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PRODUÇÃO ANIMAL

REPRODUCTIVE AND METABOLIC RESPONSES IN EWES TO DIETARY PROTEIN SUPPLEMENT DURING MATING PERIOD IN DRY SEASON OF NORTHEAST BRAZIL

RESPOSTAS REPRODUTIVAS E METABÓLICAS EM OVELHAS SUPLEMENTADAS COM DIETAS PROTEÍCAS DURANTE O PERÍODO DE ACASALAMENTO NA ESTAÇÃO SECA DO NORDESTE DO BRASIL

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Abstract:
This study evaluated the effect of food supplements with different levels of protein on reproductive and metabolic response of ewes during the mating period. Forty-one ewes were supplemented during 43 days with amount protein to meet 1.0 (diet I; n = 14), 1.7 (diet II; n = 13) and 2.1 (diet III; n = 14) times the maintenance requirements. Dry matter (DM) intake was higher ($P < 0.01$) in diet III when compared to diets I and II. Orts were lesser in diets II and III ($P < 0.05$) when compared to diet I. Intake of organic matter (OM), crude protein (CP) and ether extract (EE) was higher in diet III ($P < 0.05$), but NDF and ADF intake was superior in diet I ($P < 0.05$). In diet III, a higher frequency of female mated was observed ($P < 0.05$). The prolificity and twinning rate was higher in ewes of diet II ($P < 0.05$). Greater birth weight of lambs ($P < 0.05$) was verified in diet III. The progesterone levels were affected by diets II and III ($P < 0.05$). In conclusion, the supplementation of ewes with intermediate level of protein improves their reproductive response.
Keywords: estrus synchronization; mating; protein; sheep.

Resumo:
Este estudo avaliou o efeito de suplementos alimentares com diferentes níveis de proteína sobre a resposta reprodutiva e metabólica de ovelhas durante o período de monta. Quarenta e uma ovelhas foram suplementadas durante 43 dias com proteína em quantidade para satisfazer 1,0 (dieta I; n = 14), 1,7 (dieta II; n = 13) e 2,1 (dieta III; n = 14) vezes as exigências de manutenção. O consumo de matéria seca (MS) foi superior ($P < 0,01$) na dieta III quando comparada com as dietas I e II. As sobras foram menores nas dietas II e III ($P < 0,05$) quando comparada à dieta I. O consumo de matéria orgânica (MO), proteína bruta (PB) e extrato estéreo (EE) foram superiores na dieta III, porém a ingestão de NDF e ADF foi maior na dieta I ($P < 0,05$). Na dieta III foi observada uma

maior frequência de fêmeas montadas (P < 0,05). A taxa de prolificidade e gemelaridade foi maior em ovelhas da dieta II (P < 0,05). Verificou-se um maior peso ao nascimento de cordeiros (P < 0,05) na dieta III. Os níveis de progesterona medidos após o acasalamento foram afetados pelas dietas II e III (P < 0,05). Em conclusão, a suplementação de ovelhas com nível intermediário de proteína melhora sua resposta reprodutiva.
Palavras-Chave: monta; ovinos; proteína; sincronização do estro.

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Introduction

In the Northeast region of Brazil, small ruminants' rearing is based on native vegetation pasture systems, which have the advantage of low production cost; however, extreme climatic situations, such as prolonged droughts, occur with a cyclic frequency, influencing the reproductive performance of these animals. Unfortunately in the Northeast region of Brazil there is still relative little information about the recommended protein levels necessary to meet the requirements of the local ovine breed. Adequate levels of protein, together with a good profile of amino acids, have been pointed out as essential especially for the functioning of specific mechanisms in reproductive processes. This is needed in order to improve the energy balance of the animal (reducing the synthesis of urea), as well as enhancing the synthesis of lipoproteins, essential agents for the transport of cholesterol which is necessary for steroidogenesis[(1)], besides participating in the direct stimulation of IGF-I secretion by luteal cells[(2)]. Based on the understanding that foods rich in degradable nitrogen in the rumen and soluble carbohydrates stimulate the synthesis of microbial protein, as well as non-degradable crude protein, contributing to the requirements of metabolizable protein[(3)], corrective measures such as diet supplementation work as an effective alternative, particularly when carried out in specific periods of the reproductive cycle, such as before and during the mating season[(4)], in the last third of gestation and in the postpartum period[(5)].
Therefore, this study was conducted to evaluate the effect of food supplements with different levels of protein on reproductive and metabolic response of ewes during the mating period in the Northeast of Brazil.

Materials and Methods

This study was conducted at the Rio Formoso Experimental Farm, belonging to the State University of Ceara, located in the municipality of Guaiuba-CE, situated at 4º 2' 23'' S and 38º 38' 14'' W, in the period of December to January, during the dry season. The area, characterized by a constant photoperiod regimen, has a warm, tropical, sub-humid climate with a mean annual rainfall and temperature of 904.5 mm and 26 – 28 ºC, respectively, with two distinct seasons: rainy from February to May and dry from June to January.
Forty-one adult ewes of the Santa Ines breed, cyclical and pluriparous, were divided into three lots, homogeneous in weight, body score condition and age (41.77 ± 0.87 kg; 2.8 ± 0.03 and 27.88 ± 0.95 months; p>0.05; overall average, respectively). The ewes were housed in three pens with free access to an open paddock area and received diet daily (Table 1) providing 5.40% (Diet I; n = 14),

9.52% (Diet II; n = 13) and 11.61% (Diet III; n = 14) of dry matter, respectively, 1, 1.7 and 2.1 times of the protein requirement for maintenance of live weight[6] during 43 days.

Table 1: Diet's ingredients and composition

Constituents	Diet			Silage	DCAB	Concentrate
	I	II	III			
Ingredients (% DM)						
Sorghum Silage	73.56	44.00	19.19	-	-	-
Dried Cashew Apple Bagasse	24.04	43.12	56.43	-	-	-
Concentrate – based supplements	2.40	12.89	24.38	-	-	-
Composition (% DM)						
Organic Matter	94.21	95.46	96.16	98.59	95.84	96.97
Crude Protein	5.40	9.52	11.61	4.02	14.95	16.67
Ether Extract	1.82	2.84	3.78	2.70	6.64	12.04
Ash	6.04	4.74	4.03	1.41	4.45	3.03
Neutral Detergent Fiber	62.82	57.69	55.76	64.92	81.78	-
Acid Detergent Fiber	38.18	32.38	29.50	38.50	33.07	-
Ingredient composition of the concentrate – based supplements (% DM)						
Ground corn	54.19					
Cashew nut brun	27.00					
Wheat brun	8.00					
Soybean meal	5.00					
Vitamin mineralized premix	4.00					
Urea	1.00					
White salt	0.81					

DCAB: Dried Cashew Apple Bagasse; DM: Dry Matter.

Water was supplied ad libitum. Seven days after the introduction of the experimental diet, estrus was induced in the ewes using a controlled intravaginal drug release (CIDR®) device impregnated with 0.33 g progesterone (Eazi-Breed CIDR®, InterAg, Hamilton, New Zealand), which was left in the cranial portion of the vagina for 5 days. Upon removal of the device (time zero), the ewes received 1 ml $PGF_{2\alpha}$ (Lutalyse®, Upjohn, Kalamazoo, USA), and 24 h after removal of the device they were exposed to rams of proven fertility, equipped with marker pigtails, which remained among the females for 72 consecutive hours. Diets were maintained up to the diagnosis of gestation, using a Pie Medical Scanner (Falco 100, Pie Medical equipment B.V., Maastricht, Netherlands) attached to a 6.0/8.0 MHz linear array transducer at 30 days after mating, and at this moment, orts were collected and weighed daily to determine the extent of acceptance of the dietary supplement. Samples of feed ration and orts were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash and organic matter (OM), according to AOAC[7], and acid detergent fiber (NDF) and neutral detergent fiber (ADF) were analyzed following procedures described by Van Soest et al.[8].

At the start of nutritional treatment and every three days after mating up to the 12th day, blood samples were taken before grazing (7 AM) and were collected in heparinized tubes by venipuncture. Plasma progesterone and insulin were assessed by RIA kit (IBL International GmbH, Flughafenstr, Hamburg, Germany)[9]. The interval of blood sampling after mating was determined based on the elevation of progesterone levels during the initial phase of embryonic development in

sheep[10]. Enzymatic methods were used to determine plasma concentrations of urea, glucose and creatinine by commercial kits (Wiener Laboratórios, Rosário, Argentina).

All data were analyzed using the SAS program software (SAS, Inc., Cary, NC, USA). For live weight, orts, intake, metabolite and plasma progesterone concentration, effects of protein level (Diet I, II and III), interval of assessment considered (time) and interaction, were analyzed using GLM procedures. For the number of marked sheep and reproductive response variables, effects were analyzed by the NPAR1WAY procedure. Differences between diet groups were determined by Duncan's test. Comparison between numbers of sheep was performed using the chi-squared test ($P < 0.05$).

Results

There was a significant effect of dietary supplementation ($P < 0.01$) on dry matter intake and of other dietary nutrients expressed in g/kg $W^{0.75}$, as well as on the percentage of orts (Table 2). The same parameters were not affected by the duration of diet, nor was there an interaction between diet and time ($P > 0.05$). The intake of dry matter (Table 3) was greater ($P < 0.01$) by ewes of group III when compared to the diet intake of groups I and II. The amount of orts from the feed did not differ between animals that received diet II and III and it was lower than the orts of diet I ($P < 0.05$). The intake of the dietary nutrients OM, CP, EE (Table 2) differed among the three diets ($P < 0.05$), being the highest in ewes on diet III, while NDF and ADF were comparatively higher ($P < 0.05$) in ewes that received diet I. The type of diet, time and interaction showed no significant effects ($P > 0.05$) on plasma levels of glucose, insulin and creatinine (Table 2). Due to the high complexity of the results and the close relationship with protein metabolism, the plasma urea levels were shown separately (Figure 1).

Table 2: Body weight, orts, intakes and metabolites in ewes supplemented during the mating

Attributes	Diet			Effects		
	I	II	III	Diet	Time	Interaction
No. of ewes exposed	14	13	14			
Body weight (kg)	42.15 ± 1.71	42.72 ± 1.44	40.39 ± 1.37	ns	-	-
Orts %	2.01 ± 0.18a	1.40 ± 0.23b	1.44 ± 0.23ab	*	ns	Ns
Intake (g/kg $W^{0.75}$)						
Dry Matter	56.77 ± 0.11a	56.52 ± 0.13a	58.63 ± 0.13b	***	ns	Ns
Organic Matter	55.83 ± 0.10a	56.33 ± 0.13b	58.09 ± 0.15c	***	ns	Ns
Crude Protein	2.99 ± 0.01a	5.64 ± 0.01b	7.19 ± 0.01c	***	ns	Ns
Ether Extract	1.08 ± 0.01a	1.69 ± 0.01b	2.33 ± 0.01c	***	ns	Ns
Neutral Detergent Fiber	37.14 ± 0.07a	33.95 ± 0.09b	34.22 ± 0.09c	***	ns	Ns
Acid Detergent Fiber	22.57 ± 0.05a	19.03 ± 0.06b	18.05 ± 0.06c	***	ns	Ns
Hormone and Metabolites						
Insulin (µU/mL)	11.92 ± 0.63	10.49 ± 0.32	11,62 ± 0.53	ns	ns	Ns
Glucose (mg/dL)	77.45 ± 12.40	75.41 ± 12.20	70.04 ± 12.05	ns	ns	Ns
Creatinine (mg/dL)	0.99 ± 0.27	0.92 ± 0.14	1.36 ± 1.85	ns	ns	Ns

[a,b,c] $p < 0.05$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns not significant.

Urea concentrations were significantly influenced by time and by interaction effects ($P < 0.01$). The animals fed diets II and III showed an increase ($P < 0.05$) in plasma urea on days 0 and 3 after mating (Figure 1), corresponding, respectively, to 13 and 16 days of dietary treatment. For these diets peak levels occurred on days 3 and 6 after mating, but for diet I, the increase in plasma urea was gradual, where values were higher ($P < 0.05$) than in other treatments from day 6 to 12 days after mating ($P < 0.05$) or 25 days after the start of dietary treatment.

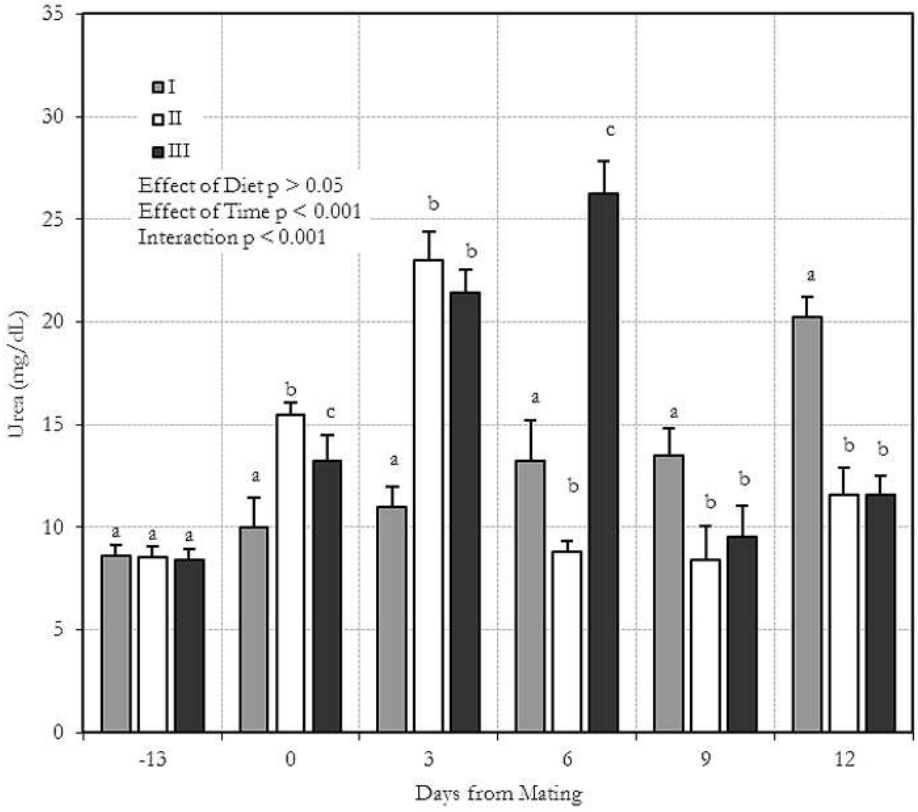

Figure 1: Urea level measured in ewes supplemented during mating. Values are given in means ± SEM. [a,b,c] $p < 0.05$ comparisons between diets in each period. Statistically significant effect of Diet, Time of treatments and Interaction are given in the figure.

Figure 3 illustrates the concentrations of progesterone within twelve days after mating in pregnant ewes fed the three diets. There was a significant effect for dietary supplementation ($P < 0.01$), time ($P < 0.01$) and interaction between these factors ($P < 0.05$). Regarding the response to estrus synchronization, it did not differ in the total number of marked animals for any of the diets (Table 2 and Figure 2). The frequency of the markings was higher for diets I and II, at 24 and 48 h after removal of the CIDR ($P < 0.05$), while for diet III, 100% of animals were marked at 48 h ($P < 0.05$) (Figure 2). The weights determined upon CIDR insertion and at delivery, pregnancy rate, parturition rate, duration of gestation and mortality rate (Table 3), were similar with the three diets ($P > 0.05$), while the parameters prolificity and twinning rates were significantly higher with diet II in relation to the other diets ($P < 0.05$). In lambs, birth weight was affected by supplementation ($P < 0.05$), as well as the weight of male lambs and singleton deliveries ($P < 0.01$). In these, diet III produced the greatest live weight ($P < 0.05$), while there was no difference between diets I and II ($P > 0.05$).

Table 3: Reproductive response and lambs birth weights of ewes supplemented during the mating

Attributes	Diet			Effect of Diet
	I	II	III	
No. of ewes exposed	14	13	14	
Body weight changes (kg)				
CIDR Insertion	41.90 ± 2.49	43.14 ± 1.74	41.03 ± 1.98	Ns
Parturition	44.47 ± 3.20	44.39 ± 2.07	41.76 ± 2.39	Ns
Reproductive Response				
Ewes marked	100% (14/14)	92.31% (12/13)	100% (14/14)	Ns
Pregnancy rate	78.57% (11/14)	91.67% (11/12)	100% (14/14)	Ns
Lambing rate	64.29% (9/14)	83.33% (9/13)	64.29% (9/14)	Ns
Litter size	1.00 ± 0.10a	1.44 ± 0.17b	1.11 ± 0.11a	*
Twinning rate	00.00 % (0/0)a	44.44 % (4/9)b	11.11% (1/9)a	*
Gestation length (days)	147.89 ± 1.37	143.78 ± 4.54	149.56 ± 0.67	Ns
Total Lamb Mortality	35.71% (5/14)	30.77% (4/13)	35.71% (5/14)	Ns
Birth weight (kg)	2.77 ± 0.16a	2.71 ± 0.12a	3.29 ± 0.13b	*
Male	2.60 ± 0.10a	2.87 ± 0.10a	3.45 ± 0.12b	**
Female	2.81 ± 0.21	2.35 ± 0.30	3.05 ± 0.25	Ns
Single	2.77 ± 0.16a	2.94 ± 0.10a	3.44 ± 0.09b	**
Twins	-	2.57 ± 0.18	2.70 ± 0.30	Ns

[a,b,c] $p < 0.05$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns not significant.

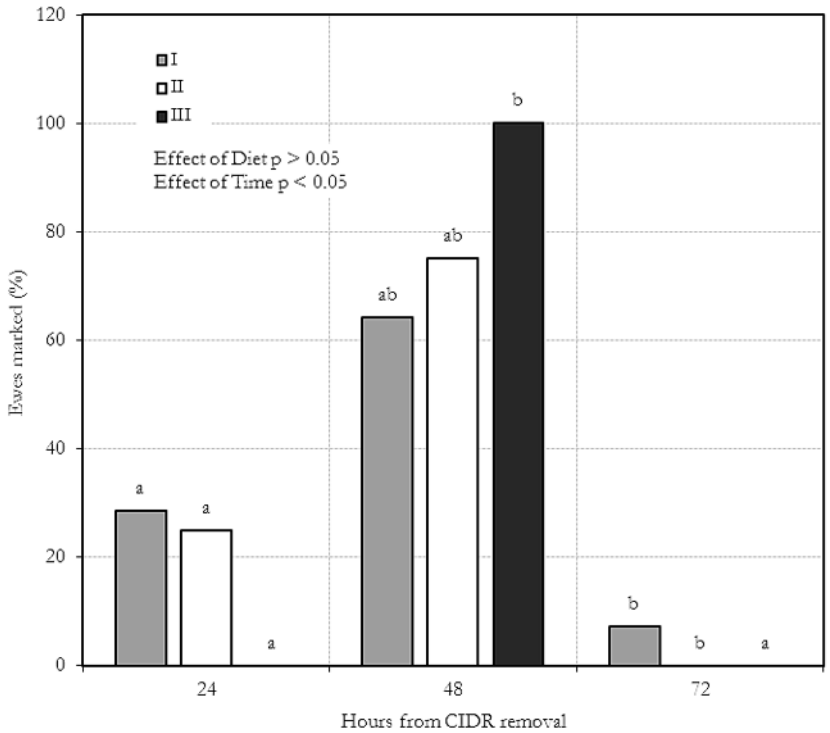

Figure 2: Distribution of ewes marked (n = 40), according to the time (hours) from CIDR removal. [a,b] $p < 0.05$ comparisons among interval in each group. Statistically significant effect of Diet and Time of treatments are given in the figure.

The three experimental diets showed increasing plasma progesterone levels during the sampling interval with values over 1 ng/mL starting on the 6th day after mating in group I, and starting on the 3rd day with diets II and III, indicating the presence of a functioning corpus luteum. On days 3 and 6 after mating, diet III produced higher progesterone concentrations compared to group I ($P < 0.05$), while on days 9 and 12, animals on diet II had higher values ($P < 0.05$) than did those fed diet I (Figure 3).

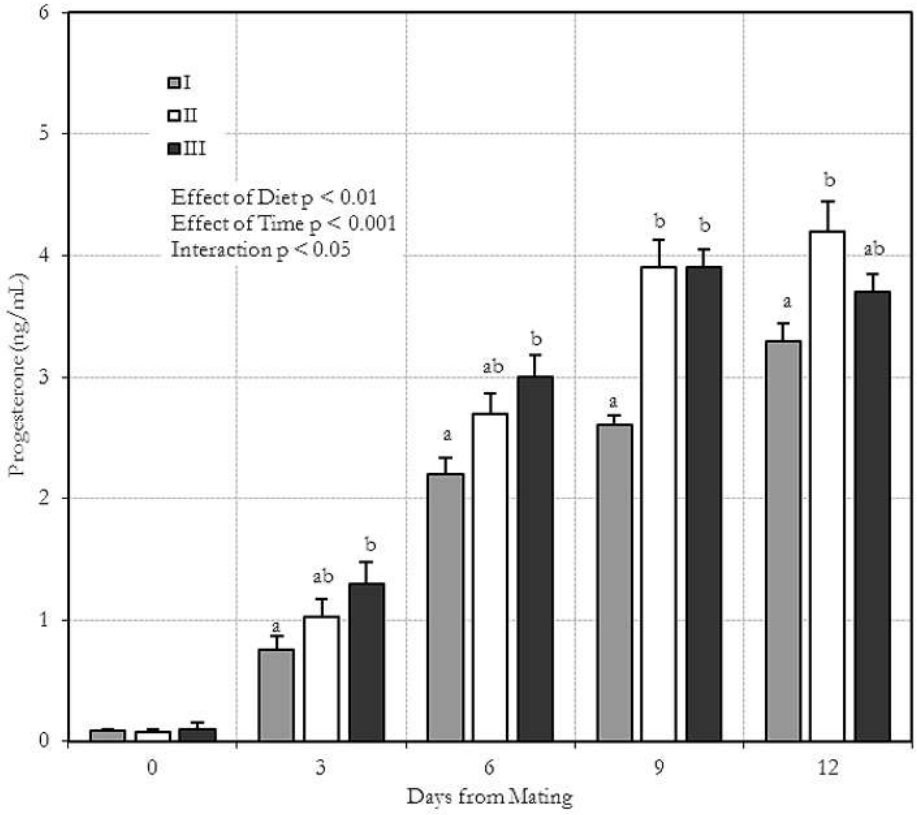

Figure 3: Progesterone level measured in ewes supplemented during the mating. Values are given in means ± SEM. [a,b] $p < 0.05$ comparisons between diets in each period. Statistically significant effect of Diet, Time of treatments and Interaction are given in the figure.

Discussion

The quantity of daily dietary orts was taken as the degree of acceptance on the part of the ewes, and showed that group II was better consumed, because there was no inhibitory effect on intake by the animals with the maximal increment in protein in diet III, which corresponded to a greater inclusion of cashew. Despite the intake of nutrients, the animals that received the greatest amount of proteins generally ingested the most readily available nutrients and the least quantity of NDF, favoring consumption. Ferreira et al.[11] compared the intake of nutrients and production performance of sheep fed diets based on elephant grass silage with or without pineapple, west indian cherry and apple cashew byproducts, and found that the consumption by animals fed silage containing apple cashew byproduct was greater than that of those fed the other silages.

In ewes supplemented with the greatest protein amount (Diet II and III), plasma peak levels for urea were positively related to the increase in dietary protein, since blood urea concentrations can undergo transient changes, even during the day or after eating[12]. The urea levels we found were below the 17 to 42 mg/dL range described for sheep by Kaneko[13]. Ribeiro et al.[14] determined plasma urea levels in Border Leicester x Texel ewes reared extensively on natural pastures, finding values of 7.61 ± 1.8 mg/dL and 7.08 ± 1.7 mg/dL during the initial period of gestation and in nonpregnancy, respectively. Although blood urea concentration is directly related to the protein supplement in the feed ration and to the energy/protein ratio, it is necessary to consider the capacity of homeostatic adaptation of the liver to changes in protein levels in the diet and the capacity of this organ to synthesize urea from absorbed ammonia, causing recycling via saliva or excretion of the excess through the urine. An elevated level of creatinine is a sensitive indicator of renal injuries and muscle depletion since it is formed from phosphocreatine in the muscles[15]. In this study, the values of this metabolite were relatively lower than those cited as reference for sheep (1.2 to 1.9 mg/dL[13], indicating that the ewes did not lose weight over the course of the experiments. Therefore, animals with different body conditions and different proportions of muscle and fat can excrete different amounts of creatinine per unit of live weight[16].

In this study the alimentary treatment time was not chosen aiming to verify the effects of the diets on the entire goat folliculogenesis. Traditionally, the treatments intended to have an acute change of nutrition effect[17] on the follicular pools that are usually subjected to recruitment waves, i.e., tertiary follicles. It has been shown that short period (< 7 days) of feeding before[17] or after ovulation[18] can have an extensive influence on follicular development and on the quality of the oocyte and embryo development, since it is the duration of the follicle of 2 mm to develop to ovulatory stage[19]. On the other hand, the utilization of feeding treatments of at least two weeks before the mating is an established practice, whose effects have been widely documented in the specialized literature[20]. For instance, flushing can be done between two and four weeks before the mating season, depending on the nutritional state of the animals[21].

The response to estrus synchronization observed in this study was as expected. Oliveira et al.[22] reported that the CIDR-PGF2α hormone protocol is a highly efficient treatment in synchronizing oestrus in goats. The administration of exogenous progesterone during the anoestrus period sensitizes the hypothalamus-hypophysis-ovarian axis directly or indirectly, besides unblocking the release of gonadotrophins and activating the behavioral centers, thereby stimulating the expression of oestrus in a large proportion of animals[23]. Currently, it has been suggested that the efficiency of oestrus synchronization treatments is subjected to the nutritional status of the animal before the hormone protocol [21]. In addition, several studies[24-26] have observed that protein level in the diet do not change the frequency of natural or synchronized oestrus in ewes, but it can increase the progesterone levels[25]. This information is consistent with our results, demonstrating that an increase in the protein level in diet for ewes maintained in adequate body condition did not show any significantly effect on number of animals in oestrus, but may affect the moment of estrus manifestation.

Dietary supplementation before and during the mating period makes it possible to significantly increase the reproductive parameters of ewes in virtue of the increased rate of ovulation[4]. Abecia et al.[24] recorded a pregnancy rate of 100% in ewes submitted to flushing 15 days after the start of mating, while this rate was 40% in those without supplementation. Additionally, it has been reported that flushing leads to a greater number of fertilized oocytes due to the increased rate of

ovulation[4] and lower embryonic mortality[27]. Adequate levels of protein can improve the energy balance of the animal, and also promote the synthesis of lipoproteins[28] which are essential agents for the transport of cholesterol, a molecule necessary for steroidogenesis, besides participating in the direct stimulation IGF-I secretion by luteal cells[2]. Various mechanisms, by which the increase in the energy level of the diet elevates the number of follicles, appear to be mediated by alterations in blood concentrations of glucose[29], and insulin[30]. Insulin is the principal hormone controlling the utilization of glucose by extra-hepatic tissues in ruminants and, as in other species, shows a wide range of activities, with influence also on protein and lipid metabolism[31]. Glucose is apparently the specific mediator of nutrition effects on animal reproduction[32], and it can indicate faults in the homeostatic mechanism that balances glucagon and insulin levels[33]. In this study, we observed glucose levels within the reference range cited for sheep (50-80)[13], and close to those reported by López e Stumpf Junior[34], when evaluating the influence of different levels of sorghum grain as an amide source for sheep.

The ewes that received diet II showed the best prolificity rates (number of lambs born/number of lambing females) among the three groups of ewes. Mori et al.[35] evaluated over two consecutive years the reproductive performance of ewes that received, before and during the mating period, ground corn or soybean meal + ground corn as dietary supplementation, and observed parturition levels of 90.48% and 77.55%, and prolificity of 1.22 and 1.00 for the first and second year, respectively. Mexia et al.[36] evaluated the reproductive behavior of Santa Ines ewes fed basically on pasture and residues of cassava starch and supplemented at different stages of gestation (initial third and last third) with soybean grain husk and found prolificity rates that varied from 1.19 to 1.32 with 25% twin rate and 75% single rate, comparatively lower than that observed in the group of animals that received diet II in the present study. In the three diets, loss of newborns was similar. The type of parturition is one of the factors of great influence on the survival of lambs because it is directly related to birth weight and amount of milk available[37]. The nutritional state of the female in the peri-partum period determines, to large extent, the vigor of the lamb at birth, the amount of milk that can be produced and the maternal ability of the female[38]. The lambs born to ewes fed diets I and II had similar weights while those born in the group III were significantly heavier, which can be explained by the greater number of heavier lambs born in this group that were males and of single births, while among the female newborns there were no differences observed in weight, nor among the newborns from twin births.

Diet supplementation clearly influenced the levels of circulating peripheral progesterone. Considering that plasma progesterone levels higher than 3 ng/mL characterize the gestation phase[39], the ewes in group I reached these levels by the 12th day after mating, while those of group II by the 9th day and those who received the highest level of added protein by the 6th day. This behavior does not exclude the influence of exogenous progesterone, as in the case during the synchronization of estrus[40]. However, considering that the corpus luteum is an original structure of the remaining cells of the ovulatory follicle, it is possible that their presence had occurred primarily in those females supplemented with the highest levels of protein, probably in response to the increased proliferation of granulosa cells of the ovulatory follicle. Apparently, there is a positive correlation between follicle size and luteal volume[41]. Lammoglia et al.[42] found that the number of follicles classified as large (> 8 mm) also increased when Brahman cows were supplemented with rice bran.

Conclusions

We concluded that the utilization of different levels of dietary protein for ewes in the mating season have important implications for the metabolic and reproductive responses of these animals. Although the diet tested apparently made a sufficient contribution to nutritional requirements, since there was no weight loss, inhibition of intake or nutritional/metabolic alterations that could indicate the contrary, the increase in protein contribution in the diets induced an increase in the quality of the corpus luteum following mating. However, only the 9% of protein supplementation stood out with respect to multiple and twin births, and therefore, it is regarded as the supplement most indicated for ewes reared under experimental conditions tested during periods of dry season in Northeast Brazil.

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