Ciência Animal Brasileira
Brazilian Animal Science
e-ISSN 1809-6891

Ciência Animal Brasileira

DOI: 10.1590/1809-6891v23e-72333E

Section: Animal science
Research article

Effect of stocking management and nitrogen supplementation on pasture milk yield

Efeito do manejo do pastejo e suplementação nitrogenada sobre a produção de leite a pasto

Rita Kelly Couto Brandão Dias[1*], Fábio Andrade Teixeira[2], Daniela Deitos Fries[2], Fabiano Ferreira da Silva[2], Daniel Lucas Santos Dias[1], Jeankarlo Penalva dos Santos[2], Fernando Oliveira Barreto[2], Sinara Sales de Oliveira Rodrigues[2], Murilo de Almeida Meneses[2]

[1]Universidade Estadual de Feira de Santana (UEFS), Feira de Santana, Bahia, Brazil.
[2]Universidade Estadual do Sudoeste da Bahia (UESB), Itapetinga, Bahia, Brazil.
[*]Correspondent: kelly_brandao@hotmail.com

Abstract
This study aimed to evaluate the effect of the first and last stocking strategies combined with a partial substitution of the protein from the supplement for urea nitrogen on nutrient intake and digestibility, milk composition, and nitrogen balance of primiparous Girolando cows. The cows were allocated to a double 4 × 4 Latin square composed of four animals and four treatments in a 2 × 2 factorial arrangement. Supplements were formulated to provide an intake of 0.6% body weight, with and without the inclusion of 21% urea nitrogen in their composition. The first stocking management method improved nutrient intake and digestibility. Supplementation with urea led to a 47% higher excretion of urine N (g/day) than the urea treatment. The combination of the supplement without urea and the first stocking provided higher intake and retention of nitrogen and higher retained-N levels (%digested N). The combination of a supplement containing 21% urea nitrogen and the first stocking can be used without compromising the nutritional and productive parameters of the cows.
Keywords: xaraes grass; tip; repast

Resumo
Objetivou-se avaliar os efeitos dos manejos do pastejo ponta e repasse combinados a substituição parcial da proteína do suplemento por nitrogênio ureico sobre o consumo e digestibilidade dos nutrientes, composição do leite e balanço de nitrogênio de vacas primíparas da raça Girolanda. As vacas foram alocadas em dois quadrados latinos 4 x 4 compostos de quatro animais cada e quatro tratamentos em um esquema fatorial 2 x 2. Os suplementos foram formulados para um consumo de 0,6% do peso corporal: sem utilização de nitrogênio ureico; ou com 21% de nitrogênio ureico em sua composição. O manejo do pastejo de ponta melhorou o consumo e a digestibilidade dos nutrientes. O suplemento contendo ureia excretou 47% a mais de N urina (g/dia) em comparação ao suplemento sem ureia. A combinação entre o suplemento sem ureia e o manejo de pastejo ponta apresentou maior ingestão e retenção de nitrogênio e maior N retido (%N digerido). A combinação entre o suplemento contendo 21% de nitrogênio ureico e o pastejo de ponta pode ser utilizada sem prejuízos aos parâmetros nutricionais e produtivos das vacas.
Palavras-chave: capim xaraés; ponta; repasse

Received: March 23, 2022. Accepted: July 5, 2022. Published: August 15, 2022.

Introduction

In Brazil, forage is a basal feed resource for dairy herds. However, climatic conditions during the rainy period accelerate the growth of grasses, which reach their optimal quantity and quality when the dossel reaches the recommended height for grazing. When properly conducted and optimal light interception as the management target is maintained, the intermittent grazing system provides rest to the pasture, respects its regrowth without degrading it, and allows high-quality forage to be made available to animals(1).

Considering the behavior of grazing animals as a selection criterion, as they enter the paddocks, they tend to choose the most palatable and nutritious plant parts, performing 'more selective' grazing that result in decreased availability of these components and consequent consumption of older leaves and stems. In intensive pasture-based milk production systems, two management targets stand out: maximum intake and consequent performance of cows at their lactation peak and grazing up to an adequate height for their exit to promote regrowth. Distinct management strategies are required to attain these goals. In this regard, the first and last stocking methods are used to meet these demands, thus increasing the efficiency and precision of intermittent grazing.

Despite the large forage production of tropical grasses, the grazing performance of animals is reduced due to the limited intake caused by their high level of fiber and usually low protein. Therefore, it is necessary to supplement forage to adapt to the crude protein requirements of cattle. One alternative is to partially replace the total dietary protein in soybean meal with urea as a source of urea nitrogen.

This study was undertaken to investigate the effects of the first and last stocking methods combined with a partial substitution of the protein from the supplement for urea nitrogen in the diet of lactating cows on their intake, nutrient digestibility, milk production, composition, and nitrogen balance.

Material and Methods

The experiment was conducted at Bela Vista Farm, located in the southwest region of Bahia, Brazil, between January 10 and April 4, 2015. All experimental procedures were reviewed and approved by the Committee of Ethics in Animal Use (CEUA/UESB) under Document 131/2016.

Animals, diets, and experimental design

Eight primiparous Girolando cows with an average age of three years, and an average initial BW of 381.3 ± 44.1 kg, in the middle third of the lactation period, were allocated to a double 4 × 4 Latin square composed of four animals and four treatments, in a 2 × 2 factorial arrangement, consisting of two types of supplements (without addition of urea nitrogen [W/o urea] and replacement of 21% of the total protein with non-urea nitrogen [W/urea]), and two stocking managements (first-and-last stocking). In the first stocking, the first group of animals spent two days in the paddock, which was fenced for approximately 21 d for forage regrowth. The last stocking consisted of the access of a second group of animals in the same paddock after leaving the first group and remaining for two days to consume the remaining forage.

The experimental period consisted of four 21-d periods that included 16 d of adaptation and five days of collection. Cows were kept in fifteen 3,000-m^2 paddocks of *Brachiaria brizantha* cv. Xaraés and were given free access to rest areas and water in an intermittent-grazing system. Cows were offered 3 kg of supplement/cow/d after morning milking in individual covered stalls.

Evaluation of the pasture

The pasture was evaluated every 21 d before the first group was allowed to graze, and after the last group exited, at the start of each period. The dry mass (Table 1) availability was determined by collecting three representative samples of forage collected at 5 cm from the soil in the entire area of a square of measuring 0.49 m^2. All samples were weighed, homogenized, and divided into two representative subsamples: one was used to estimate the availability of the dry mass of each paddock, and another to obtain the leaf/stem ratio, which was separated into leaf blade, stem, and dead material. Forage allowance (FA) was calculated using the following formula: , where FA = forage allowance, in dry matter (DM)/100 kg BW/day; DMA = dry matter availability, in kg DM/ha; BW = total body weight of the animals, in kg/ha; and OP = occupation period. The potentially digestible dry matter (pdDM) and availability of potentially digestible dry matter (pdDMA) of the forage were calculated according to Paulino et al.(2).

Digestibility trials and urine and blood collections

The digestibility trial began on the ninth day of each experimental period and lasted 12 d. Chromic oxide was administered orally at a dose of 10 g/animal/d for 11 consecutive days. Feces and grass (simulated grazing by hand plucking) samples were collected over the last five days. Samples of feces were collected individually, on the floor of the corral, carefully so as not to contaminate the material which was then stored at –20 ºC. Simulated grazing was performed as recommended by Johnson(3).

Table 1. Structural traits, total dry matter availability (DMA), availability of potentially digestible dry matter (pdDMA), and forage allowance (FA) of Xaraés grass

Structural trait	Stocking management	
	First	**Last**
Height (cm)	39.00	24.40
Leaf/stem ratio	0.84	0.52
Forage production		
DMA (kg DM/ha)	3210.10	2358.10
pdDMA (kg DM/ha)	2224.40	2095.50
FA (% BW)	13.00	9.00

Spot urine samples were collected on the 19th day of each experimental period, approximately 4 h after morning feeding, during spontaneous urination. A 10-mL aliquot of urine was diluted in 40 mL of sulfuric acid (0.04 N). The samples were stored at –20 °C for subsequent analysis of creatinine, urea, allantoin, and uric acid. On the same occasion, blood samples were collected from each animal by puncturing the mammary vein, using heparin as the anticoagulant. The samples were immediately centrifuged at 1.500 rpm for 15 min. The resulting plasma was stored at –20 ºC for later determination of urea content.

Milk production and composition

Milk production was evaluated from the 17th to the 21st day of each experimental period and weighed immediately after manual milking on a 30 kg digital scale. Two 300 mL milk samples were collected on day 18: one to determine milk composition (protein, fat, lactose, and total solids) using a Lactoscan® digital device and another for further laboratory analysis. The corrected milk yield to 3.5% fat was estimated according to the model proposed by the NRC[4].

Laboratory analyses

Samples of forage (hand-plucked), concentrate (Table 2), and pre-dried feces were ground using a Willey mill to 1-mm particles for chemical analyses. Dry matter (DM; method INCT-CA G-003/1), organic matter (OM; method INCT-CA M-001/1), crude protein (CP; method INCT-CA N-001/1; ether extract (EE; method INCT-CA G-005/1); insoluble neutral detergent fiber corrected for ash and protein (NDFap; methods INCT-CA F-002/1, INCT-CA M-002/1), and INCT-CA N-004/1), and insoluble acid detergent fiber (ADF; method INCT-CA F-004/1) contents were determined according to the techniques described by Detmann et al.[5]. Total carbohydrate content was calculated using the equation proposed by Sniffen et al.[6].

Table 2. Ingredient and chemical composition of experimental diets and simulated-grazing

Ingredient (%)	Supplement (g/kg DM)[a]			
	W/o urea	**W/urea**		
Forage	700	700		
Corn	180	228		
Soybean meal	111	56		
Urea	---	7.3		
Mineral mixture (lactation)	6.0	5.7		
Limestone	3.0	3.0		
Chemical composition			First stocking	Last stocking
Dry matter (g/kg)	947	949	257	245
Crude protein (CP)	258	272	104	94
Acid detergent fiber (ADF)	361	375	361	375
NDIN[b]	270	346	388	482

[a] W/o urea, supplement without urea; W/urea, supplement with urea. [b]NDIN, insoluble neutral detergent nitrogen as percent of total N

For the urea-containing diet, the non-fibrous carbohydrate (NFC) content was estimated according to Hall[7], while the equation recommended by Detmann et al.[8] was used for the diet without the substance and total digestible nutrients (TDN), using the NDF corrected for ash and protein[9]. Total digestible nutrients (TDN) in the supplement and forage samples were estimated using the equation proposed by Cappelle et al.[10]. To estimate the voluntary intake of roughage, the indigestible neutral detergent fiber (iNDF) internal marker was used, obtained after 288 h of ruminal incubation using the INCT-CA F-009/1 method [5]. Apparent digestibility of nutrients was determined as described by Silva and Leão[11].

The excretion of total purines (TP) was estimated as the sum of the amounts of allantoin and uric acid excreted in the urine and allantoin secreted in the milk. The amount of microbial purines absorbed (mmol/day) was estimated using the equation proposed by Verbic et al.[12]. The intestinal flow of microbial nitrogen (g MN/day) was estimated from the amount of purines absorbed (mmol/day) according to Chen and Gomes[13]. Microbial CP (MCP) synthesis was estimated by multiplying MN by 6.25, while the efficiency of microbial protein synthesis was determined by the following equation: $EMCP\ g/kg = MCPg/TDNIkg$, where TDNI is the total digestible nutrient intake. The balance of nitrogen compounds was obtained as the difference between the total N ingested and the total N excreted in the feces, urine, and milk. Total N in feces and urine was determined according to the methods described by Silva and Queiroz[14].

Statistical analyses

The effects of the type of supplement and grazing management were compared using the F test at a probability level of 0.05, using the PROC GLM statistical procedures of the Statistical Analyses System (SAS, 2001). The interaction was decomposed (or not) according to significance. The following statistical model was used:

$$Yijkl = m + P\,i + Aj + Bk + Fl + (BxF)kl + \varepsilon ijkl$$

where Yijk is the dependent variable, m = average of all experimental units, Pi = effect of period, Aj = effect of animals, βk = effect of supplement, Fl = effect of grazing management, $(B\times F)_{kl}$ = effect of supplement × grazing management interaction, and εijk = residual error.

Results

Nutrient intake and digestibility coefficients

Supplementation did not influence intake-related variables. The first stocking increased nutrient intake (Table 3). No interaction effect was observed between supplementation and stocking management on the apparent digestibility coefficients of the total diets. The first stocking strategy improved the digestibility coefficients for DM, OM, NDFap, and TDN. There was an interaction effect between supplement and stocking management on the intake of CP and TDN (Table 4).

Table 3. Intake and digestibility of nutrients by lactating cows supplemented on pasture

Variable[a]	Supplement[b]		Stocking		CV%	Significance[c]		
	W/o ureia	W/ureia	First	Last		S	G	S x G
Intake (kg/d)								
DM	9.8	9.4	10.3	8.9	11.0	0.3521	0.0014	0.0509
OM	8.9	8.6	9.4	8.1	8.5	0.3523	0.0014	0.0592
NDFap	5.3	5.0	5.6	4.7	14.2	0.3502	0.0016	0.0502
NFC	1.9	1.9	2.0	1.8	7.8	0.3792	0.0153	0.1442
Coefficient of digestibility (g/kg)								
DM	694	690	700	684	5.2	0.3541	0.0348	0.1511
OM	719	707	722	704	7.6	0.3313	0.0455	0.1917
CP	694	682	689	688	6.5	0.8223	0.7120	0.2264
NDFap	700	683	707	676	7.4	0.3494	0.0326	0.1847
NFC	751	774	756	769	5.1	0.1059	0.3541	0.3623
TDN	672	674	681	665	5.8	0.5961	0.0353	0.1813

[a]DM, dry matter; OM, organic matter; CP, crude protein; NDFap, ash-free NDF and protein; NFC, non-fibrous carbohydrates and TDN, total digestible nutrients. [b]W/o urea, supplement without urea; W/urea, supplement with urea. [c] S, supplement; G, stocking management; S × G, interaction between supplement and stocking management at 5% probability by the F test.

Table 4. Intakes of crude protein (CP) and total digestible nutrients (TDN) as a function of supplementation and stocking method by lactating cows supplemented on pasture

	CP (kg/dia)			CV%[b]
	First stocking	Last stocking	Mean	7.6
W/o ureia[a]	1.57aA	1.31bA	1.44	
W/ureia[a]	1.53aA	1.39aA	1.46	
Mean	1.55	1.35	1.45	
	TDN (kg/dia)		Mean	12.9
W/o ureia	7.54aA	5.69bA	6.61	
W/ureia	6.56aB	6.13aA	6.34	
Mean	7.05	5.91	6.48	

[a]W/o urea, supplement without urea; W/urea, supplement with urea. [b]CV%: coefficient of variation. Means followed by the same uppercase letters in the column and lowercase letters in the row do not differ according to the F test at 5% probability.

Milk production and composition

The production of first-stocking milk was 16.2% higher than that of the last stocking. There was no interaction effect between supplement type and stocking management, or an isolated effect of each factor on milk composition.

Table 5. Milk yield and composition by lactating cows supplemented on pasture

Variable	Supplement[b]		Stocking		CV%[c]	Significance[d]		
	W/o ureia	W/ ureia	First	Last		S	G	S x G
Production (kg/day)[a]	7.9	8.0	8.6	7.4	8.7	0.2544	0.0008	0.1422
Composition (g/kg)								
Crude protein	0.032	0.032	0.032	0.032	2.4	0.5335	0.5335	0.9287
Fat	0.049	0.052	0.052	0.050	8.9	0.0808	0.1361	0.3632
Lactose	0.048	0.048	0.048	0.048	2.1	0.7217	0.9054	0.4890
Total solids	0.136	0.139	0.139	0.136	3.8	0.1960	0.1716	0.5137

[a] 3.5% fat corrected milk. [b]W/o urea, supplement without urea; W/urea, supplement with urea.[c]CV% - coefficient of variation. [d] S, supplement; G, stocking management; S × G, interaction between supplement and stocking management at 5% probability by the F test.

Nitrogen Balance

Data pertaining to nitrogen balance are presented in Table 6. The first stocking increased the concentration of N in milk (g/day) and provided a greater retention of nitrogen as a function of the percentage of ingested nitrogen (retained N/% ingested N). The last stocking increased the proportion of digested N (g/day) and digested N as a function of the percentage of ingested N (digested N/% ingested N). W/o urea increased the retained N/% ingested N ratio, while supplementation with urea resulted in 47% more urine (g/day) than the W/urea treatment. No effect of supplementation or grazing management was observed on the amount of N excreted in feces (N feces, g/day).

Table 6. Balance of nitrogen compounds and concentrations of nitrogen in the milk and urine of lactating cows supplemented on pasture

Balance of nitrogen compounds	Supplement[a]		Stocking		CV%[b]	Significance[c]		
	W/o ureia	W/ ureia	First	Last		S	G	S x G
Milk N (g/dia)	39.4	40.7	42.6	37.5	9.0	0.3173	0.0009	0.1548
Retained N (g/kg N intake)	47.5	42.9	46.4	44.0	12.6	0.0429	0.2415	0.1401
Urinary N (g/dia)	21.5	31.6	25.3	27.9	42.7	0.0219	0.5241	0.7805
Faecal N (g/dia)	58.0	58.8	61.7	55.1	17.3	0.5400	0.1738	0.6194
Digested N (g/dia)	176.9	183.3	177.9	182.3	7.2	0.0572	0.1962	0.7597
Digested N (g/kg Nintake)	79.0	80.5	74.1	85.4	10.2	0.5065	0.0009	0.2390

[a]W/o urea, supplement without urea; W/urea, supplement with urea. [b]CV% - coefficient of variation; [c]S, supplement; G, stocking management; S × G, interaction between supplement and stocking management at 5% probability by F test.

An interaction effect between supplementation and grazing management was observed for ingested N, retained N, and retained N as a function of the percentage of digested N (retained N/% digested N) (Table 7). The combination of the supplement without urea and the first stocking led to higher ingestion and retention of N and higher levels of retained N (% digested N).

Table 7. Decomposition of the interaction for the balance of nitrogen compounds as a function of supplementation and stocking method by lactating cows supplemented on pasture

Variable[a]	Intake N[b] (g/dia)			CV (%)[c]	Significance
	First stocking	Last stocking	Mean	6.6	0.0357
W/o ureia	249.7aA	209.3bB	229.5		
W/ureia	237.3aA	223.1aA	230.2		
Mean	243.5	216.2	229.9		
	Retained N (g/dia)		Mean	15.6	0.0390
W/o ureia	127.4aA	93.7bA	110.5		
W/ureia	100.3aB	97.9aA	99.1		
Mean	113.9	95.8	104.8		
	Retained N (g/kg N digested)		Mean	15.6	0.0454
W/o ureia	73.0aA	53.1bA	63.0		
W/ureia	56.6aB	52.4aA	54.5		
Mean	64.8	52.7	58.8		

[a]W/o urea, supplement without urea; W/urea, supplement with urea. [b]N – nitrogen [c]CV% Coefficient of variation. Means followed by the same uppercase letters in the column and lowercase letters in the row do not differ according to the F test at 5% probability.

There was no interaction effect (Table 8) between supplementation and stocking management on the concentration of urea nitrogen in plasma (PUN) and milk (MUN). The urea-containing supplement increased the PUN.

Table 8. Concentrations of urea N in lactating primiparous cows supplemented on pasture

Variable[a]	supplement[b]		Stocking		CV%[c]	Significance[d]		
	W/o ureia	W/ureia	First	Last		S	G	SxG
Concentrations of urea N (mg/dL)								
NUP	12.0	13.7	12.8	12.9	14.0	0.0193	0.8373	0.5064
MUN	11.8	11.5	11.4	11.9	22.2	0.2282	0.2942	0.7942

[a]NUP, nitrogen in the plasma; MUN, nitrogen in the milk; [b]W/o urea, supplement without urea; W/urea, supplement with urea.; [c]CV%, coefficient of variation ; [d]S, supplement; G, stocking management; S × G, interaction between supplement and stocking management at 5% probability by the F test.

Microbial protein synthesis

No interaction effect or isolated effect of stocking management was detected on the synthesis of nitrogen and microbial proteins or on microbial efficiency (Table 9). Supplemente without urea increased the synthesis of nitrogen and microbial proteins and improved microbial efficiency.

Table 9. Syntheses of microbial nitrogen and protein and microbial efficiency of lactating cows supplemented on pasture

Variable	Supplement[a]		Stocking		CV%[b]	Significance[c]		
	W/o ureia	W/ureia	First	Last		S	G	SxG
Syntheses of microbial N and CP (g/day)								
Microbial N	63.8	59.2	62.9	60.2	33.3	0.0021	0.6417	0.8967
Microbial CP	377.1	360.0	371.8	365.3	33.3	0.0021	0.6415	0.8968
Microbial efficiency								
g CP/kg TDN	61.3	57.6	58.5	60.5	27.1	0.0003	0.4015	0.4837

[a]W/o urea, supplement without urea; W/urea, supplement with urea. [b]CV% - coefficient of variation. [c]S, supplement; G, stocking management; S × G, interaction between supplement and stocking management at 5% probability by F test.

Discussion

Nutrient intake and digestibility coefficients

Replacing part of the protein content originating from soybean meal with urea is a viable strategy, since the supplement W/urea did not influence nutrient intake. The total DM intake of the animals in the first stocking exceeded the intake of those eating the last stocking of the forage by 16%, because the former group had the opportunity to enter the paddock that had been closed for

21 d and had more available forage (13% BW) and higher leaf/stem ratios (Table 1) than the forage allowance of 9% BW obtained by the last stocking group. Consequently, the former group could select the more nutritious and less fibrous part of the forage, the leaves, thereby influencing their intake.

The lower intake of nutrients by the animals in the last stocking was due to the physical limitation caused by the greater NDF content of the forage consumed by the cows in this group. As the leaf/stem ratio was lower when they grazed, the animals might have consumed more stems, which, in general, have inferior fiber quality compared to green leaves, ultimately compromising the digestibility of the consumed nutrients. Supplement W/urea probably did not affect the population of rumen bacteria to maximize the degradation of fiber from the forage. Leaves have a higher CP content than stems. Coupled with the consumption of the supplement W/o urea, this elevated CP intake by the animals in the first stocking of the pasture, since the CP from soybean meal has a better biological value than urea, improves the rumen environment, and promotes the development of cellulolytic bacteria. The higher TDN intake for the combination of no urea + first stocking resulted from the higher intake of all nutrients observed in the first stocking group.

Milk production and composition

Primiparous cows require approximately 20% more nutrients than their maintenance needs to meet the requirements for body development[15]. Milk production was satisfactory because it matched the productive capacity of these animals and presented forage intake. Diet had little impact on milk composition. Fat is the component most strongly affected by nutrition; however, there is a negative correlation (–0.25) between milk fat content and milk yield[16].

Nitrogen Balance

Cows in the first stocking had 13.6% more N excreted in their milk as a consequence of the higher CP and lower NDIN content (Table 2) in the grass available to them, which resulted in more protein in the rumen to improve forage utilization. The higher concentration of soybean meal in the supplement W/o urea stimulated microbial growth and increased the supply of metabolizable protein to the intestine, improving the utilization of protein by the animals, which in turn contributed to an increased amount of retained N as a function of ingested N.

The lower CP and higher NDIN content in the forage of the last stocking group contributed to a lower N intake, leading to less recycling, coupled with a higher N intake via supplement, which improved forage digestion efficiency (digested N/% ingested N). These facts, associated with the lower rate of passage of ingested material, improved the digestibility of this fibrous material. As stated by Vasconcelos et al.[17], a positive N balance indicates fixation of protein in the animal body, providing conditions for the animal to gain weight, and suggesting that the protein levels in the diet were satisfactory to meet the animals' requirements.

Because the excretion of N in the urine was higher in the cows that consumed the supplement containing urea, it is assumed that there was a lack of synchronism in the release of N from this supplement since urea is rapidly degraded in the rumen. Therefore, the energy source could be partially replaced by a rapidly degradable source such as molasses to promote the growth of rumen microorganisms, or the feed supplied could be split into more provisions, preventing excess rumen ammonia in a single period, thus minimizing N loss via urine and reducing feed costs without affecting milk production. The combination of the consumption of supplement without urea and first stocking led to higher ingestion and retention of N as a result of the higher intake of TDN and CP (Table 3) by the cows in that group, associated with the greater efficiency of utilization of soybean meal and the highly degradable starch source. This maximized fermentation in the rumen generates an adequate environment for the growth of microorganisms, especially those that degrade cellulose.

In the pastures grazed by the second (last stocking) group, the CP and NDIN contents (Table 2) contributed to lower N retention, irrespective of the supplement consumed, revealing that 4.9% of the crude protein present in the forage consumed from the last stocking of the pasture was available to the animals. The higher amount of retained N (% digested N) for the association between W/o urea and first stocking demonstrates better utilization of the ingested N, since N ingestion by the cows was a result of the sum of the CP from the forage and from the supplement. Therefore, there was better synchronism between energy and protein in the W/o urea supplement group.

The higher PUN content found in cows supplemented with urea was a result of its rapid degradation. According to Vasconcelos et al.[17], elevated PUN values are associated with diets containing high levels of rumen-degradable proteins and a deficiency of rumen-fermentable organic matter. There was possibly a slight lack of synchronism between the protein and energy from the supplement W/urea, suggesting that this is more prone to cause N losses from the organism, although the PUN content was below the limit proposed in the literature. Excess urea not used by rumen microorganisms is excreted in the urine or diffused through the tissues, elevating urea N levels in the plasma and milk[18]. Urine urea N concentration is positively correlated with PUN content, indicating efficiency in the utilization of ingested N[1].

The mean values for MUN obtained in this study for both factors (supplementation and grazing management) were within the interval of 10 to 17 mg/dL, as determined in the literature [19, 20]. According to Guliński et al.[21], in determining the MUN value, information pertaining to the diet and factors such as milk yield, cow age, lactation stage, breed, and other variables should be considered, since they can influence the results.

Microbial protein synthesis

Microbial protein synthesis does not depend only on the N and carbohydrate sources of the diet; it is also influenced by the dilution rate in the rumen, feeding frequency, roughage to concentrate ratio, ionophores, and minerals such as P, S, and Mg in the diet[22]. The microbial efficiency values obtained in this study were below those reported by Ramos et al.[23]. The values obtained in the current study are explained by the total DM and production levels of the cows. According to Aguiar et al.[24], microbial production efficiency is one of the factors influencing the amount of microbial protein that reaches the small intestine and is inversely proportional to the permanence of microorganisms in the rumen.

Conclusion

The combination of a supplement containing 21% urea N and the first stocking can be used without compromising the nutritional and productive parameters of the cows.

Conflict of interests

The authors declare no conflict of interest

Author Contributions

Conceptualization: R.K.C.B. Dias and F.A. Teixeira; *Investigation:* R.K.C.B. Dias, D.L.S. Dias, J.P. dos Santos, F.O Barreto, S.S. de O. Rodrigues and M. de A. Meneses; *Methodology*: R.K.C.B. Dias and F.A. Teixeira; *Writing (original draft):* D.D Fries and F. F. da Silva.

Acknowledgments

We thank the Foundation for Research of the State of Bahia (FAPESB) for financial support.

References

1. Lima MLP, Simili FF, Giacomini A, Roma-Junior LC, Ribeiro EG, Paz CCP. Rotational stocking management affects the structural and nutritional characteristics of Guinea grass swards and milk productivity by crossbred dairy cows. Anim. Feed Sci. Technol. 2013; 186(1): 131–138. Available from: http://dx.doi.org/10.1016/j.anifeedsci.2013.10.002

2. Paulino MF, Detmann E, Valadares Filho SC. Suplementação animal em pasto: energética ou proteica? In: Simpósio sobre manejo estratégico da pastagem - SIMFOR. Viçosa; MG.; 2006. p.359-392.

3. Johnson AD. Sample preparation and chemical analysis of vegetation. In: Manetje LT (ed.) Measurement of grassland vegetation and animal production. Aberustwyth: Commonwealth Agricultural Bureaux. 1978; p 96–102.

4. National Research Council - NRC. Nutrient requirements of dairy cattle. 7 th.ed. Washington, D.C.: National Academic Press; 2001. p 381.

5. Detmann E, Souza MA, Valadares Filho SC, Queiroz AC, Berchielli TT, Saliba EOS, Cabral LS, Pina DS, Ladeira MM, Azevedo JAG. Métodos para análise de alimentos. 1th ed. Suprema, Visconde do Rio Branco; 2012. p 214.

6. Sniffen CJ, O'Connor, JD, Van Soest PJ, Fox DG, Russell JB. A net carbohydrate and protein system for evaluating cattle diets: II- Carbohydrate and protein availability. Journal of Animal Science. 1992; 70(11): 3562-3577. Available from: https://doi.org/10.2527/1992.70113562x

7. Hall MB. Challenges with non-fiber carbohydrate methods. Journal of Animal Science. 2003; 81(12): 3226–3232.

8. Detmann E, Valadares Filho SC. On the estimation of non-fibrous carbohydrates in feeds and diets. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2010; 62(4): 980-984. Available from:http://dx.doi.org/10.1590/S0102-09352010000400030.

9. Weiss WP. Energy prediction equations for ruminant feeds. In: Cornell nutrition conference for feed manufacturers, 61. Ithaca. Proceedings Ithaca: Cornell University; 1999 P.176–185.

10. Cappelle ER, Valadares Filho SC, Silva JFC, Cecon PR. Estimates of the Energy Value from Chemical Characteristics of the Feedstuffs. Revista brasileira de zootecnia. 2001; 30(6): 1837-1856. Available from: http://dx.doi.org/10.1590/S1516-35982001000700022.

11. Silva JFC, Leão MI. Ruminat nutrition fundamentals. Piracicaba: Livro ceres; 1979. 380p.

12. Verbic J, Chen XB, Macleod NA, Ørskov ER. Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivative excretion by steers. Journal of Agricultural Science. 1990; 114(1): p.243-248. Available from: https://doi.org/10.1079/BJN19900098

13. Chen XB & Gomes MJ. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives an overview of technical details. Bucksburnd: Rowett Research Institute, International Feed Resources Unit; 1992. 21p.

14. Silva DJ, Queiroz AC. Análise de alimentos (métodos químicos e biológicos). 3.ed. Viçosa, MG: Editora UFV; 2002. 235p.

15. Porto PP, Deresz F, Santos, GT, Lopes FCF, Cecato U, Cóser AC. Produção e composição química do leite, consumo e digestibilidade de forragens tropicais manejadas em sistema de lotação intermitente. Revista Brasileira de Zootecnia. 2009; 38(8): 1422-1431. Available from: https://doi.org/10.1590/S1516-35982009000800005

16. Reis AM, Costa, MR, Costa, RG, Suguimoto HH, Souza CHB, Aragon-Alegro LC, Ludovico A, Santana EHW. Effect of the racial group and number of lactation on the productivity and composition of bovine milk. Semina: Ciências Agrárias. 2012; 33(6): 3421-3436. Suplemento 2. Available from: http://dx.doi.org/ 10.5433/1679-0359.2012v33Supl2p3421.

17. Vasconcelos, A.M., Leão, M.I., Valadares Filho, S.C., Valadares, R.F.D., Dias, M., Morais, DAEF. Ruminal parameters, nitrogen compound balance and microbial production in dairy cows fed soybeans and their by-products. Revista Brasileira de Zootecnia. 2010; 39(2): 425-433. Available from: http://dx.doi.org/10.1590/S1516-35982010000200028

18. Leão GFM, Neumann M, Rozanski S, Durman T, Santos SK, Bueno AVI. Nitrogênio ureico no leite: aplicações na nutrição e reprodução de vacas leiteiras. ACSA – Agropecuária Científica no Semi-Árido. 2014; 10(2): 23-28.

19. Jonker JS, Kohn RA, Erdman RA. Milk urea nitrogen target concentrations for lactating dairy cows fed according to national research council recommendations. Journal of Animal Science. 1999; 82(6): 1261-1273. Available from: https://doi.org/10.3168/jds.S0022-0302(99)75349-X

20. Dall-Orsoletta AL, Almeida JGR, Oziemblowski MM, Ribeiro-Filho HMN. Corn supplementation on milk urea nitrogen content of dairy cows grazing on temperate annual pasture. Ciência Rural. 2020; 50(2): e2019007. Available from: https://doi.org/10.1590/0103-8478cr20190077

21. Guliński P, Salamończyk E, Młynek K. Improving nitrogen use efficiency of dairy cows in relation to urea in milk – a review. Animal Science Papers and Reports. 2016; 34(1): 5-24.

22. Pereira KP, Veras ASCV, Ferreira MA, Batista AMV, Marques KA, Fotius ACA. Balanço de nitrogênio e perdas endógenas em bovinos e bubalinos alimentados com níveis crescentes de concentrado. Acta Scientiarum. Animal Sciences. 2007; 29(4): 433-440.

23. Ramos AO, Ferreira MA, Santos DC, Véras ASC, Conceição MG, Silva EC, Souza ARDL, Salla LE. Associação de palma forrageira com feno de maniçoba ou silagem de sorgo e duas proporções de concentrado na dieta de vacas em lactação. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2015; 67(1): 189-179. Available from: https://dx.doi.org/10.1590/1678-6537

24. Aguiar MSMA, Silva FF, Donato SLR, Schio AR, Souza DD, Meneses MA, Lédo AA. Síntese de proteína microbiana e concentração de ureia em novilhas leiteiras alimentadas com palma forrageira *Opuntia.* Semina: Ciências Agrárias. 2015; 36(2): 999-1012. Available from: https://doi.org/10.5433/1679-0359.2015v36n2p999