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Nutritional and productive performance of grazing system beef heifers receiving multiple supplementation in the dry season

Desempenho nutricional e produtivo de novilhas de corte em sistema de pastejo recebendo suplementação múltipla na estação seca

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Highlights

Provisión of multiple supplement for beef heifers in grazing system was evaluated.
Supplementation increased the total nutrient digestibility.
Our study not evidence differences in productive performance.
Supplementation improved nitrogen statuses in the animals.

Abstract

This research was conducted to evaluate the effects of supplementation multiple on intake, digestibility, and microbial protein synthesis and, productive performance of grazing system receiving multiple supplementation in the dry season. Twelve Nellore heifers (averaging 214.6 ± 7.3 kg initial BW and 8.0 ± 0.10 months) were distributed to a completely randomized design in two treatments: unsupplemented (only mineral) and; supplemented (1 kg per animal day^{-1} of multiple supplement). Only the crude protein (CP) intake was increased by supplementation ($P < 0.05$). In addition, the supplementation increased the digestibility of organic matter (OM), CP, ether extract (EE), non-fibrous carbohydrates (NFC) and dietary content of digested OM (DOM) ($P < 0.05$). The supplementation decreased microbial nitrogen to ingested nitrogen ratio (MICNR) ($P < 0.05$) and increased the serum urea nitrogen (SUN) concentration and urinary nitrogen excretion (UNE) ($P < 0.05$) in the animals. Finally, average daily gain (ADG) and final BW (FBW) were not affected by supplementation ($P > 0.05$). In summary, the provision 1 kg per animal day^{-1} of multiple supplement for beef heifers in grazing system does not improve productive performance in dry season when there is adequate qualitative and quantitative availability of forage mass. However, provision of supplement increases the total nutrients digestibility and nitrogen statuses in the animals.

Key words: Digestibility. Microbial protein. Nellore. Ruminant nutrition. Supplement. Pasture.

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Resumo

Esta pesquisa foi conduzida para avaliar os efeitos da suplementação múltipla sobre o consumo, digestibilidade e síntese de proteína microbiana e, desempenho produtivo de novilhas de corte em sistema de pastejo na época seca. Doze novilhas Nelore (média de 214,6 ± 7,3 kg de peso corporal inicial e 8,0 ± 0,10 meses) foram distribuídas em delineamento inteiramente casualizado em dois tratamentos: não suplementado (apenas mistura mineral) e; suplementado (1 kg por animal dia^{-1} de suplemento múltiplo). Apenas o consumo de proteína bruta (PB) aumentou com a suplementação ($P < 0,05$). Além disso, a suplementação aumentou a digestibilidade da matéria orgânica (MO), PB, extrato etéreo (EE), carboidratos não fibrosos (CNF) e teor dietético de MOD ($P < 0,05$). A suplementação diminuiu a relação nitrogênio microbial nitrogênio ingerido (MICNR) ($P < 0,05$) e aumentou a concentração de nitrôgeno ureico no soro (NUS) e excreção urinário de ureia (ENU) ($P < 0,05$) nos animais. Finalmente, o ganho médio diário (GMD) e o peso corporal final (PCF) não foram afetados pela suplementação ($P > 0,05$). Em resumo, o fornecimento de 1 kg por animal dia^{-1} de um suplemento múltiplo para novilhas de corte em sistema de pastejo não melhora o desempenho produtivo na época seca, quando existe uma adecuada disponibilidade qualitativa e quantitativa da massa de forragem. No entanto, o fornecimento do suplemento múltiplo aumenta a digestibilidade total dos nutrientes e status de nitrogênio dos animais.

Palavras-chave: Digestibilidade. Nellore. Nutrição de ruminantes. Pastagem. Proteína microbiana. Suplemento.

Introduction

Tropical grasses are the main source of nutrients for cattle production in the tropics, standing out from other means of feeding due to the low cost of production and high practicality (Detmann et al., 2014a). However, tropical pastures in grazing should be understood as a highly complex basal nutritional resource, since its capacity to supply substrates for animal production varies qualitatively and quantitatively throughout the year, mainly due to the influence of climatic variables (Detmann et al., 2014a). For this reason, tropical pastures rarely constitute a balanced diet in the sense that their organic and inorganic constituents are present in the concentrations and proportions that satisfy the animals' needs. Thus, cattle generally suffer from multiple deficiencies in protein, energy, minerals, and vitamins (Paulino et al., 2012).

In the dry season, tropical forages are considered low-quality as a result of reduced crude protein (CP) contents and higher lignification of insoluble fiber. The CP contents are usually below the minimum values for the rumen microorganisms to be present in adequate capacity for fiber degradation (<7 to 8% of CP in the dry matter [DM]), which constrains the bacterial growth and, consequently, limits the use of fibrous carbohydrates from basal forage by ruminal microorganisms (Detmann et al., 2014b). Thus, the effects of multiple or nitrogen supplementation in the dry season has the main role of improving the microbial activity in the rumen (Detmann et al., 2014b). Additionally, supplementation increases the performance in the beef heifers (Martins et al., 2016; Silva et al., 2017; Almeida et al., 2018). However, when considering the combination of supplemental nitrogen and energy

compounds that can maximize the positive interaction between forage and supplement components, it is important to remember that this combination can lead to increase the retention of nitrogenous compounds in the animal body, reflected by greater weight gain in the dry season (Lazzarini et al., 2016; Franco et al., 2021).

In this context, cattle supplementation programs are tools for providing supplementary resources to reduce or eliminate nutritional and/or metabolic constraints and the achievement of animal production goals (Detmann et al., 2014b). In this sense, supplementation for grazing cattle promotes changes in forage intake and digestibility, improving the availability of energy and nitrogen compounds in the diet and, consequently, animal performance (Paulino et al., 2012).

We hypothesized that multiple supplementation of beef heifers would improve their nutritional and productive performance. This research was conducted to evaluate the effects of multiple supplementation on voluntary intake, digestibility, microbial protein synthesis and productive performance in grazing system beef heifers in the dry season.

Materials and Methods

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV No. 10/2016), according to the ethical principles of animal experimentation established by the National Council of Animal Experimentation Control.

Location and weather conditions

This experiment was carried out at the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil (20°45'S 42°52'W; 657 m de altitude), between July and September during dry season. The experimental period lasted for 84 d into three 28-day periods. Average temperature and precipitation of 17.7 ºC and 17.6 mm were observed during the experimental period.

Experimental design and treatments

For this study, twelve Nellore heifers averaging 214.6 ± 7.3 kg initial BW and 8.0 ± 0.10 months of age were assigned to a completely randomized design in two treatments and six replicates.

The treatments evaluated were: unsupplemented (only mineral mix) and supplemented (1 kg per animal day^{-1} of multiple supplement). The supplement was composed of corn meal, sorghum meal, soybean meal, and mineral mix and formulated to contain 300 g CP kg^{-1} (as fed) (Table 1). The supplement amounts accounted to approximately 45 and 22% of the dietary requirements of CP and TDN, respectively, for Zebu heifers under grazing with BW of 250 kg and expected gain of 0.5 kg d^{-1} (S. C. F. Valadares et al., 2016).

Table 1
Ingredients and chemical composition of supplement and forage consumed by the animals during the experimental period

Item	Supplement	*U. Decumbes*[2,4]	*U. Decumbens*[3,4]
Ingredient % (as fed)			
Soybean meal	53.80	-	-
Corn meal	20.80	-	-
Sorghum meal	20.40	-	-
Mineral mix1	5.00	-	-
Chemical composition (% DM)			
Dry matter	84.18	91.82 ± 1.17	91.12 ± 0.29
Organic matter	91.44	92.31 ± 0.40	92.61 ± 0.43
Crude protein	30.83	9.39 ± 1.97	9.69 ± 0.49
Ether extract	1.31	1.73 ± 0.31	2.05 ± 0.13
NDFap	18.54	57.73 ± 2.04	55.75 ± 0.66
Non-fibrous carbohydratesd (NFC)	40.75	31.14 ± 1.60	25.11 ± 0.46
Indigestible NDF (iNDF)	0.48	13.70 ± 1.83	12.64 ± 0.46

[1]Centesimal composition: 50.0% dicalcium phosphate, 47.2% sodium chloride, 1.5% zinc sulfate, 0.7% copper sulfate, 0.05% cobalt sulfate, 0.05% potassium iodate, and 0.5% manganese sulfate.
DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; apNDF neutral detergent insoluble fiber corrected for ash and protein.
[2]Samples obtained from a grazing simulation during the digestion trial
[3]Samples obtained from a grazing simulation during the experimental period
[4]Means ± standard error of the mean.

Animal handling

The animals were subjected to 14 days of adaptation to the diet and experimental area, in this period, all animals received 0.5 kg of multiple supplement. Animals were housed to one of two paddocks of 2.5 ha (one for each treatment), uniformly covered with Urochloa decumbens, and equipped with water dispensers and feeders. In the experimental period, the supplement was offered daily at 10h00 (1 kg per animal day^{-1} of multiple supplement). Animals were weighed every 28 days without fasting and always in the morning (6h00) in the order monitor the performance and welfare of animals. In order to minimize potential effects of the paddocks on experimental treatments, animals were rotated across the four pastures every 7 days with each group staying on each plot the same length of time. All animals were subjected to the control of ecto- and endoparasites at the beginning of the experiment and over its course

Experimental procedures and sampling

Forage samples

Forage samples were assessed by hand-plucked samples collected every 14 days to evaluate the pasture chemical composition (Table 1). A second pasture sample was

collected every 28 days to estimate the total availability of DM and potentially digestible dry matter (pdDM). Four subsamples were randomly collected in each plot by cutting it close to the ground using a metal square of 0.5 m × 0.5 m. Samples were oven-dried at 60 °C for 72 h and ground in a Wiley mill (model 3; Arthur H. Thomas, Philadelphia, PA) to pass through a 2-mm screen. After that, half of each ground sample was ground again to pass through a 1-mm screen.

Voluntary intake and digestibility

A 9-day digestibility trial was carried out, starting at the 42th day of the experimental period to evaluate of nutrient intake and digestibility. The first six days were used for the adaptation of animals to the markers (stabilization of markers excretion). Chromium oxide (Cr_2O_3) was used as an external marker to estimate fecal excretion in the amount of 10 g per animal. Titanium dioxide (TiO_2) was used to estimate individual intake of supplement mixed in the supplement at the proportion of 10 g kg^{-1} of supplement. The indigestible neutral fiber (iNDF) was used as an internal marker to estimate forage DM intake. Fecal samples were collected immediately after defecation or taken directly from the rectum of animals at amounts of approximately 200 g, on the last 4 days of the trial at different times according to the following schedule: day 6-18 h00, day 7-14 h00, day 8-10 h00, and day 9-06 h00. Fecal samples were identified, oven-dried at 60 °C for 72 h, and ground as previously described.

Additionally, in the last day of trial, spot urine samples (10 mL) were collected from spontaneous micturition 4 hours after supply of supplement to evaluate microbial protein synthesis of the heifers. Urine samples were diluted in 40 mL of H_2SO_4 (0.036 N) and frozen (-20°C). After urine collection, blood samples were collected by jugular vein puncture, using vacuum tubes with separator gel (BD Vacutainer ® SST 137 II Advance), and centrifuged at 3000 × g for 15 minutes. The plasma was then frozen (-20°C) to evaluate for serum urea nitrogen (SUN).

Productive performance

For performance evaluation, the animals were weighed at the beginning and end of the experiment after 14 h of solids fasting.

Analytical procedures

Samples of forage, feces, and supplement ground through 1-mm sieves were analyzed for DM (dried overnight at 105 °C; method INCT-CA number G-003/1), ash (complete combustion in a muffle furnace at 600 °C for 4 h; method INCT-CA number M-001/1), N (Kjeldahl procedure; method INCT-CA number N-001/1), ether extract (Randall procedure; method INCT-CA number G-005/1), and neutral detergent fiber corrected for ash and protein (NDFap; using a heat-stable α-amylase, omitting sodium sulfite and correcting for residual ash and protein; method INCT-CA number F-002/1) as recommended by Detmann et al. (2012). The content of iNDF in samples of feces, forage, and supplement (ground through 2-mm sieves) was estimated as the residual NDF remaining after 288 h of ruminal in situ incubation using F57 filter bags (Ankom Technology Corp., Mac edon, NY) as recommended by Valente et al. (2011).

The non-fibrous carbohydrates (NFC) were quantified, according to Detmann and Valadares (2010).

$$NFC = 100 - (CP + EE + NDFap + MM)$$

where MM = mineral matter; EE = ether extract; NDFap = neutral detergent fibre corrected for ash and protein residue; CP = crude protein

Fecal samples were also analyzed for chromium concentration by atomic absorption spectrophotometry (method INCT-CA M-005/1) and titanium dioxide by colorimetry (method INCTCA M-007/1), as recommended by Valente et al. (2011).

The pdDM in forage available on pasture was estimated using the following equation described by Paulino et al. (2008):

$$pdDM = 0.98 \times (100 - NDF) + (NDF - iNDF)$$

The fecal DM excretion was estimated using the chromic oxide marker, based on the ratio between the amount of chromium supplied and its concentration in the feces. Individual supplement intake was estimated (SI) by relation of excretion of TiO_2 in feces and marker concentration in the supplement. Dry matter intake (DMI) was estimated by using iNDF as na internal.

Blood and urinary urea were quantified by the enzymatic kinetic method (Ref. Número K056-1, Bioclin® Quibasa, Belo Horizonte, Brazil). Serum urea N (SUN) was estimated as 46.67% of total serum urea (Velloso, 1984). This metabolite was analyzed in accordance with an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China).

Daily urinary volume was calculated using the relationship between the daily creatinine excretion (CE), taking as reference the equation proposed by Costa e Silva et al. (2012), and its concentration in the spot samples:

$$CE\ (g\ d^{-1}) = 0.0345 \times BW^{0.9491}$$

where: BW = body weight

Excretion of the purine derivatives in urine was calculated by the sum of the allantoin and uric acid excretions, which were obtained by the product between their concentrations in urine by the daily urinary volume. Absorbed purines were calculated from the excretion of purine derivatives as recommended by Chen and Gomes (1992).

$$AP = X - 0.301 \times BW^{0.75} / 0.80$$

where AP = absorbed purines (mmol/d), X = excretion of purine derivatives (mmol d^{-1}), 0.8 = recovered absorbed purines. The 0.301 × $BW^{0.75}$ value = endogenous excretion of purine derivates.

Ruminal of nitrogen compounds production was calculated as a function of the absorbed purines using the equation described by Barbosa et al. (2011).

$$MICN = 70 \times AP / 0.93 \times 0.137 \times 1.000$$

where MICN= microbial nitrogen production (g d^{-1}), AP = absorbed purines (mmol d^{-1}), 70 = purine N content (mg mol^{-1}), 0.93 = purine digestibility and 0.137 = relation of purine N:total N of microorganisms.

Efficiency of protein microbial synthesis (EMS) was estimated by dividing protein microbial production by the DOM intake.

Statistical analyses

The experiment was analyzed according to completely randomized design. All statistical procedures were conducted using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The intake, digestibility, production of microbial protein, average daily gain (ADG) and final BW (FBW) and SUN were submitted to analysis of variance, adopting the initial body weight as covariate. Statistical significance was considered at P≤ 0.50.

Thus, the experiment was analyzed according to the model:

$$Yij = \mu + Ti + e(i)j$$

where: Yij = average observation between individuals taken in the experimental unit j subjected to treatment I; μ = general constant; Ti = fixed effect of treatment i; e(i)j = random error, non-observable associated with each experimental unit, assumption NID (0, σ2).

Results and Discussion

Mean forage availability on pasture of total DM and pdDM was 5.34 and 3.40 t/ha, respectively, representing an availability of 64 g pdDM kg^{-1} of BW for animals. Forage samples obtained by the hand-plucked method had an average CP content of 9.69 % in the DM (Table 1). According to Paulino et al. (2008), the availability of 40 to 50 g of pdDM kg^{-1} BW from forage promotes satisfactory intake and performance in cattle in a grazing system. In this study, the average availability of pdDM was 64 g kg^{-1} BW during experimental period, this indicates the forage mass available was not a limiting factor of feed intake and animal performance.

In general, the voluntary intake in kg day^{-1} of DM, forage DM, organic matter (OM), EE, NDFap, iNDF, digested OM, digested NDF and dietary tenor of TDN were not affected (P>0.05) by supplementation. However, the supplementation increased (P<0.05) of CP intake in the animals (Table 2). Evaluating the intake in g kg^{-1} BW, the supplementation did not affect (P>0.05) DM, OM, DMF and NDFap intake in the animals (Table 2). The supplementation increased the CP intake by heifers (Table 2). This greater intake is attributed to the supply of protein provided by the supplement, as it contains a higher concentration of CP than forage.

The supply of nitrogenous compounds from supplementation to animals fed low-quality forage provide substrates to rumen and, thus, improves the growth of fibrolytic bacteria; as a consequence, there would be an increase in the degradation of fibrous carbohydrates and improvement of voluntary forage intake (Doyle et al., 2005; Detmann et al., 2014b). Although positive effects for the supply of nitrogenous compounds were not observed on forage intake and fiber digestibility (Table 2 and Table 3, respectively).

Table 2
Effect of multiple supplementation on voluntary intake in grazing system beef heifers in the dry season

Item	Treatments		SEM	*P* - Value
	Unsupplemented	Supplemented		
	kg day^{-1}			
DM	4.64	4.73	0.320	0.851
DMF	4.64	3.89	0.320	0.126
DMS	-	0.84	-	-
OM	4.30	4.35	0.296	0.909
CP	0.46	0.70	0.033	<0,001
EE	0.10	0.10	0.007	0.724
NDFap	2.58	2.26	0.177	0.236
NFC	1.16	1.28	0.080	0.283
iNDF	0.57	0.48	0.039	0.132
DOM	2.60	2.90	0.208	0.332
DNDF	1.57	1.48	0.123	0.640
TDN	2.59	2.91	0.210	0.304
	g kg^{-1} BW			
DM	19.7	18.7	1.40	0.614
DMF	19.7	15.3	1.39	0.051
OM	18.2	17.2	1.29	0.569
NDFap	10.9	8.9	0.77	0.095

DM: dry matter; DMF: dry matter from forage; DMS; dry matter from supplement; OM: organic matter; CP: crude protein; EE: ether extract; apNDF: neutral detergent insoluble; NFC: non-fibrous carbohydratesd; iNDF: indigestible NDF; DOM digested organic matter, DNDF:digested neutral detergent insoluble fiber; TDN: total digestible nutrients; SEM: standard error of the mean.

The total digestibility coefficients of OM, CP, EE, NFC, and dietary content of DOM were improved (P<0.05) by supplementation. However, the total digestibility of DM and NFDap were not affected (P>0.05) when the supplement was supplied (Table 3). The greater digestibility of OM, CP, OM, EE, NFC in supplemented heifers is attributed to the intake of easily digestible nutritional compounds from multiple supplements. This pattern resulted in an increase in the DOM (Table 3).

Table 3
Effect of multiple supplementation on apparent digestibility coefficients in grazing system beef heifers in the dry season

Item	Treatments		SEM	*P* - Value
	Unsupplemented	Supplemented		
	g g^{-1}			
DM	0.575	0.603	0.0112	0.105
OM	0.606	0.663	0.0128	0.010
CP	0.619	0.669	0.0149	0.040
EE	-0.087	0.083	0.0440	0.021
NDFap	0.609	0.651	0.0154	0.085
NFC	0.651	0.724	0.0107	<0,001
	g kg^{-1} DM			
DOM	560.4	611.7	14.79	0.030

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; apNDF: neutral detergent insoluble; NFC: non-fibrous carbohydratesd; DOM digested organic matter; SEM: standard error of the mean.

The MICN, EMS was not affected (P>0.05) by supplementation. In contrast, the supplementation decreased (P<0.05) microbial nitrogen to ingested nitrogen ratio (MICNR) (Table 4). Increases in N intake by supplementation led to an increase (P<0.05) of SUN concentration and urinary N excretion (UNE) in the animals. The higher SUN concentration for heifers that received of supplement is due to higher CP intake (Table 2). In support of this rationale, Van Soest (1994) indicates that SUN concentration is positively associated with CP intake. In beef heifers, the optimal SUN concentrations range from 13.52 to 15.15 mg/dL (Valadares et al., 1997), which correspond to the maximum microbial efficiency that would probably represent the maximum point from which there would be loss of protein in zebu cattle fed diets with approximately 62.5% total digestible nutrients. In this sense, the higher concentration observed for the supplemented animals (17.0 mg dL^{-1}; Table 4), indicating a possible protein in excess in the diet or deficiency of degradable organic in the rumen, promoted greater UNE in the supplemented animals.

Table 4
Effect of multiple supplementation on nitrogen synthesis and excretion in grazing system beef heifers in the dry season

Item	Treatments		SEM	*P* - Value
	Unsupplemented	Supplemented		
MICN (g day^{-1})	56.0	58.0	6.54	0.835
MICNR (g g^{-1} N)	0.777	0.525	0.0785	0.046
EMS (g CP kg^{-1} DOM)	137.78	130.2	18.46	0.778
SUN (mg dL^{-1})	11.0	17.0	1.21	0.005
UNE (g day^{-1})	17.3	41.8	2.24	<0.001

MICN: microbial nitrogen production; MICNR: microbial nitrogen to ingested nitrogen ratio; EMS: efficiency of microbial protein synthesis; UNE: urea nitrogen excretion in the urine; SUN: serum urea nitrogen; SEM: standard error of the mean.

The greater UNE for supplemented heifers can be indicative of a fraction of N that is not being used efficiently. In support of this rationale, Bento et al. (2015) suggest that when protein degradation rate exceeds the rate of carbohydrate degradation, the absorption of ammonia and oxidation of amino acids in the rumen increase. Therefore, an imbalance between protein and carbohydrate sources may decrease nitrogen use efficiency and limit animal performance (Detmann et al., 2014b). The results obtained in this study are similar to the reported by Batista et al. (2016), Ortega et al. (2020) and Franco et al. (2021) who supplemented grazing beef heifer with protein and/or energy supplement in tropical conditions.

According to Clark et al. (1992), ruminal availability of energy and nitrogen are the nutritional factors that most affect microbial growth. Thus, the lack of effect on MICN and EMS between treatments (Table 4), indicates that that there was no deficiency of nitrogen compounds and energy to optimize growth of ruminal microorganisms. However, the higher estimate of MICNR for non-supplemented heifers (Table 4) indicates a much greater dependence on nitrogen recycling to maintain the growth of ruminal microorganisms. In support this rationale, Detmann et al. (2014a), suggests that the supplementation with nitrogen compounds reduces the recycling of nitrogen towards the rumen mainly when the dietary CP concentration is less than 8% DM, which is the value required to sustain microbial growth from dietary nitrogen and minimize the dependency on nitrogen recycling. Similar results were obtained by Cabral et al. (2014), Ortega et al. (2016) and Sotelo et al. (2018), when supplemented grazing beef heifer with protein and/or energy supplement in tropical conditions

Supplementation did not affect (P>0.05) ADG and FBW in the animals (Table 5). The absence of difference in the productive performance of the animals from the different treatments may be due to the percentage of CP from forage intake by animals was close to 10% (Table 1) being reported by Sampaio et al. (2009) as the level that optimizes the use of energetic substrates of the forage, and thus justifies the supplementation with nitrogen compounds to optimize the use of the forage and, consequently, the animal performance.

Table 5
Effect of multiple supplementation on productive performance in grazing system beef heifers in the dry season

Item	Treatments		SEM	*P* - Value
	Unsupplemented	Supplemented		
FBW (kg)	241.9	245.3	2.76	0.430
ADG (kg day^{-1})	0.340	0.351	0.0324	0.804

FBW: final body weight; ADG: average daily gain; SEM: standard error of the mean.

Conclusions

Provision of 1 kg per animal day^{-1} of multiple supplement for grazing system beef heifers does not improve productive performance in dry season when there is adequate qualitative and quantitative availability of forage mass. However, provision of supplement increases the total nutrient digestibility and the nitrogen status in the animals.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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