), respectively.

The births all took place between 20 June and 23 June, giving a three-day difference in the age of the lambs. The lambs were weighed at birth on an electronic hook scale with a capacity of 50 kg and an interval of 20 grams, and were kept with their mothers in a cultivated pasture of black oats (Avena strigosa) and ryegrass (Lolium multiflorum), at a stocking rate of 4.5 ewe-lamb pairs per ha. At 60 days of age, the lambs were again weighed and then weaned. The lamb production index was calculated by associating the weight of the lambs at weaning and the weaning rate of the ewes, with the result expressed as kilograms of lamb produced per ewe, using a methodology adapted from Vaz et al. (2010).

In early December, 160 days after lambing, the ewes were shorn using the traditional hammer method and the dirty fleeces were weighed.

The experiment was conducted in a completely randomised design. The data were first tested for normality (Shapiro-Wilk) and submitted to analysis of variance (P< 0.05) to check the effects of supplementation during the different phases of gestation using the SAS 9.0 statistical software. The mean values were compared by Tukey's test, and the non-parametric data by the Kruskal-Wallis test, both at a significance level of 5%. The statistical analysis was carried out using the academic version of the SAS statistical package.

Results and Discussion _____

Ewes of the STG group presented higher weights and body condition scores during the prepartum period and did not differ from the SFG ewes (Table 1). Nutritional support during pregnancy and especially during the final third of pregnancy is essential, as there are important physiological adjustments that, if not met, predispose the female to metabolic disease (Araújo et al., 2014). At the end of pregnancy under non-ideal nutritional conditions, ewes need to use their body reserves to meet their physiological needs, which determines a lower weight and body condition score (Greenwood et al., 2010).

There was no loss in weight or body condition in ewes supplemented during the final third of pregnancy, since during the critical period of pregnancy their nutritional level was higher (Cranston et al., 2017), showing that it is not necessary to remove energy from the organism to maintain pregnancy and secretion of the mammary gland (Joy et al., 2014), or remove nutrients intended for the growth of wool fibre for other functions of the organism (Silveira et al., 2015).

Even with no difference in weight at weaning, lambs from ewes that were better nourished at the end of gestation showed greater survival, determining a higher lamb production index: a parameter that estimates the probable production of a herd, associating not only the weight of the lamb, but also survival and production in kilograms of lamb per ewe exposed to breeding. The maintenance of weight and body condition score determines higher rates of lamb production and survival, generating direct economic benefits, as well as improving animal welfare and meeting society's demands for ethical livestock production (Ferguson et al., 2014). In sheep, supplementation and proper nutrition are necessary, since most of the plasma glucose comes from hepatic gluconeogenesis, a result of the propionate produced by rumen microorganisms providing adequate nourishment for the different functions (Macedo et al., 2018).

STG ewes produced heavier fleeces than ewes kept exclusively on natural pasture during pregnancy; ewes supplemented during only one of the pregnancy stages presented intermediate fleece weights, and did not differ from the other animals, whether supplemented or not during pregnancy. Regarding birth weight, only lambs born to STG ewes were heavier at birth than those born to SMG ewes. The higher nutritional level resulting from supplementation during the final third or throughout pregnancy is important for providing higher birth weights (Corner-Thomas et al., 2015; Rooke et al., 2015) and, consequently, higher survival rates for the lambs. (Thompson et al., 2011). In the region of the present study, 15% to 40% of lambs are unable to survive due to the starvation/hypothermia complex, which are both of nutritional and not infectious origin.

Table 1

Mean values, coefficient of variation and standard error for the development characteristics during pregnancy and parturition of ewes and their lactating lambs, submitted to supplementation at different stages of pregnancy

	Period of Supplementation						
Characteristics	NS	SIG	SMG	SFG	STG	CV	SE
Number of ewes	11	11	11	11	9		
Initial Weight, kg	41.7	41.3	41.4	41.3	41.6	4.2	1.65
Prepartum Weight, kg	47.3 ^b	44.1°	46.4 ^b	49.4 ^{ab}	52.3ª	5.78	2.73
ADG first third of pregnancy, g/day	39	50	25	36	64	17.53	2.58
ADG middle third of pregnancy, g/day	37	5	67	44	73	22.53	3.65
ADG final third of pregnancy, g/day	35	5	8	85	80	24.65	4.01
Prepartum BCS, points	2.31 ^b	2.70 ^b	2.50 ^b	3.65 ^a	3.95ª	16.17	0.50
Fleece Weight, kg	3.31 ^b	3.60 ^{ab}	3.85 ^{ab}	3.96 ^{ab}	4.03ª	13.86	0.52
Weight at Birth, kg	3.67 ^{ab}	3.50 ^{ab}	2.75 [⊳]	3.48 ^{ab}	4.13ª	10.27	4.84
Weight at 60 days, kg	16.88	17.85	13.25	16.07	13.93	22.49	2.01
Lamb Survival, %	63,6	63,6	63,6	90,9	100,0	-	-
Number of lambs	7	7	7	10	9		
Lamb production index, kg	10.74 ^{bc}	11.35 ^{bc}	8.43°	14.60ª	13.93 ^{ab}	14.47	1.47

NS - no supplementation. SIG - initial third supplementation. SMG - middle third supplementation. SFG - final third supplementation. STG - supplementation throughout pregnancy. ADG - average daily gain. BCS - body condition score. * lamb survival up to 15 days of age. * lamb production index - Lamb weight at weaning × lamb survival/100 = kg weaned lamb/100 kg ewe. ^{a,b,c} on the same line differ P<0.05.

The lamb survival rate up to 15 days postpartum was higher for lambs born to STG and SFG ewes. There was no difference between these groups, which showed a superior survival rate than lambs born to SMG, SGI and NS ewes, with the latter groups showing no difference from each other. There are contradictory reports in the literature regarding the nutritional level imposed on the ewes during the prepartum period and its effects on lamb performance, with favourable results for better nutrition (Geraseev et al., 2006; Kenyon et al., 2014), or even showing no effects on the rate of lamb mortality from the use of supplementation during the final third of the pregnancy (Corner-Thomas et al., 2015).

Lamb weight at weaning (60 days) did not differ between groups. Ewes from the SFG and STG groups had a higher lamb production index than those from the NS, SIG and SMG groups, which did not differ from each other. The contradictory responses regarding nutritional levels are due to genetic and environmental variations that determine different results when carrying out the studies, in addition to the earlier nutritional condition of the animals (Cranston et al., 2017). With haematological parameters indicating a negative energy balance, as in the present study, Guyoti et al. (2015) found that when comparing pre- and postpartum ewes, there was a higher lamb birth weight and consequently a higher growth rate during the first weeks of life, highlighting a reduction in the risk factors that contribute to perinatal mortality.

Blood glucose levels did not differ (P>0.05) between the feeding systems as a function of the use or period of supplementation (Table 2). The average value of 27.30 mg/dL for glucose concentration found with the blood tests of the sheep in the present study shows that they are hypoglycaemic compared to the reference parameters of 50-80 mg/dL (González & Silva, 2006). When evaluating the performance of shorn and unshorn Corriedale ewes during mid-pregnancy maintained on an extensive grazing system, Guyoti et al. (2015) found values of 51.10 mg/dL and 51.5 mg/dL, respectively, which were also less than the reference values. Research into the energy, protein, mineral and enzymatic metabolic parameters of sheep raised in Brazil gives results that are generally lower than the international values used as reference, and show no signs of pregnancy toxemia (Silva et al., 2020; Varanis et al., 2021).

Rapid foetal growth during the final weeks prepartum, and the uptake of glucose by the mammary gland for lactose synthesis, physiologically reduces the concentration of this metabolite by the end of the pregnancy. This may explain the low values found in this experiment, as collections were carried out two days before lambing, when all the above effects were intense. The feeding restriction measured between 100 and 140 days of pregnancy in the ewes determined the sensitivity of the glucose levels as a function of diet and stage of pregnancy (Macedo et al., 2014), reducing the blood glucose concentration when comparing non-pregnant to pregnant ewes, with further reductions during pregnancy (Akraeim et al., 2021). Oscillations in haemato-biochemical parameters need monitoring, in some cases requiring the use of supplementation in pregnant ewes to ensure successful lamb production (Akraeim et al., 2021).

Table 2

Mean values, coefficient of variation and standard error for the components of the metabolic profile of ewes submitted to different nutritional levels during pregnancy based on the use or not of supplementation

Metabolites	NS	SIG	SMG	SFG	STG	CV	SE
Glucose, mg/dL	29.21ª	28.14ª	26.01ª	27.71ª	25.45ª	19.17	5.22
BHB, mmol/L	1.27ª	1.10 ^{ab}	1.19 ^{ab}	0.31 ^b	0.47 ^b	16.62	0.63
Total Protein, g/dL	7.15ª	6.95ª	7.43ª	6.59ª	6.57ª	11.24	0.78
Albumin, g/dL	2.39ª	2.37ª	2.32ª	2.37ª	2.36ª	13.11	0.79

NS - no supplementation. SIG - initial third supplementation. SMG - middle third supplementation. SFG - final third supplementation. STG - supplementation throughout pregnancy.



The beta-hydroxybutyrate (BHB) behaved contrary to the body condition score between the feeding systems, i.e. ewes with better body condition scores had lower concentrations of the metabolite. On the other hand, albumin and total proteins did not differ between the feeding systems. Only ewes from the STG and SFG groups (0.47 and 0.31 mmol/L, respectively) had values within the reference range for BHB, which is less than 0.60 mmol/L (Kaneko et al., 1997). The increase in BHB concentration in the groups that received supplementation during the initial and middle third of pregnancy, and in those that received no supplementation, may be associated with the energy demand of the animals for maintaining foetal development. The moderate positive correlation between non-esterified fatty acids (NEFA) and BHB shows that the mobilisation of body fat leads towards a moderate increase in BHB concentration, and when, during the final third of pregnancy, the negative energy balance is not so intense, allows for complete oxidation of the mobilised NEFA compared to partial oxidation during the ketogenesis cycle (Araújo et al., 2014).

Oliveira et al. (2016), working with the metabolic profile during different peripartum stages of Santa Inês ewes in the Western Amazon, found values for BHB within reference levels, attributing this to the genetic and adaptive characteristics of the animals, to the breeding system and to feeding, together with a body condition score of 3.0-3.5 for the ewes, allowing energy reserves.

BHB is considered a better energy indicator than glucose, as it does not have such tight homeostatic control and is stable in

serum (Feijó et al., 2014). The increase in BHB concentration during pregnancy and lactation coincides with a period of accelerated foetal growth and udder development, as well as a greater demand for glucose for synthesising milk (Joy et al., 2014). Low plasma glucose and high BHB concentrations suggest that the animals are in a state of negative energy balance, and as such, mobilising body reserves (Ribeiro et al., 2003). In situations with a marked reduction in blood glucose, there is a considerable increase in the degree of gluconeogenesis and, in some cases, the development of ketosis, with an increase in the levels of plasma BHB, acetoacetate and acetone (González, 2000). In the present study, a deficient nutrient supply was clearly seen in some feeding systems; however, for each system, serum BHB concentrations increased in hyperglycaemic animals.

Even with no difference between the periods of supplementation during pregnancy, total protein concentrations remained within the described physiological parameters of 6.6 to 9.0 g/dL (Contreras et al., 2000). Albumin also showed no difference between the periods of supplementation (average of 2.36 g/dL), however did not maintain the required concentration of 2.4 to 4.2 g/dL (Contreras et al., 2000). Albumin is important as it is the most abundant protein in plasma (35%-50% of the total proteins), contributing to 80% of blood plasma osmolarity, and is also considered an important protein reserve, responsible for the transport of numerous substances in the body. (Hoffman et al., 2001).

If pathological causes are excluded, lower levels of total proteins in the metabolic profile of ewes during pregnancy, when found,

are due to dietary deficiencies (Brito et al., 2006), or even under favourable nutritional conditions, to protein requirements in the face of the greater physiological demands imposed by foetal growth and udder development, which both take place during this period (Ribeiro et al., 2003; Joy et al., 2014). When studying the inclusion of lipid sources with different degradation sites on the metabolic profile of pregnant ewes, Sousa et al. (2020) found lipid supplementation to be efficient in maintaining the energy, hepatic and protein metabolism of the animals by altering the concentration of betahydroxybutyrate (BHB) during the middle and final third of pregnancy.

Conclusions .

The use of better nutritional levels during pregnancy by means of supplementation during the final third and throughout the pregnancy determines greater weight for the ewe and lamb at birth, with a consequent increase in the survival rate of the lambs and optimisation of the wool production of the sheep; whereas supplementation during earlier periods has no effect on the characteristics that determine the productivity of sheep herds. Therefore, evaluated from an economic point of view, supplementation only during the final third of pregnancy is the best option for producers for higher lamb production.

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