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UREA CAN REPLACE SOYBEAN MEAL IN SHEEP DIET WITHOUT ALTER INTAKE, DIGESTIBILITY AND RUMINAL PARAMETERS

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ABSTRACT

This study aimed to evaluate the replacement of soybean meal by urea on ruminal parameters, nutrient intake and digestibility in sheep fed with spineless cactus based diets. Four rumen fistulated sheep, non-emasculated, without defined breed were used. Samples were collected for determination of nutrient intake and digestibility.Ruminal fermentation pattern was determinatethrough the collection of ruminal liquid immediately prior to feeding and at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 h post-feedingfor determination of pH, ammoniacal nitrogen (N-NH_3) and short-chain fatty acids (SCFA). The replacement of soybean meal by urea did not influence the intakes of dry matter, crude protein, ether extract, organic matter, mineral matter, total carbohydrates, non-fibrous carbohydrates and neutral detergent fiber. The replacement of soybean meal by urea did not influence the apparent digestibility coefficients of dry matter, crude protein, ether extract, neutral detergent fiber, total carbohydrates and non-fibrous carbohydrates. Ruminal parameters evaluateddid not influence the levels of urea replacement by soybean meal on any of the observed variables. Urea can replace soybean meal up to 24.0 g/kg in the diet of sheep fed on spineless cactus based diets without altering intake, nutrient digestibility and ruminal parameters.

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INTRODUCTION

The protein ingredients commonly used for formulating feed are the most expensive ones, which often make the development of the sector unfeasible. Therefore, it is necessary to search for means that enable a safe and efficient replacement of the true protein source by a source of non-protein nitrogen (NPN). Urea ($(NH_2)_2CO$) is a solid organic compound of fast water solubility and hygroscopic. According to the chemical classification, it is an amide, therefore it is not a true protein, and belongs to the group of non-protein nitrogenous compounds. According to NRC (2001), urea has 45% N, corresponding to the equivalent of 281% crude protein with high rumen degradability. Basically, urea is included in ruminant diets in order to reduce costs, since the potential production of microbial protein from NPN is very considerable, which allows the breeder to substitute true protein sources, which are more onerous. In addition, urea exerts beneficial functions related to ruminant nutrition. Ruminants like sheep can take advantage of non-protein nitrogen through the action of the microorganisms present in the rumen, which are capable of transforming nitrogen from some NPN compounds into high nutritional protein, which are the microbial proteins. Thus the use of urea in the diet of these animals presents itself as a saving method, allowing to conserve inputs, such as soybean meal, normally used in human food and other non-ruminant animals. The high rumen hydrolysis rate of urea causes the rapid release of ammonia due to accumulation of N-NH_3 in the rumen. The excess intake or lack of energy readily available for NH_3use by ureolytic bacteria can lead to intoxication, which will culminate in a high expenditure of ATP for the excretion of this surplus, decreasing the energy availability for the animal (Azevedo et al., 2008). Due to the large amount of ammoniacal nitrogen (N-NH_3) present in the rumen immediately after the feed containing urea, it is essential that there be carbon skeletons to form the microbial proteins, in addition to the sulfur supplementation for the formation of sulfur amino acids.

The supply of foods rich in soluble carbohydrates of rapid availability in the rumen is essential for the use of urea, as it will be the necessary input of carbon skeletons, maximizing the use of N and avoiding intoxication caused by the excess of N-NH_3 absorbed by the ruminal membrane and consequently circulating in blood plasma. The spineless cactus is an excellent alternative for consortium with urea, as it is a plant widely cultivated in semi-arid zones; its adaptability and constitution make it one of the main foods for ruminants in these regions. According to Ferreira (2009), the high non-fibrous carbohydrates (NFC) content of spineless cactus has aroused the interest for its use in replacing energy concentrates and also its association with NPN sources, notably urea. In view of the above, it becomes evident the need to deepen the technologies related to the use of urea in diets based onspineless cactus, since it is essential to know beyond the productive parameters of these diets, those of degradation, physiology and metabolism, which will optimize the use of nutrients and bring more reliability in the association of spineless cactus with urea. The present experiment was intended to evaluate the replacement of soybean meal by urea on nutrient degradation, ruminal and physiological parameters, intake and digestibility of nutrients from sheep fed with spineless cactus.

MATERIALS AND METHODS

This study was carried out in strict accordance with the recommendations of the Guide of the National Council for the Control of Animal Experimentation (CONCEA). The field phase of the experiment was conducted at the Experimental Station of Small Ruminants in the municipality of São João do Cariri-PB, belonging to the Agricultural Sciences Center of the Federal University of Paraíba - CCA / UFPB. The municipality of São João do Cariri is located in the Cariri Oriental region of Paraíba, between coordinates 7º 29 '34 "South Latitude and 36º 41' 53" West Longitude. This location has a semiarid climateand average annual temperatures around 26º C, rainfall of 400 mm per year, in the last ten years with irregular distribution, observing dry season with duration of more than eight months, and humidity relative to the air around 68%. The laboratory analyzes were performed at the Laboratory of Analysis of Food and Animal Nutrition of the Department of Animal Science (CCA/UFPB). For the execution of the experiment 150 days were required, 60 days were for adaptation of sheep to the ruminal fistula and approximately 88 days for the experiment which were divided in four periods of 22 days, each phase consisting of 15 days of adaptation and seven for data collection. The animals used in the experiment were four castrated Santa Inês sheep, with an average body weight of 50 kg, and had 15-mo age. The animals were wormed, vaccinated against clostridiosis, identified and confined in individual stalls with a 3.75 m^2 area made of wood on beaten floor, oriented east-west with clay tile. The litter was cleaned daily and the animals were weighed weekly (BL300pro Balance). The feedstuffswere offered (*ad libitum*) as a complete mixtureand occurred twice daily (at 08:00 and 16:00 h).Leftovers were collected and weighed daily at 6:30 am and the intake was adjusted daily allowing 10% remains. The water was available daily, clean and of good quality.

The experimental rations were composed of Tifton hay, spineless cactus (*Opuntiaficus-indica* Mill), ground corn, soybean meal, urea and sulfur. The diets were formulated, based on the nutritional requirements of growing sheep, according to NRC (2007). The percentages of urea were increasing from 0 to 2.4 g/kg, while soybean meal decreased from 187.0 to 0 g/kg; the amount of corn in the feed was also increased due to the energy deficiency of urea, while the sulfur increased proportionally to urea in a 9:1 ratio. The values of the food base (spineless cactus), hay and mineral sourcedid not change (Table 1). Samples were collected from the diets and leftovers, which were pre-dried in an oven under forced ventilation at 55ºC until a constant mass was obtained. They were identified and stored in a freezer at -20ºC and ground in a Wiley mill in sieves with 1mm diameter for further determination of the chemical composition. For this purpose an individual composite sample of leftovers and feeding stuffs supplied throughout the experimental period was prepared per animal for the determinations of dry matter (DM)(method 967.03), mineral matter (MM) (method 942.05), crude protein (CP) (method 988.05), and ether extract (EE) (method 920.29) according to the AOAC (1990). The neutral detergent fiber corrected for ash and protein (apNDF) and acid detergent fiber (ADF) were obtained according to Van Soest*et al.* (1991) using alpha-amylase as recommended by AOAC (1990).The equations proposed by Sniffen, Hall (2000), and Weiss (1999) were used to estimate total carbohydrates (TC) and non-fibrous carbohydrates (NFC), and total digestible nutrients (TDN), respectively. In order to estimate the apparent digestibility in vivo, collection bags made from unbleached cotton coated with nappa were utilized. The sheep spent four days of adaptation with the bags and the data collection was performed in the first five days of each collection period. The feces were collected at 07:30 and 15:30 h. The total feces obtained per animal/day was weighed and a fraction of 20% was withdrawn from it; at the end of the fifth day, a composite sample of the five days collected per treatment was made; these were properly packaged, identified and frozen for analysis of DM, CP,NDF,ADF, MM, and EE. Ruminal liquid samples were collected during the first six days of collection, so that on each day were collected two times. The first time was collected immediately prior to feeding and at: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 h post-feeding, totaling 12 hours collected per period and animal, so that the times were equidistant. The ruminal fluid was extracted directly from the rumen through the fistula, from which a fraction of the extrudate was removed to represent the whole ruminal environment, being immediately strained with the support of a cloth strainer for pH determination with the aid of the potentiometer. The ruminal fluid was placed in three universal collectors identified by each treatment and schedule, being one stereo, the second one with 20% metaphosphoric acid (4ml of acid for 20ml of liquid) and another with 20% sulfuric acid (H_2SO_4 was added until the pH is reduced to 2.5) and were frozen until the time of processing. For the analysis ruminal liquid was gradually thawed and centrifuged so that the supernatant was pipetted in 1.5 ml eppendorffs. By means of the acquisition of commercial kits, the N-NH_3 analysis was carried out in the pure supernatant.In thoseuniversal collectors containing metaphosphoric acid were quantified the fatty acids of short chain in the Laboratory of Nutrition of the Federal University of Minas Gerais, in Viçosa-MG.

The experimental design used was the Latin square (4x4), using four animals, four diets and four data collection periods, totaling four replicates per treatment.The statistical model was:

$$Yijk = \mu + Ti + Pj + Ak + \varepsilon ijk, \quad (1)$$

where:

Yijk= observation of animal k, in the period, receiving treatment i;
μ = average effect of the general;
Ti = effect of treatment i, where i = 1, 2, 3 and 4;
Pj = effect of period j, where k = 1, 2, 3 and 4;
Ak = effect of animal k, where l = 1, 2, 3 and 4;
εijk = random error associated with each observation *Yijk*.

The data were interpreted by PROC GLM for ANOVA using Statistical Analysis Systems software version 9.1 (SAS Institute, Cary, North Carolina, USA), with $\alpha = 0.05$ as the critical level of probability. The comparisons between the different levels of the urea (0, 8, 16 and 24 g/kg) were conducted by decomposing the treatment sum of squares into contractions relative to the linear and quadratic, with subsequent adjustment of regression equations. Contrast analysis was performed on CONTRAST-encoded SAS. Data normality (Shapiro-Wilk at 5% probability) was verified by the UNIVARIATE procedure (PROC UNIVARIATE) of SAS. The mean standard error was obtained from the original data.

RESULTS AND DISCUSSION

Intake of dry matter, organic matter, crude protein, neutral detergent fiber, non-fibrous carbohydrates and total digestible nutrients were not influenced by the substitution of soybean meal by urea in diet of sheep fed rations based on spineless cactus (Table 2). This fact can be explained by the similarity between the chemical composition of the diets, since they were isoproteic and isoenergetic, to meet the nutritional requirements of a growing sheep. Thus, can be considered that the replacement of soybean meal by urea up to the level of 24.0 g/kg, did not alter the intake of nutrients by the animals. Similar to that found by Carmo et al. (2005) and Silva (2007), the authors did not verify difference in dry matter intake when they replaced soybean meal with urea in diets for cows and lactating goats, respectively. Additionally, studies evaluating the replacement of soybean meal by urea up to 15.0 g/kg of dry matter in lactating cows showed no effect on dry matter intake (Aquino et al., 2007; Guidi et al., 2007). Protein plays a key role in ruminant nutrition, not only providing amino acids for the animal, but also as a source of nitrogen for microbial protein synthesis. According to the National Research Council (1996), estimates of protein requirements were subdivided into animal and microbial components. It is known that synthesis of microbial protein is important for ruminants, and for this to happenit is necessary that there degradable protein in the rumen (DPR), in the quantity and quality in order to obtain maximum efficiency. It is worth noting that soybean meal presents a lower fraction of DPR when compared to urea, and the balance of nitrogen (N) in the rumen may be negative with the use of soybean meal. In the rumen, the fiber-fermenting bacteria use ammonia as the only source of N and are highly impaired when there is N deficiency in the rumen, leading to less fiber disappearance, reducing the passage rate and, consequently, decreasing dry matter intake (Russell et al., 1992; Tedeschi et al., 2000). In this way, it can be stated that the use of urea as a source of ammonia, supplied the needs of N of the animals, since the nutrient intake was not altered by the replacement of urea by soybean meal. Rumen microorganisms require a minimum amount of N for their maintenance. In addition, there is a need to synchronize the availability of N and energy within the rumen. Although urea has high solubility in the rumen, the necessary energy input was guaranteed by the use of spineless cactus and ground corn, providing an adequate environment for the synthesis of microbial protein.

The substitution of soybean meal by urea did not alter the digestibility coefficients of dry matter, organic matter, crude protein, neutral detergent fiber, total carbohydrates, non-fibrous carbohydrates and total digestible nutrients. It happened because soybean meal can be substituted for urea up to the level of 24.0 g/kg without changing the nutrient digestibility coefficient (Table 3). Similar results were observed by Silva et al. (1994) in sheep, and by Carmo (2001), in dairy cows. These authors used the same nitrogen sources as the present work. Silvaet al. (2002) did not observe differences in the digestibility of DM and OM when using soybean meal (which has medium degradability protein) and urea (source of NPN) in steers. Likewise, digestibility of OM did not differ between treatments (P>0.05). Similar results were found by Silva et al. (1994), when supplementing sheep with starea (urea + extruded corn), urea and soybean meal. NDF digestibility was also not affected by the treatments. In some cases, the use of soybean meal in the diet may result in lower digestibility of NDF due to the lack of ruminal ammonia, which may impair fiber fermenting bacteria, which may lead to a reduction in the passage rate and, consequently, to DM intake.Some authors (Silva, 1994; Carmo, 2001) verified alterations in the digestibility of crude protein in sheep. Treatments that had sources of non-protein nitrogen had higher coefficient of digestibility of crude protein. However, in the present work, this behavior was not found. The fact that soybean meal is also used in human and other animal feed makes this ingredient more expensive to produce ruminants. Taking into account the current price of the ingredients being replenished (soybean meal and urea) the use of urea in the feed of these animals reduces food costs, which correspond to up to 70% of production costs, without altering the performance of animals. The ruminal pH values observed did not differ (P>0.05) for the different levels of soybean meal replacement by urea in the diet of sheep fed diets based on spineless cactus (Table 4).The pH is influenced by the type of feed consumed and its stabilization is due in large part to saliva, which has a high buffering power (Van Soest, 1994).

Table 1. Treatments and experimental diets

Ingredients (g/kg)	Levels of urea (g/kg)			
	0	8.0	16.0	24.0
Groundcorn	53.00	106.7	160.4	213.6
Soybeanmeal	187.0	124.5	62.0	0.000
Tifton grasshay	300.0	300.0	300.0	300.0
Spineless cactus	450.0	450.0	450.0	450.0
Urea	0.000	8.0	16.0	24.0
Sulfur	0.000	0.8	1.6	2.4
Mineral mix	10.0	10.0	10.0	10.0
Nutrients	Chemical composition (g/kg)			
Dry matter (g/kg FM)[a]	304.6	304.4	303.9	305.0
Organic matter	925.3	928.7	932.0	935.3
Ashes	74.7	71.3	68.0	64.7
Crude protein	135.2	133.0	130.6	128.5
Etherextract	16.1	16.9	17.6	18.4
Neutral detergent fibercorrected for ash and protein	406.3	407.0	407.6	408.3

[a]FM: grams per kilogram of fresh matter

Table 2. Nutrient intake in urea-fed sheep in replacement of soybean meal in spineless cactus based diets

Items	Urea Levels (g/kg)				SEM	Ŷ	Contrast	
	0	8.0	16.0	24.0			Linear	Quadratic
Dry matter								
Intake (kg/day)	2.20	2.09	2.22	2.16	0.1448	2.16	0.9945	0.7719
% Bodyweight	4.23	4.19	4.33	4.27	0.2217	4.26	0.7658	0.9598
Organic matter								
intake (kg/day)	2.03	1.94	2.06	2.02	0.1351	2.01	0.8115	0.7396
Ashes								
Intake (kg/day)	0.1625	0.1475	0.1500	0.1350	0.0103	0.15	0.0511	1.0000
Crude protein								
Intake (kg/day)	0.3375	0.3150	0.3250	0.3075	0.0217	0.3212	0.1607	0.8305
Neutral detergent fiber								
Intake (kg/day)	0.9025	0.8575	0.9125	0.8825	0.0589	0.8887	0.9726	0.8180
% Bodyweight	1.73	1.72	1.78	1.75	0.0879	1.75	0.7527	0.8876
Total carbohydrate								
Intake (kg/day)	1.66	1.59	1.70	1.67	0.1124	1.65	0.6426	0.7381
Non-fibrouscarbohydrate								
Intake (kg/day)	0.7600	0.7325	0.7875	0.7875	0.0544	0.7668	0.3716	0.6810
Total digestiblenutrients								
(Kg/day)	1.76	1.65	1.76	1.74	0.1080	1.73	0.8308	0.5305

SEM: standard error of the mean

Table 3. Nutrient digestibility coefficient in urea-fed sheep in replacement of soybean meal in spineless cactus-based diets

Items (g/kg)	Levels of urea (g/kg)				EPM	Ŷ	Contrast	
	0	8.0	16.0	24.0			Linear	Quadratic
Dry matter	836.27	819.10	823.87	832.32	8.99	827.89	0.8820	0.2562
Organic matter	849.95	835.40	837.57	845.37	8.54	842.07	0.7909	0.2756
Crude protein	895.80	875.85	881.12	887.77	6.96	885.13	0.7403	0.3142
Neutral detergent fiber	794.20	784.30	767.52	781.27	12.10	781.82	0.4043	0.4259
Total carbohydrate	842.42	829.22	829.62	837.37	9.03	834.66	0.7386	0.3093
Non-fibrouscarbohydrate	899.72	881.70	902.12	900.17	8.29	895.93	0.4665	0.2468
Total digestiblenutrientes	802.48	792.15	800.02	812.37	8.19	801.76	0.3999	0.2674

SEM: standard error of the mean

The proportion of fibrous and non-fibrous carbohydrates in the experimental diets was maintained in order to provide normal ruminal health conditions, since rumen bacteria are adapted to multiply in medium with pH between 5.5 and 7.0 (Hoover and Stokes, 1991). According to Smith et al. (1972), in the rumen the maximum activity of cellulolytic organisms would occur at a pH ranging from 6.0 and 6.8. The diet offer in the form of complete ration contributed to the maintenance of ruminal pH within the range considered ideal by Hoover and Stokes (1991) and Smith et al. (1972). According to Pessoa et al. (2004), in diets based on spineless cactus, the complete form supply allows a more constant pattern of ruminal fermentation. Santos et al. (2010), feeding a diet with 73% of spineless cactus associated with tifton hay as a complete mixture for sheep, observed a mean ruminal pH of 6.49, values similar to those found in the present study (mean: 6.42).

The concentration of N-NH_3 in the ruminal liquid observed did not differ (P>0.05) for the different levels of soybean meal replacement by urea in the diet of sheep fed on spineless cactusdiets (Table 4). Rumen microorganisms degrade protein sources producing ammoniacal nitrogen (N-NH_3) which is used for incorporation and growth. The growth of ruminal flora and fauna, in turn, plays a fundamental role in the degradation of the fiber, being larger as there is a higher concentration of microorganisms in the rumen. Wanderley et al. (2012) observed ammonia concentrations in the ruminal liquid of sheep receiving diets with 61% of spineless cactusvery close to those observed in the present study (mean of 12.38mg/dL). Bispo et al. (2007) observed ammonia concentration of 12.4 mg/dL in diets with 56% of spineless cactusfor sheep. Similar to the values found in the present study (mean: 12.38).

Table 4. Rumen parameters (pH, N-NH_3 and SCFA) of sheep fed urea to replace soybean meal in spineless cactus-based diets

Items	Urea Levels (g/kg)				EPM	Ŷ	Contrast	
	0	8.0	16.0	24.0			Linear	Quadratic
Ph	6.42	6.37	6.40	6.42	0.0266	6.41	0.8864	0.3559
N-NH3, mgdL	11.27	13.89	12.48	11.79	1.2058	12.38	0.9663	0.0657
Acetate (C2)								
Mmol/ml	16.16	16.89	16.22	16.56	0.2960	16.46	0.7554	0.6230
%	71.08	71.79	71.34	72.20	0.3901	71.60	0.0582	0.8021
Propionate (C3)								
Mmol/ml	4.91	4.88	4.80	4.78	0.1122	4.85	0.3585	0.9646
%	21.58	20.80	21.16	20.80	0.3628	21.09	0.2474	0.5739
Butyrate (C4)								
Mmol/ml	1.67	1.74	1.70	1,61	0.0299	1.68	0.3556	0.1136
%	7.34	7.41	7.49	7.01	0.0832	7.31	0.1316	0.0567
Total								
Mmol/ml	22.74	23.52	22.73	22.96	0.3783	22.99	0.9540	0.5898

According to Leng (1990), concentrations of ammonia in ruminal fluid between 10 and 20mg/100mL are required, aiming to promote adequate nutrient utilization in forage-based diets with reduced nitrogen content. When nitrogen is provided to ruminant animal with inadequate amount of energy available in the rumen, there is an increase in ammonia concentration in ruminal fluid (Van Soest, 1994). In the present study, the adequacy in the association of spineless cactuswith urea is verified, since the mixture presupposes an adequate synchronization between the energy and nitrogen supply for the ruminal microorganisms, considering the high concentration of soluble carbohydrates in the spineless cactus, which facilitates the incorporation of supplemental nitrogen into the microbial protein, the main source of metabolizable protein for the host animal (Pessoa et al. 2009). The concentrations of total SCFA in the ruminal fluid observed did not differ (P>0.05) for the different levels of soybean meal replacement by urea in the diet of sheep fed on spineless cactusdiets (Table 4). SCFA concentrations are influenced, among other factors, by diet composition. According to Hall et al. (1985), in addition to diet, other factors that affect the concentration of SCFA are the absorption and passage of SCFA, the amount of ruminal liquid to which the mass of SCFA is diluted, as the inflow and outflow of water dilutes or concentrates the ruminal solutes. The similarity between diets may explain the fact that the treatments did not influence the total concentration of SCFA.

According to Lópes et al. (1994), SCFA concentrations are a consequence of SCFA production and removal rates (absorption and passage) in the rumen and the latter can be influenced by factors such as pH, rumen volume and osmotic pressure. As can be seen in Table 4, the pH values were not influenced by the substitution of soybean meal for urea. This fact may explain the fact that there was no difference in the concentration of SCFA, since pH is one of the factors which may alter the concentration of SCFA in the rumen. Due to the high percentage of fast digestion carbohydrates present in the spineless cactus, could increase the microbial activity and the concentration of SCFA, resulting in the drop in pH. In this way, it can be affirmed that the supply of energy and nitrogen to the ruminal microorganisms was attended, thus guaranteeing a ruminal condition favorable for the development of ruminal microorganisms. N rumen is required so that there is no limitation in rumen carbohydrate degradation. Therefore, in diets with similar carbohydrates, changes in molar concentration and SCFA ratio are expected only if there is ruminal N deficiency (NocekandTamminga, 1991). Thus, it can be stated that in the present study there was no ruminal N deficiency among the treatments. There was no difference (P>0.05) in the molar concentration of acetate, propionate and butyratethat is, the concentrations of these SCFA did not differ for the different levels of soybean meal replacement by urea in the diet of sheep fed on spineless cactus diets. Although urea is a source of non-protein nitrogen and soybean meal is a source of true protein with high ruminal degradability, providing a greater synthesis of microbial protein, the substitution of soybean meal for urea did not influence the concentration of these proteins SCFA.

Conclusion

Urea can replace soybean meal by up to 24.0 g/kg in the diet of sheep fed diets based on spineless cactus without altering intake, digestibility and ruminal parameters.

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Authors' contributions

Conception and supervision of project by FFRC andANM.Design of experiments and data analysis byEJOS and FFRC.GFCP, AOR, MVM, GLCB and RSNconducted the experiment and data collection. GFC, FFRC and EJOS participated in interpretation of results, writing and integration of the manuscript. All authors approved the final manuscript.

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