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INCREASED NITROGEN FERTILIZATION OF MARANDU GRASS AND PROTEIN SUPPLEMENTATION: HERBAGE INTAKE, FEEDING BEHAVIOR AND DIGESTION OF NELLORE HEIFERS

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ABSTRACT

This study aimed to evaluate the efficiency of increased nitrogen via supplementation or fertilization in Marandu-grass pastures on the productive performance of Nellore heifers. The experiment was conducted in an area dominated by *Brachiaria brizantha* 'Marandu'. Animals were randomly distributed in 16 paddocks of 0.6 ha under continuous stocking in a completely randomized design, with four repetitions and a 2 × 2 factorial design, with two doses of nitrogen and two types of supplements. This study evaluated animal performance, nutrient digestibility and intake, mean daily weight gain, feed conversion, and the levels of urea in blood plasma. An interaction between nitrogen fertilization and protein supplementation was observed for the parameters herbage intake and coefficient of crude protein digestibility and the levels of urea in the blood plasma, in which higher values were obtained for the combination of pastures fertilized and supplemented. Daily mean gain ($P \leq 0,05$) was superior for animals that received supplementation. The results show positive effects of nitrogen fertilization in pasture, maximizing the production efficiency of Nellore heifers raised on pasture. Satisfactory effects were obtained from the protein supplement used on the animals under pasture.

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INTRODUCTION

The main peculiarity of tropical grasses is the broad capacity of light energy interception that occurs through their high photosynthetic rate, impacting the storage of this energy in the plant tissue as latent energy, mainly in the fibrous carbohydrates existing in the cell wall. This energetic substrate is a very low-cost component in pasture cattle rearing systems, although it presents important nutritional limitations. As the main hurdle, the restricted availability of nitrogen (N) (Detmann *et al*. 2009) leads to low usage of the cellular wall, which is potentially degradable by the ruminal microorganisms, thereby compromising pasture intake and animal performance. Studies conducted in tropical conditions have shown that the supplementation that uses nitrogen compounds as a priority tool to increase the use of low-quality forage by grazing animals allows the necessary increase in protein levels associated with intensive pasture handling (Viana *et al*. 2011). The main effects of nitrogen applied to pastures are an increase in forage productivity, shoot characteristics and foliage expansion, as well as protein content. However, not much attention has been given to the interaction of the nitrogen provided as pasture fertilizer and protein supplementation. Thus, the increase in research looking at information on blood parameters is essential in evaluating the diet (Alberghina *et al*. 2011), as it allows the visualization of nitrogen mobility within the metabolism of animals. The aim of this study was to evaluate the effects of increasing nitrogen through protein supplementation and/or nitrogen fertilization of Marandu grass pastures on the production performance of the animals.

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MATERIALS AND METHODS

Statement of Animal Rights: All procedures were performed according to principles of animal experimentation approved by protocol 97/2015 by the Ethics Committee on Animal Experimentation of the State University of Southwest Bahia, Brazil.

Location, Animals, Diet and Fertilization

The experiment was conducted on Boa Vista farm, Macarani/BA, in south-central Bahia, located at 15° 33′ 46″ south latitude and 40° 25′ 38″ west longitude, altitude 402 m, from 8, Nov. 2013 to 22 June 2014; and in the Forage and Pasture Laboratory at the State University of Southwest Bahia (Laboratório de Forragicultura e Pastagem da Universidade Estadual do Sudoeste da Bahia), at the Itapetinga Campus, Itapetinga, BA. Blood analyses were performed at the Center of Chromatographic Analysis of the State University of Southwest Bahia.The region's weather is classfied, according to the new Köppen classification, as type As, which is defined as tropical with a dry season (Álvares *et al*. 2013). The soil of the experimental area is eutrophic equivalent red-yellow podzolic (EMBRAPA 1999). Soil samples were collected before nitrogen fertilization and chemical analysis at a depth of 0–20 cm is presented in Table 1.

Table 1. Chemical analysis of the soil of the experimental area

Treatment	pH	mg/dm³	cmol/dm³ of soil						%	mg/dm³
	(H_2O)	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+	SB[5]	V[6]	OM[7]
N100+PS[1]	5,75	1	0,49	1,45	1,07	0,20	2,65	3,0	51	3,93
N100+MS[2]	5,62	1	0,45	1,40	1,05	0,26	2,66	2,9	52	4,03
N0+PS[3]	5,68	1	0,46	1,46	1,10	0,25	2,56	3,0	56	3,97
N0+MS[4]	5,72	1	0,43	1,53	1,11	0,23	2,48	3,12	57	3,83

[1]N100+PS = Pasture fertilized with 100 kg/ha of N and protein supplement.[2]N100+MS = Pasture fertilized with 100 kg/ha of N and mineral supplement.[3]N0+PS = Pasture non-fertilized and protein supplement.[4]N0+MS = Pasture non-fertilized and mineral supplement [5]SB = Sum of bases.[6]V = Saturation per base.[7]OM = Organic Matter.

Table 2.Chemical analysis of protein supplement and of simulated grazing (%DM) of *brachiariabrizantha* cv. Marandu fertilized with nitrogen

Componente	Protein Supl.	PSA[9]	PA[10]
DM[1]	86,93	30,70	29,01
CP[2]	31,13	7,39	9,29
EE[3]	1,34	2,16	2,29
NDFcp[4]	22,85	69,91	68,13
ADF[5]	6,75	-	-
MM[6]	9,0	7,31	6,97
NFC[7]	37,92	13,22	13,32
TC[8]	60,77	83,13	81,45

[1]DM = Dry matter. [2]CP = Crude protein. [3]EE = Ether extract. [4]NDFcp= Neutral detergent fiber corrected for ash and protein. [5]ADF = Acid detergent fiber. [6]MM = Mineral matter. [7]NFC = non-fibrous carbohydrate. [8]TC = Total carbohydrate. [9]PSA = Pasture non-fertilized.[10]PA = Pasture fertilized with 100 kg/ha of N.

data collection, which consisted of seven periods of 28 days, plus a 30-day period of adaptation. The supplements were balanced in order to meet maintenance requirements and gains of 0.6 kg/day according to NRC (1996) and were used as a source of protein, supplementary to urea and soybean meal. Supplementation was offered daily at 1000 h in plastic communal troughs with no lids, and the individual supplement consumption was estimated from the fecal production in one animal from each treatment, verified with the help of chromium oxide (Cr2O3) as an external indicator and indigestible neutral detergent fiber (NDFI) as an internal indicator. Chromic oxide was supplied daily at 0900 h in a single dose of 10.0 g, for 11 days, with seven days for adaptation and regulation of the excretory flow of the marker, and five days for collection of feces. Mineral supplementation consisted of 233 g of Ca/kg, 80 g of P/kg, 5 g of Mg/kg, 48 g of Na/kg, 25 mg of Co/kg, 380 mg of Cu/kg, 25 mg of I/kg, 1080 mg of Mn/kg, 3.75 mg of Se/kg, and 1722 mg of Zn/kg. Based on the chemical analysis of the soil, the dosage of 60 kg.ha^{-1} of P_2O_5 was used for the entire experimental area. The use of K_2O was not necessary. For nitrogen fertilization, urea was used (45 % of N) two months before the animals entered the experiment site.

The total area of the experiment was composed of 10 hectares of *Brachiaria brizantha* 'Marandu', established around 10 years ago. The treatments were distributed following a completely randomized design with four repetitions and factorial design of 2 × 2: the site consisted of a control pasture with no nitrogen fertilization (N0) and a pasture with nitrogen fertilization of 100 kg.ha^{-1} of N (N100), and two types of protein supplements: protein (PS) and mineral (MS), which were randomly distributed across 16 paddocks of 0.625 ha each, separated by an electric fence, each containing a communal drinking fountain and trough. Protein supplementation (Table 2) provided to the animals corresponded to 0.3 % body weight, and mineral supplementation was offered ad libitum. Treatments one, two, three, and four contained 400.0, 291.76, 324.93, and 233.17 g rumen-degraded protein (RDP) and 60.56, 51.45, 57.89, and 53.81 g non rumen-degraded protein (RDNP), respectively. The experimental period lasted for 226 days of grazing and

Evaluation and Procedures

In order to evaluate the performance, 60 Nellore heifers at a mean age of nine months and initial body weight of 195 ± 26.67 kg were used. Heifers were identified with an ear tag and dewormed before the beginning of the experiment using 1 % Ivermectin®. The animals were randomly distributed into the experimental units, and the grazing method used was one of continuous stocking, with variable stocking rate, according to the "put-and-take" technique (Mott & Lucas 1952). Each treatment received two test animals and a variable number of regulators, according to the mass of forage. Two intermediate weighings were conducted for the stocking rate adjustments, and at the end of the experiment the average stocking rate was 2.1 AU/ha. Table 2 shows the chemical composition of the forage consumed by the animals obtained through simulated grazing, which was performed according to Johnson (1978).

The samples were collected after a careful observation period, in which the grazing behavior of the animals, as well as the area, height, and parts of the plant that were being consumed were observed. After observation, samples were taken by the same observer, manually, in an attempt to obtain a portion of the plant similar to that selected by the animals. Heifers were weighed at the beginning and end of the experimental period, after fasting for 12 hours. Two intermediary weighings were performed in order to adjust stocking rate, the amount of supplement provided, and animal performance. To determine the content of crude protein (CP), the method described by Silva and Queiroz (2002) was used. For dry matter (DM) content and the apparent digestibility coefficients from NDFi, the sequential procedure as described by the methodology of Casali *et al*. (2008) was used. Nutrient intake was determined as forage DM, supplement DM, CP, NDF, TC, NFC and TDN in kg/day and DM and NDF as a percentage of body weight. Apparent digestibility (AD) of the nutrients was determined as described by Silva and Leão (1979). For the digestibility assay, chromic oxide was used as an external indicator, and provided daily at 0900 at a unique dose of 10 g for 11 days, in which seven days were an adaptation period and regulation of excretion flow of the marker, and five days were for fecal collection. An amount of 300 g of feces were collected daily in a rectal ampoule and stored in a freezer at -10 ºC. The samples were thawed, homogenized and pre-dried in a forced-air circulation oven at 60 °C for 96 hours, and then processed in a Wiley mill with a 1 mm mesh. The samples were then analyzed by atomic absorption spectroscopy (AAS) for chrome dose determination, as described by Williams *et al*. (1962). The collection of urine was performed on spot during the spontaneous urination of four animals per treatment every 27 days, four hours after the supplement was provided, as described by Barbosa (2007). Samples of 10 ml were diluted in 40 ml of 0.036 N sulfuric acid (H_2SO_4). The pH of the samples was adjusted to less than three, in order to prevent bacterial destruction of the purine derivatives and precipitation of uric acid, and then stored in -20 °C conditions for further analysis of creatinine and purine derivatives, urea and total N. The balance of the N compounds was calculated as the difference between the total ingested N and the total N excreted in the feces and urine. Blood samples were collected in four animals from each treatment every 27 days, four hours after the supplement was provided, through puncture of the external jugular vein and manual vein tourniquet, in vacuum containers. The blood samples were then centrifuged at 1500 rpm for 15 min in order to separate the plasma, which was frozen in an Eppendorf tube until further analysis. Plasma and urine urea concentrations and creatinine and uric acid concentrations in urine were determined using commercial kits (Bioclin®), according to the manufacturer's guidelines.

Statistical analysis: The results were subjected to analysis of variance, considering the nitrogen fertilization, protein supplement and the interaction among these factors as sources of variation. A 2 × 2 factorial analysis was performed according to the statistic model below. Data were statistically interpreted using Tukey's Test at 5 % probability. Statistical analyses were performed using Sistema de AnálisesEstatísticas e Genéticasversão 9.1 – SAEG (Ribeiro Junior 2001).

$$Y_{ijk} = \mu + N + S + N{*}S_{ij} + \varepsilon_{ijk}$$

Y_{ijk}= observed value in relation to the nitrogen fertilization *i*, to the supplementation *j* and the repetition *k*;
μ = general mean;
N_i = nitrogen fertilization, with *i* = 1 and 2;
S_j = supplements used, with *j* = 1 and 2;
$N{*}S_{ij}$= effect between nitrogen fertilization *i* and supplementation *j*;
ε_{ijk} = random error, associated to each observation.

Table 3. Mean of total dry matter intake (TDMI % BW), crude protein intake (CPI), and coeficiente of crude protein digestibility (CPD) in Nellore heifers receiving protein supplements in comparison to nitrogen fertilization of Marandu-grass pasture

Total dry matter intake (TDMI % BW)							
N0[2]	N100[3]	Mineral Supl.	Protein Supl.	CV[1] (%)	P-N[4]	P-S[5]	P-NxS[6]
2,37	2,55	2,24b	2,67a	13,74	0,15	0,001	0,77
	Protein Content (% of DM)						
	N0[2]		N100[3]	CV[1] (%)			P-NxS[6]
Mineral Supl.	7,21bB		9,43aA	15,65			0,04
Protein Supl.	9,34aA		10,99aA				
	Crude Protein Intake (CPI kg.day^{-1})						
	N0[2]		N100[3]	CV[1] (%)			P-NxS[6]
Mineral Supl.	0,40bB		0,62aA	12,67			0,03
Protein Supl.	0,64aA		0,65aA				
Coefficient of crude protein digestibility (CPD %)							
	N0[2]		N100[3]		CV[1] (%)		P-NxS[6]
Mineral Supl.	69,32aB		69,05aB		1,19		<0,01
Protein Supl.	74,38aA		75,68aA				

Different lower case letters in lines and capital letters in columns differ significantly by the Tukey test (P≤0,05)
[1]CV: Coefficient of variation.[2]N0 = non-fertilized pasture.[3]N100 = Pasture fertilized with 100 kg N/ha.[4]P-N = Probability of error referring to the fertilization.[5]P-S = Probability of error referring to the supplementation.[6]P-NxS = Probability of error referring to the interaction between fertilization and supplementation.

Table 4. Mean balance of nitrogen compounds, nitrogen digested, and nitrogen retained in Nellore heifers supplemented in pastures of *Brachiariabrizantha* cv. Marandu fertilized with nitrogen over 84 days of evaluation

Balance of nitrogen compounds (g/day)								
N Balance	N0[2]	N100[3]	Mineral Supl.	Protein Supl.	CV[1](%)	P-N[4]	P-S[5]	P-NxS[6]
N ingested	93,94	103,36	82,82b	104,4a	13,53	0,19	0,01	0,14
N feces	60,95	67,51	66,45	62,01	15,52	0,16	0,14	0,62
N urine	3,22	3,82	3,24	3,80	56,01	0,32	0,46	0,79
N digested	67,14	74,93	57,30b	79,34a	12,91	0,21	0,01	0,07
N retained	29,77	32,03	13,13b	38,59a	13,80	0,21	0,01	0,08

Different lower case letters in lines and capital letters in columns differ significantly by the Tukey test (P ≤0,05)
[1]CV: Coefficient of variation. [2]N0 = non-fertilized pasture. [3]N100 = Pasture fertilized with 100 kg N/ha. [4]P-N = Probability of error referring to the fertilization. [5]P-S = Probability of error referring to the supplementation. [6]P-NxS = Probability of error referring to the interaction between fertilization and supplementation.

RESULTS

No effect (P ≥ 0.05) on the interaction among treatments was observed for the total intake of dry matter (% BW), although an isolated effect (P ≤ 0.05) was observed in the animals that received protein supplementation. Significant interaction (P < 0.05) was observed for the crude protein of forage, and the results showed that pastures fertilized with 100 kg.ha^{-1} of N and animals that received protein supplementation presented higher values. There was an effect (P ≤ 0.05) on the interaction between urea concentration in the plasma, which was influenced by nitrogen fertilization and supplementation (Table 5). Lower values were obtained for the combination of non-fertilized pastures and mineral supplementation.

Table 5. Mean urea concentration (mg.dL^{-1}) in the plasma of Nellore heifers supplemented on pastures of *Brachiariabrizantha* cv. Marandu fertilized with nitrogen

	Urea Concentration (mg/dL)			
	N0²	N100³	CV¹ (%)	P-NxS⁴
Mineral Supl.	12,96bB	25,52aA	30,43	0,04
Protein Supl.	20,77bA	27,78aA		

Different lower case letters in lines and capital letters in columns differ significantly by the Tukey test (P≤0,05) ¹CV: Coefficient of variation. ²N0 = non-fertilized pasture. ³N100 = Pasture fertilized with 100 kg N/ha. ⁴P-NxS = Probability of error referring to the interaction between fertilization and supplementation.

Table 6. Mean Performance of Nelore heifers supplemented in pastures of *Brachiariabrizantha* cv. Marandu fertilized with nitrogen

Performance	N0⁵	N100⁶	Mineral Supl.	Protein Supl.	CV⁴%	P-N⁷	P-S⁸	P-NxS⁹
MDG¹ (kg)	0,52	0,54	0,47b	0,61a	23,77	0,79	0,03	0,37
Total FC²	12,67	11,29	10,66b	13,29a	25,10	0,17	0,01	0,55
PGA³ (kg.ha^{-1})	148,32	201,45a	138,95b	210,81a	20,22	0,01	0,02	0,23

Means followed by different letters, lower case in rows refer to the nitrogen and supplement dose differing by the F test (p<0,05). ¹MDG = Mean daily gain. ²Total FC = Total feed conversion. ³PGA = Weight gain by area. ⁴CV: Coefficient of variation. ⁵N0 = non-fertilized pasture. ⁶N100 = Pasture fertilized with 100 kg N/ha. ⁷P-N = Probability of error referring to the fertilization. ⁸P-S = Probability of error referring to the supplementation. ⁹P-NxS = Probability of error referring to the interaction between fertilization and supplementation.

No effect (P ≥ 0.05) on animal performance was observed, between the variables of mean daily weight gain, feed conversion, and weight gain by area. Protein supplementation promoted additional gains of 29.8 % in daily weight gain and 51.71 % in weight gain per area, and a reduction of 19.8 % in feed conversion.

DISCUSSION

The adjustment of the protein to energy ratio optimized forage digestibility in the studied conditions. It is probable that the increment of nitrogen compounds derived from the higher concentration of CP in the fertilized pastures and from the protein supplement acted as a catalyst in maximizing total dry matter intake of forage and CP intake. As the contribution of nitrogen increased, there was an increased availability of this compound in the soil, allowing the plant to store higher amounts of this nutrient in the tissue, thereby leading to an increase of CP. The increase in availability of protein fractions in the forage is important in correcting the deficiencies of this nutrient in animal feeding. It also optimizes the performance and favors high ruminal degradability due to the accumulation of nitrogen in relation to the availability of energy (Reis *et al.* 2009). The results of this study present superior protein content for pastures fertilized with nitrogen compared to unfertilized pastures, and corroborate with those observed by Abbasi *et al.* (2012) who reported the positive effect of nitrogen fertilization on the harvest date and nutritive value of *Amaranthushypochondriacus*. Considering results obtained in similar climatic conditions to Brazil, Detmann and Huhtanen (2013) estimated that the voluntary intake of forage was stimulated at CP concentrations close to 145 g.kg^{-1} DM. The concentrations of CP in the conditions of this study ranged from 73.6 to 108.2 g.kg^{-1} of DM, for treatments with mineral and protein supplements, respectively. Although the values of this study were lower than those suggested by the authors, the increase in CP through protein supplementation was sufficient to stimulate voluntary forage intake. The combination with nitrogen fertilization might have been a determining factor in obtaining these results. The increase in concentration of plasma urea may be justified by the increase in total dry matter intake, as this variable is related to the amount of protein ingested. In this way, the fact that there was no loss of excreted nitrogen through feces and urine, and an increase in the values of nitrogen digested and retained, reveal the effects of the increase from both fertilization and supplementation methods. The increases direct the compound to the body tissues, and therefore convert them into muscle gain. This fact corroborates the results found in this study and those reported by Azevedo *et al.* (2014), who observed a significant increase of intake in hay and concentrate throughout the experimental period, followed by increased concentrations of urea. The levels of urea in the blood are directly correlated to the amount of protein or non-protein nitrogen in the diet, as well as liver and kidney functions (Haga *et al.* 2008). In this experiment, pathological causes for the increase in urea were dismissed, as no signs of kidney disease were observed in the experimental animals. The positive balance of nitrogen provided through diet supplementation indicates an adequate balance between protein and energy, with a consequent lack of mobilization of body reserves, thereby leading to better use of the nutrients (Goes *et al.* 2015). Similar results to those discussed above were observed in this study. As the nitrogen was offered in the diet through fertilization of the pasture, there was no need to mobilize protein from the body reserves. In this way, the animals presented satisfactory mean gains for their age and species, evidencing the importance of the positive balance of nitrogen. Therefore, no influence was observed in the use of protein supplementation when the pasture was fertilized on the concentration of urea in the plasma, which can be justified by the availability of nitrogen from the fertilization process. However, in non-fertilized pasture, the animals receiving protein supplementation presented higher urea levels, showing the influence of nitrogen sources in the diet or fertilization, and the response of the nutritional physiology of the animals. Fertilization of the forage did not directly influence the proportion of organic matter digestible in the rumen, probably due to the increase in water soluble carbohydrate that are promptly fermented to aid ruminal digestibility. Thereby, the amount of fatty acids produced is not significantly affected by the level of nitrogen fertilization (Peyraud and Astigarraga1998). These facts corroborate the results of this study, which revealed no loss of nitrogen in the feces and urine, probably due to the use of this compound in the digestion process. Additionally, animals that received protein supplementation presented higher averages for ingestion, digestion and retention of nitrogen.

A significant mean daily gain of 0.610 kg.day^{-1} was achieved by animals that received protein supplementation, showing the efficiency of using supplements, as well as meeting the expected result when formulated according to NRC (1996). The results show the relationship between the intake of protein supplementation and total dry matter. Animals receiving protein supplements presented values of total dry matter intake superior to those that received mineral supplements, which impacted on the increased mean daily gain. Fertilization showed no observed influence on this variable, although animals treated with the fertilized pasture presented higher protein content. A study evaluating the effect of the frequency of the supplementation on the intake, behavior and performance of cows kept on Marandu-grass pastures observed a similar mean daily gain of 0.69 kg/day. The authors observed that up to a level of reduction in the supplementation there was no effect on animal performance, showing the need to estimate and adjust these values (Morais *et al*. 2014). Protein supplementation promoted a decrease in feed conversion by 19.8 %. This parameter was different in the animals who received protein compared to mineral supplementation; animals that received the protein supplementation presented better feed conversion in comparison to those who received mineral supplementation. Better conversion of ingested forage into animal product in fertilized pastures was probably the result of greater daily forage intake and protein provided through supplementation, which indicates the adequacy of the balance between protein and energy. Such balance directly aids pasture use, increases total dry matter intake (TDMI), and therefore results in better feed conversion.

In a study evaluating the performance and feed conversion of beef heifers on Tanzania grass, the cows were subjected to two stocking intensities of rotational grazing. The study observed that animals kept in pastures handled with residue at 50 cm presented better feed conversion in comparison to those maintained on pastures with residue at 25 cm. These results are probably related to the greater total matter intake and greater availability of well-handled forage, which are contrary to the results obtained in this study; this study did not reveal significant differences between feed conversion of animals grazing in fertilized pastures with greater forage availability and the feed conversion off animals in non-fertilized pastures (Difante *et al*. 2010). The use of concentrate supplementation is an alternative source that can be used in order to circumvent the limitations that occur due to the exclusive use of pastures (Couto *et al*. 2010). According to Reis *et al*. (2009), multiple supplementations, such as the strategy used in pasture handling, enables mean gains of 500 to 600 g per day, which is essential in allowing animals to be slaughtered at 20 months old. When observing data on weight gain by area, it was observed that protein-energy supplementation in the wet season (0.3% of PV) allowed for greater weight gain compared to animals receiving only mineral supplementation. These results can be justified by the chemical composition of the forage, which presented 8% of CP and 69% of FDNcp, a high leaf-blade supply (2.2 kg / 100 kg CP), allowing selective grazing by the animals and nutrients.

Implications

According to results obtained in the experiment, the use of nitrogen in animal supplementation and fertilization of forage is indicated. The data showed that animals receiving protein supplementation and fed with fertilized pasture presented better intake of dry matter and supplement as well as better digestibility and individual performance. This may be because the nitrogen supplied is a source of protein for ruminal microorganisms in the digestive system, which need protein for better utilization of the forage.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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