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Supplementation strategies for dairy cows kept in tropical grass pastures

Estratégias de suplementação para vacas leiteiras mantidas em pastos de gramíneas tropicais

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Abstract

This study aimed to evaluate the supplementation strategies on forage intake and nutrient digestibility, feeding behavior, milk production and composition, feed efficiency, nitrogen balance and body weight change of dairy cows kept in Tanzania grass pastures (*Panicum maximum* Jacq. cv. Tanzania) in the dry season. We used eight crossbred Holstein and Zebu cows in the final third of lactation, with an average weight of 505±44 kg and initial milk production of 9.0±1.44 kg day^{-1}, in a double 4X4 Latin square design. The experiment consisted of four experimental periods of 17 days each, with nine days for initial adaptation and eight days to collect data. Treatments consisted of mineral mix and supplements (energy, protein or multiple) provided in the amount of 2 kg per cow day^{-1}. Supplements promoted increased consumption of dry matter and nutrients ($P < 0.05$), without changing the forage intake in relation to the mineral mixture. The treatments did not affect the feeding behavior of animals ($P > 0.05$). There was an increase ($P < 0.05$) of 19.51% in milk production in the supplemented animals and productive responses approximate marginal 0.64 kg of milk for each kg of supplied supplement, however, the different supplements promoted a drop in the milk fat. There were higher net energy values of animal lactation for protein-energy supplementation and mineral mixture, but no change was observed in feed efficiency and energy efficiency. The performance of dairy cows kept in tropical grass pasture can be improved with the use of a concentrate supplementation of 0.40% of body weight per day^{-1} without compromising the forage intake of animals.
Key words: Concentrate. Digestibility. Forage intake. Milk production.

Resumo

Objetivou-se avaliar estratégias de suplementação sobre o consumo de forragem e nutrientes, digestibilidade dos nutrientes, comportamento ingestivo, produção e composição do leite, eficiência alimentar, balanço de compostos nitrogenados e variação de peso corporal de vacas leiteiras mantidas

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em pastos de capim Tanzânia (*Panicum maximum* Jacq. cv. Tanzânia) no período seco do ano. Foram utilizadas oito vacas mestiças HolandêsXZebu no terço final de lactação, com peso médio de 505±44 kg e produção de leite inicial de 9,0±1,44 kg dia^{-1}, em delineamento quadrado latino 4X4 duplo. O experimento consistiu de quatro períodos experimentais de 17 dias cada, sendo nove dias inicias para adaptação e oito dias para coleta de dados. Os tratamentos consistiram de mistura mineral e suplementos (energético, proteico ou *múltiplo) fornecidos na quantidade de* 2 kg por vaca dia^{-1}. Os suplementos promoveram aumento no consumo de matéria seca e nutrientes ($P < 0,05$), sem alterar o consumo de forragem em relação a mistura mineral. Os tratamentos não alteraram o comportamento ingestivo dos animais ($P > 0,05$). Foi observado aumento ($P < 0,05$) na produção de leite em 19,51% para os animais suplementados e respostas produtivas marginais aproximadas de 0,64 kg de leite para cada kg de suplemento fornecido, no entanto, os diferentes suplementos promoveram queda ($P < 0,05$) no teor de gordura do leite. Foram observados maiores valores ($P < 0,05$) de energia líquida de lactação dos animais mantidos no suplemento múltiplo em relação ao energético, contudo não foi observado alteração ($P > 0,05$) na eficiência alimentar. O desempenho de vacas leiteiras mantidas em pastagem de gramínea tropical pode ser melhorado com o uso de suplementação concentrada de 0,40% do peso corporal dia^{-1}, sem comprometer o consumo de forragem dos animais.
Palavras-chave: Concentrado. Consumo de forragem. Digestibilidade. Produção de leite.

Introduction

The performance of dairy cows in tropical pastures is limited, due to restrictions on the consumption and supply of nutrients by forage, requiring the use of supplements to meet the nutritional requirements of cows with an average milk production potential. Santos et al. (2003) revised the literature and observed average milk production of 9.10 kg day^{-1} in systems based exclusively on pasture, ranging from 5.0 to 13.7 kg day^{-1}. In the dry season, issues related to the reduction of forage supply and a decrease in its nutritional value further limit the productive response of animals with average milk production potential. However, in well-managed pastures and with the use of high-biomass forage, mass available in the dry season may be appropriate, requiring supplementation for most limiting nutrients, according to the chemical composition of the forage.

Although the use of protein supplements is commonly suggested to correct nitrogen deficiencies in forage in the dry season in order to meet the protein requirements of rumen microbial and animal (maintenance and milk production), this suggestion cannot be generalized since it depends on the nutritional value of forage, particularly on its crude protein content, and the production potential of the animal. According to NRC (2001), cows with a production potential of around 7 to 9 kg of milk per day^{-1}, have protein requirements ranging from 11 to 12% of diet dry matter (DM), values that can be observed in forage from well-managed pastures, even in the dry season. In this way, the question would be about the type of supplement to be used to increase the performance of cows kept under these conditions in order to maximize the production of milk per cow and per area, and to optimize costs.

Thus, the use of concentrated in Brazil for dairy cows is based on the use of mathematical models requirement for a defined production (NRC, 2001). However, these criteria do not consider the animal's response to the use of nutrients, the stage of lactation and the cow's body condition (SEMMELMANN, 2007), or the type of concentrate used and the interaction with the pasture factors, such as the amount of available forage mass, dry matter intake of pasture and the nutritional value of the forage (HOFFMAN et al., 1993).

According to Vilela et al. (1980), the productive response to the use of a concentrated supplement for dairy cows on pasture ranges from 0.50 to 0.90 kg of milk per kg of concentrated in the rainy season and 0.80 to 0.95 in the dry season. The lower variation in the response of dairy cows to the use of supplements in the dry season can be explained by the lower incidence of negative interactions between pasture and supplement compared to the rainy season.

In this context, the goal was to evaluate the use of different supplementation strategies in the intake and digestibility of nutrients, milk production and composition, energy and nitrogen balance in dairy cows kept in Tanzania grass pastures in the dry season.

Material and Methods

The experiment was conducted in Santo Antônio do Leverger, Mato Grosso, Brazil. The climate, according to the Koppen classification system, is Cwa, indicating a tropical, seasonal climate with two well-defined seasons. The region experiences a rainy summer season from October to March, and dry winters (April to September).

Eight pluriparous crossbred Holstein x Zebu cows were used, which were in late lactation, with an average body weight of 505±44 kg and a production of initial average milk of 9.0±1.44 kg day^{-1}, in a double 4X4 Latin square design, according to production of milk and production observed in the previous lactation.

The experiment lasted 68 days, divided into four periods of 17 days each, with the first nine days used for the adaptation of the animals to the experimental diets and the final eight days used for data collection. The animals were submitted to four different supplementation strategies: i) mineral mixture *ad libitum* (control); ii) energy supplementation; iii) protein supplementation; iv) multiple supplementation (Table 1). The supplements were offered in individual feeders with 2 kg day^{-1} (approximately 0.4% of body weight, BW), divided in equal amounts, after milking in the morning and afternoon (06h30 and 15h30). The experimental area for the animals consisted of 16 paddocks of Tanzania grass (*Panicum Maximum*, Jacq. Cv. Tanzania), with 0.25 ha each, managed in a rotational grazing system. At the beginning of the experiment, the grass was managed at the entrance height of 70 cm, corresponding to 95% of light interception (CARNEVALLI et al., 2006). The height of the pasture was evaluated in pre-grazing by measuring the sward height at 20 points inside the paddock. The forage mass was estimated and the pasture structure evaluated by the cut of the forage at ground level in an area defined by a square of 0.5X0.5 m, with a height equal to the obtained average height. Then, the forage was homogenized and divided to determine the fractions of green and dry leaf (leaf blade), green and dry stem (stem + sheath), and the availability of forage mass (kg DM ha^{-1}) in each paddock at pre-grazing. Due to the dry season and climate constraints to the growth of the grass, the animals remained, on average, for one day in each paddock.

On the 10th and 12th day of each experimental period, manual grazing simulation was performed in order to obtain an estimate of the composition of the forage consumed by the animals (EUCLIDES et al., 1992).

The intake and digestibility of dry matter and nutrients were estimated through the use of internal and external markers. Thus, 15 g day^{-1} of chromium oxide (Cr_2O_3) were administered orally between day 4 and 11 in each trial period to estimate the fecal excretion of animals. Feces samples were collected directly from the rectum (about 200 g), between the 10th and 12th day of each experimental period according to the following protocol: day 10 at 17h00, day 11 at 11h00 and day 12 at 06h00, and were then kept at -20° C for further analysis. In all fecal samples, chromium concentration was determined, which was used to estimate daily excretion of dry matter based on the ratio between the amount of indicator provided and its concentration in stools (BURNS et al., 1994): Fecal Excretion (g DM day^{-1}) = QIF x 100 CIF^{-1}, where: QIF = quantity of the supplied indicator (g day^{-1}), CIF = concentration of indicator in feces (%).

The forage intake was estimated using the internal marker indigestible NDF (VALENTE et al., 2011) and the use of the equation proposed by Detmann et al. (2001): total dry matter intake (TDMI) = [(FE x IMC) – IMCS] $IMCF^{-1}$, where: FE= fecal excretion (kg day^{-1}), IMC= internal marker content in feces (kg kg^{-1}), MCS= internal marker content in the supplement (kg day^{-1}), IMCF= internal marker content in the forage (kg kg^{-1}). The DM and the nutritional component digestibility were calculated as the difference between intake and respective fecal excretion.

Table 1. Composition of Tanzania grass, mineral mixture and energy, protein and protein-energy mineral supplements.

Item	Mineral Mixture	Supplements		
		Energy	Protein	Multiple
Corn	-	95.75	-	65.20
Soybean meal	-	-	97.70	20.00
Sunflower meal	-	-	-	10.00
Limestone	-	1.00	1.30	1.10
Dicalcium phosphate	-	2.00	-	1.00
Magnesium oxide	-	0.25	-	-
Sodium chloride	-	0.80	0.80	0.70
Urea	-	-	-	2.00
Premix mineral[1]	-	0.20	0.20	0.20
Mineral mixture[2]	100.00	-	-	-
Composition	**Tanzania grass**	**Supplements**		
		Energy	Protein	Multiple
Dry matter, g kg^{-1}	327.00	847.50	858.60	853.90
Mineral matter, g kg^{-1} of DM	90.50	71.80	134.90	79.20
Organic matter, g kg^{-1} of DM	909.50	928.20	865.10	920.80
Crude protein, g^{-1} kg of DM	115.70	60.70	472.70	240.00
Ether extract, g^{-1} kg of DM	17.20	21.60	4.90	29.70
NDF, g^{-1} kg of DM	720.50	139.10	155.30	321.90
NDFap[3], g^{-1} kg of DM	572.20	119.90	129.00	222.80
NDFi[4], g^{-1} kg of DM	175.20	28.90	20.70	22.40
NFC[5], g^{-1} kg of DM	204.40	661.10	190.60	439.70
TC[6], g^{-1} kg of DM	776.60	845.90	387.50	662.60
NIND[7], % of total N	395.30	779.70	915.10	710.40

[1]Composition of premix mineral: 105 g kg^{-1} calcium, 230 g kg^{-1} sulfur, 7,300 mg kg^{-1} magnesium, 330 mg kg^{-1} de cobalt, 2,000 mg kg^{-1} copper, 160 mg kg^{-1} iodine, 2,800 mg kg^{-1} manganese, 220 mg kg^{-1} selenium, 6,800 mg kg^{-1} zinc. [2]Composition of mineral mixture: 165 g kg^{-1} calcium, 90 g kg^{-1} phosphorus, 10 g kg^{-1} sulfur, 10 g kg^{-1} magnesium, 125 g kg^{-1} sodium, 90 mg kg^{-1} cobalt, 1.500 mg kg^{-1} copper, 90 mg kg^{-1} iodine, 1,000 mg kg^{-1} manganese, 20 mg kg^{-1} selenium, 4,000 mg kg^{-1} zinc, [3]NDFap (neutral detergent fiber corrected for ash and protein), [4]NDFi (indigestible neutral detergent fiber), [5]NFC (non-fiber carbohydrates) = 100 - (%CP - %CP urea + %urea) + %NDF + %EE + %ash), [6]TC (total carbohydrates), [7]NIND (nitrogen insoluble in neutral detergent, % of N total).

The total digestible nutrients (TDN) were calculated according to the equation proposed in the NRC (2001): TDN= digestible CP + digestible NDF + digestible NFC + (2.25 x digestible EE), where: CP= crude protein, NDF= neutral detergent fiber, NFC= non-fiber carbohydrates, EE= ether extract. The values of digestible, metabolizable and net lactation energy of experimental diets were determined using equations (NRC, 2001). The digestible energy (DE), expressed as Mcal kg^{-1}, was estimated by multiplying the concentration of the estimated total digestible nutrients and its combustion values (4.409 Mcal of ED per kg of TDN), while the metabolizable energy (ME), also expresses as Mcal kg^{-1} was calculated assuming a fat content of the experimental diets feed lower than 3% by the equation ME (Mcal kg^{-1})= (1.01 x DE) – 0.45. The net lactation energy of the experimental diets was calculated using the following equation NEL (Mcal kg^{-1})= 0.703 x ME – 0.19, where was also assuming a fat content of the diets lower than 3% of dry matter basis.

Cows were mechanically milked twice daily, at 06h00 and 15h00, and milk production registered by a device connected to the milking machine, between the 11th and 14th days of each experimental period. At 12th and 13th experimental days, milk samples have been collected during morning and afternoon milking (approximately 100 mL), which were mixed and packaged in plastic flasks with preservative for the determination of fat, protein, lactose and urea-nitrogen by infrared spectrophotometry (IDF, 1996). Fat-corrected milk production (FCMP) for 3.5% was estimated (SKLAN et al., 1992) using the following equation: FCMP kg day^{-1}= 0.432 x milk production in kg^{-1} day + 16.216 x fat production kg day^{-1}.

Feed efficiency was calculated by dividing the milk production corrected to 3.5% fat by the total DM intake. Energy efficiency, expressed as Mcal of net energy of lactation excreted in milk by the consumed net energy of lactation. Energy balance (EB, Mcal day^{-1}) was calculated by subtracting the consumed lactation energy of net energy requirements for maintenance and lactation, with net energy for maintenance calculated as EL_M (Mcal day^{-1})= 0.080 x body $weight^{0,75}$ and net energy of lactation as EL_L (Mcal day^{-1}= (0.0929 x % fat + 0.0547 x % CP + 0.0395 x % of lactose) x milk production (kg day^{-1}) (NRC, 2001).

Visual observations of cows were performed to evaluate the feeding behavior at the 15th day of each trial. The animal observation occurred at intervals of 10-minute for 24 hours to determine the time spent on grazing, rumination and idleness. The time spent by the animals in the selection and apprehension of forage, including short periods of time used in the move to forage selection, was considered grazing time (HANCOCK, 1953). The rumination time corresponded to the grazing off period, but with visual observation of animal chewing activity. Idleness times were the rest periods of the animals when no chewing activity was observed (FORBES, 1988). Visual observation periods of feeding behavior were divided into diurnal, between 06h00 and 17h50, and nocturnal, between 18h00 and 05h50.

The samples of forage, ingredients, concentrate, leftovers and feces were pre-dried in a forced ventilation oven at 60±5 °C for 72 hours and milled individually in a mill with a 1 mm sieve. It was determined the contents of DM, organic matter (OM), mineral matter (MM), CP and EE according to AOAC (1990). The content of NDF was determined using α amylase without the addition of sodium sulfite and corrected (NDFap), discounting ash and protein (MERTENS, 2002; LICITRA et al., 1996). The total carbohydrates (TC) were calculated from the equation: TC= 100 - (%CP + %MM + %EE) proposed by Sniffen et al. (1992). Due to the presence of urea in the concentrate, NFC were calculated according to the equation proposed by Hall (2000): NFC= 100 - [(%CP - %CP from urea + %urea) + %NDFap + %EE + %ash] (Table 1).

The variables were analyzed in a double and simultaneous Latin square design using the MIXED procedure of SAS (2002), version 9.2, according to the following model: Y = $Y = \mu + \alpha + \beta + \gamma(\beta) + p + \varepsilon$, where μ is the general mean, α is the fixed effect of treatment, β is the random effect of square, $\gamma(\beta)$ is the random effect of animal within square, p is the random effect of period and ε is the random error.

The degrees of freedom and tests were adjusted using the Kenward–Roger option. The LSMEANS option was used to generate individual means for treatment effect. Comparisons between means were performed using orthogonal contrasts. The evaluated contrasts were: mineral supplement versus concentrate supplementation (A), energy supplement versus protein supplement (B), energy supplement versus multiple supplement (C) and protein supplement versus multiple supplement (D). Effects were declared significant when P<0.05 and tendency when P>0.05 and P<0.10.

Results and Discussion

Average heights of Tanzania grass pasture of 50.98 cm at pre-grazing were observed, with an occupation time of one day for each paddock and a grazing interval of 14.2 days with an average stocking rate of 3.73 AU ha^{-1}, allowing an average forage mass at pre-grazing of 6.74 t ha^{-1} DM. The available forage was a compound, on average, of 38.7% of leaf blade, 6.1% green stem and 55.1% of senescent material (Table 2). Based on these values, an availability of 130 kg of grass DM per animal day^{-1} was estimated, equivalent to 25% of body weight per animal day^{-1}.

Table 2. Morphological components of Tanzania grass.

	Period				Mean
	1	2	3	4	
			Ton DM ha^{-1}		
Green leaf	4.04	2.21	1.58	1.73	2.76
Green steam	4.79	3.99	1.44	1.58	2.78
Senescent	1.68	2.39	1.53	1.59	1.60
Total	10.51	8.59	4.55	4.90	7.14
			%		
Green leaf	38.45	25.74	34.82	35.30	38.67
Green steam	45.52	46.45	31.63	32.25	38.95
Senescent	16.03	27.82	33.55	32.45	22.38
Sward height (cm)	69.53	51.34	41.61	41.45	50.98

The sward height at pre-grazing of 50.98 cm was lower than the height of 70 cm, recommended by Carnevalli et al. (2006). The lower height may be due to the experimental period (dry season), characterized by lower growth rates of forage because of reduced rainfall and photoperiodism. It is noteworthy that even at this period, the pasture used presented good composition compared to the observed average during this period, with a protein content of 11.57%.

The forage mass observed is above that quoted by Santos et al. (2010) of a three to five times daily intake and the availability cited by Silva et al. (2009) of 100 to 120 g DM kg^{-1} BW day^{-1} to ensure selectivity of the animals and that their daily DM intake was not limited. Thus, according to Bargo et al. (2003), the availability of 110 kg DM per animal day^{-1} maximizes the daily DM intake. So, it can be considered that the supply of forage in this study did not limit the animal intake.

Supplements resulted in a positive difference (P<0.05) in TDMI in relation to animals supplemented with mineral mixture without, however, causing a reduction in forage intake (P>0.05), which was similar to all treatments. Thus, it can be said that the tested supplements promoted an additive effect, with increased TDMI determined by the supplement intake. There was no difference (P>0.05) between the tested supplements (energy, protein or multiple) on the TDMI. The average TDMI observed for the supplemented animals, around 12.42 kg day^{-1}, equivalent to 2.31% BW, is close to the values predicted by the NRC (2001) equations of 2.49% of BW.

The absence of a substitutive effect in the present study should be considered, as the general aim of supplementation is to optimize the intake and digestion of forages by the animal, without reducing forage intake. The substitution effect is commonly observed in cattle (beef and dairy) supplemented in pasture and its occurrence leads to a lower intake of nutrients and animal performance, which results in lower financial returns per kg of provided supplement. The substitution effect is usually associated with high levels of supplementation in pastures with high nutritional value. Thus, the average forage quality and low level of supplementation used in this study may explain the absence of this effect.

Similarly, to this study, Lima et al. (2001) observed no change in forage intake by crossbred cows kept in Tanzania grass pasture supplemented with 3 kg of concentrate per day^{-1} (average of 9.63 kg forage DM day^{-1}). Aroeira et al. (1999) also found no supplementation effect on the DM intake of elephant grass in different seasons by crossbred cows fed with 2.5 kg concentrate a day^{-1}.

Different supplements used did not promote changes in NDF intake in kg day^{-1} or % BW, with an average of 7.86 kg day^{-1} and 1.56% BW, respectively (Table 3). These NDF intakes are above the maximum daily intake limit of 1.20% BW quoted by Mertens (1987) as the maximum daily intake of NDF, above which the DM intake is limited by rumen fill. Most studies with lactating cows grazing tropical grasses show an NDF intake of up to 1.5% of BW. Papers presented by Lima et al. (2001), Lopes et al. (2004) and Sousa et al. (2008) with Brachiaria, Elephant and Tanzania grass showed an NDF intake of 1.60%, 1.99% and 1.87% of BW, respectively.

Table 3. Least squares mean for dry matter and forage intakes, and supplement and nutritional components intake of dairy cows submitted to different types of supplementation.

Variables[1]	Mineral Mixture	Supplements			SEM[2]	*p* value of contrasts[3]			
		Energy	Protein	Multiple		A	B	C	D
Forage, kg day^{-1}	9.54	10.32	10.46	11.93	1.51	0.27	0.91	0.52	0.59
Supplement, kg day^{-1}	0.09	1.64	1.85	1.85	0.12	<0.01	0.22	0.23	0.99
Total, kg day^{-1}	9.75	11.96	12.30	13.01	1.50	0.01	0.78	0.41	0.58
Total, %BW	1.94	2.39	2.44	2.49	0.26	0.02	0.82	0.70	0.87
NDF, kg day^{-1}	6.83	7.77	8.46	8.58	1.04	0.06	0.44	0.38	0.89
NDF, %BW	1.36	1.55	1.68	1.64	0.18	0.08	0.48	0.62	0.86
Crude protein, kg day^{-1}	1.13	1.32	2.10	1.76	0.23	<0.01	<0.01	<0.01	0.02
Ether extract, kg day^{-1}	0.16	0.21	0.19	0.25	0.03	<0.01	0.24	0.09	<0.01
Total carbohydrate, kg day^{-1}	7.38	9.37	8.82	9.84	1.12	0.02	0.56	0.64	0.31
NFC, kg day^{-1}	1.98	3.20	2.49	3.13	0.35	<0.01	0.01	0.79	0.03
TDN, kg day^{-1}	5.82	7.34	8.11	8.44	1.31	<0.01	0.31	0.18	0.67
Net energy, Mcal day^{-1}	13.26	16.76	19.63	21.24	3.62	0<.01	0.14	0.028	0.40

[1]NDF= neutral detergent fiber, NFC= non-fiber carbohydrates, TDN= total digestible nutrients, [2]SEM= standard error of the mean. [3]Mineral mixture versus supplement (A), energy supplement versus protein (B), energy supplement versus multiple (C), protein supplement versus multiple (D).

Considering the NDF intake and respective TDMI, it is possible to calculate that, on average, the NDF represented more than 65% of total DM consumed by the animals, which is very interesting and characteristic of milk production in Brazil. Thus, considering that the NDF is only digested in the rumen and large intestine of the animals, and the rumen is the main compartment of digestive tract, where are produced VFAs and microbial protein by microbial fermentation, which are the major source of energy and amino acids for the animal, it is imperative to optimize the digestion of these compounds in the environment.

A higher ($P<0.05$) CP intake by supplemented animals was observed. Among the supplements, there was higher CP intake for animals submitted to protein supplementation. Protein is generally the first limiting nutrient for the cattle's milk production in grazing systems during the dry season in tropical regions, with protein levels in the forage below 7% in the DM. In this study, due to the grazing management used, forage showed a better chemical composition. Thus, supplementation with concentrates that provide, in particular, other nutrients can result in an improvement in yield performance and better financial returns for the farmer. Even for the energy supplementation, CP intake was 190 g higher than that of animals fed only with forage (mineral mixture) and, additionally, the supply of rapid fermentation energy in the rumen (starch) can enhance the use by rumen microflora of nitrogen compounds from forage.

Intake of NFC was higher for supplemented animals compared to the control ($P<0.05$). The NFC intake of animal for protein supplement was lower when compared to energy and multiple. On average, the supplemented animals showed a NFC intake 0.96 kg day^{-1} higher than the control group animals, while the animals of the energy supplement showed a NFC intake 0.71 kg day^{-1} higher than the protein supplement animals. The NFC present rapid degradation and almost complete digestion in the gastrointestinal tract, providing increased availability of energy to the microflora in the rumen and an increase in the total digestion of DM and OM, thus causing benefits to the animal from a protein and energy point of view.

A higher coefficient digestibility of OM was observed and a tendency of higher coefficient of digestibility of DM with the supply of supplements compared to the use of the mineral mixture (Table 4). The higher values of OM digestibility with supplements use are related to the increase in the content of NFC in the diet, which have fast and high digestion in the gastrointestinal tract of animals. Considering that there was only a difference in digestibility of EE and NFC between supplemented and control animals and the average percentage of each one of the nutritional components in the diet is 1.69% and 21.98%, respectively, it can be inferred that NFC are the main cause of increased digestibility of DM and OM.

Pimentel et al. (2011) found an increase from 55.17% to 62.93% in the digestibility of OM and from 49.84% to 58.36% in the digestibility of DM for dairy cows kept on pasture supplemented with 2 kg day^{-1} of concentrate supplement (50% CP in fresh matter), compared to the use of mineral mixture. In the present study, digestion of the OM increased from 55.20 to 58.57%.

Table 4. Least squares mean for coefficient digestibility of dry matter and nutrients in dairy cows kept in pasture of Tanzania grass.

Variables[1] (%)	Mineral Mixture	Supplements			SEM[2]	*p* value of contrasts[3]			
		Energy	Protein	Multiple		A	B	C	D
Dry matter	51.15	52.82	55.01	55.11	4.91	0.07	0.29	0.29	0.96
Organic matter	55.20	57.30	59.07	59.34	4.17	0.04	0.36	0.31	0.89
Crude protein	70.55	67.16	80.43	74.89	4.95	0.13	<0.01	0.02	0.07
NDF	63.03	61.83	66.07	63.16	3.53	0.55	<0.01	0.34	<0.05
Ether extract	30.75	38.65	37.50	51.44	8.95	0.01	0.83	0.03	0.02
Total carbohydrate	53.33	56.18	54.23	56.78	4.03	0.17	0.35	0.78	0.25
NFC	19.45	39.88	23.84	39.84	8.52	<0.01	0.01	0.99	0.02

[1]NDF= neutral detergent fiber, NFC= non-fiber carbohydrates. [2]SEM, standard error of the mean. [3]Mineral mixture versus supplement (A), energy supplement versus protein (B), energy supplement versus multiple (C), protein supplement versus multiple (D).

Among the evaluated supplements, higher values were observed for CP digestibility for protein and multiple supplements in relation to energy, and lower NDF digestion for the energy supplement compared to the protein supplement (Table 4). There was no difference in NDF digestibility between supplemented and control animals, but there was a difference between animals supplemented with protein or receiving multiple supplements compared to animals that received the energy supplement (NFC). This can be explained considering the probable negative effects of NFC to the fibrolytic population in the rumen environment, both in terms of growth stimulation of the population that ferments NFC, which would compete for nutrients with fibrolytic organisms, and the effects on ruminal pH or the direct negative effect of sugars resulting from NFC digestion on the adherence of fibrolytic microorganisms to fibrous components in the rumen.

In contrast to what was found regarding NFC, greater digestion was observed in animals receiving the energy supplement compared to the protein one. The apparent digestibility values for NFC observed in the present study can be considered low. However, when working with cattle fistulated and cannulated in the rumen, with a diet based on 90% roughage, Cabral et al. (2006) found apparent NFC digestibility of 85.58, 65.96 and 41.86% for diets based on corn silage, elephant grass silage and Tifton 85 grass hay, respectively. However, when estimating the true digestibility subtracting the NFC metabolic excretion from NFC found in the feces, an average true digestibility of 96.6% was observed. Thus, for nutritional components with endogenous fraction, the apparent digestibility is dependent on the diet composition and is likely to get relatively low values, as observed in this study in diets with low NFC content.

The supplements did not promote changes in the feeding behavior of the animals, however there was a tendency towards an increase in diurnal rumination time and a decrease in total grazing time for the supplemented animals compared to those receiving only the mineral mix (Table 5). According to Bargo et al. (2003), the increase in supplements intake may interfere with the time spent by the animal on grazing activity and forage dry matter intake (FDMI), by reducing the nutritional needs due to the higher concentration of nutrients in the concentrate compared to the forage (SANTANA JÚNIOR et al., 2012). Papers presented by Santana Júnior et al. (2012) and Dias et al. (2014) should be highlighted, in which the authors observed a correlation of the total chewing time and rumination with the coefficient of NDF digestibility and of organic matter and nutrients digestibility.

Table 5. Least squares means of the feeding behavior of dairy cows grazing in pasture of Tanzania grass.

Variables	Mineral Mixture	Supplements			SEM[1]	*p* value of contrasts[2]			
		Energy	Protein	Multiple		A	B	C	D
Grazing									
Diurnal, %	67.14	62.70	60.48	56.65	5.19	0.12	0.68	0.27	0.48
Nocturnal, %	12.32	14.61	13.38	12.68	4.99	0.68	0.74	0.60	0.85
Total, %	37.78	34.49	35.28	33.18	3.85	0.07	0.72	0.56	0.35
Rumination									
Diurnal, %	13.10	15.76	18.75	18.95	2.80	0.06	0.30	0.27	0.94
Nocturnal, %	47.89	42.78	47.89	45.60	2.90	0.45	0.21	0.48	0.57
Total, %	31.67	32.61	34.34	33.27	1.63	0.26	0.36	0.73	0.57
Idleness									
Diurnal, %	19.76	21.57	20.77	24.40	3.70	0.45	0.84	0.49	0.37
Nocturnal, %	39.79	42.61	38.73	41.73	4.90	0.70	0.33	0.82	0.44
Total, %	30.55	32.89	30.38	33.55	3.65	0.44	0.36	0.81	0.26

[1]SEM = standard error of the mean. [2]Mineral mixture versus supplement (A), energy supplement versus protein (B), energy supplement versus multiple (C), protein supplement versus multiple (D).

Although without differences between supplements, there was a higher milk production and milk corrected to 3.5% fat for the supplemented animals compared to those receiving only the mineral mix (Table 6). McLachlan et al. (1994), Gibb et al. (2002) and Oliveira et al. (2010) demonstrated the importance of providing concentrate for dairy cows kept on pasture and its relationship to the increase in milk production. So, it is noteworthy that the exclusive use of forage does not allow the enhancement of milk production, even in cows of low average production in the final third of lactation.

Table 6. Least squares means for milk production and composition from dairy cows at late lactation receiving different types of concentrated supplements.

Variables[1]	Mineral Mixture	Supplements			SEM[2]	*p* value of contrasts[3]			
		Energy	Protein	Multiple		A	B	C	D
MP, kg day^{-1}	5.82	6.85	7.23	6.78	0.87	<0.01	0.26	0.84	0.19
FCMP, kg day^{-1}	6.37	7.13	7.51	7.19	0.67	<0.01	0.25	0.84	0.33
Fat, %	4.08	3.81	3.82	3.95	0.25	0.02	0.90	0.18	0.23
Fat, kg day^{-1}	0.24	0.26	0.27	0.26	0.02	0.02	0.28	0.61	0.56
Protein, %	3.42	3.40	3.50	3.55	0.07	0.14	0.08	0.01	0.39
Protein, kg day^{-1}	0.20	0.23	0.25	0.24	0.03	<0.01	0.09	0.65	0.21
Lactose, %	4.28	4.24	4.29	4.37	0.24	0.75	0.45	0.04	0.17
Lactose, kg day^{-1}	0.25	0.29	0.31	0.29	0.02	<0.01	0.27	0.83	0.37
N-Ureic, mg dL^{-1}	13.36	11.24	21.62	15.83	0.80	<0.01	<0.01	<0.01	<0.01
SCC, x mil mL^{-1}	337.63	447.63	429.50	354.88	298.33	0.28	0.83	0.26	0.37
$NE_{L,}$ Mcal kg^{-1}	0.74	0.71	0.72	0.73	0.03	0.07	0.44	0.02	0.10
NE_LI, Mcal day^{-1}	4.27	4.80	5.10	4.89	0.42	<0.01	0.18	0.68	0.34
FE, FCMP DMI^{-1}	0.71	0.61	0.65	0.55	0.08	0.11	0.62	0.39	0.18
EE_F, PEL_L CE_L^{-1}	0.43	0.32	0.29	0.26	0.08	0.01	0.67	0.35	0.60
EB, Mcal day^{-1}	0.55	3.43	6.02	7.77	3.47	<0.01	0.18	0.03	0.36

[1]MP= milk production, FCMP= milk production corrected for 3.5% of fat, SCC= somatic cell count, NE_L=net energy lactation, NE_LI= net energy of lactation intake, FE= feed efficiency, EE_F= feed efficiency, EB= energy net balance, [2]SEM= standard error of the mean. [3]Mineral mixture versus supplement (A), energy supplement versus protein (B), energy supplement versus multiple (C), protein supplement versus multiple (D).

The lack of difference in milk production between different supplements, especially between protein and energy supplementation, can be explained by the average CP content of the forage observed throughout the study (11.51% of DM), which is considered "too good" for tropical pastures in the dry season. Thus, it can be inferred that by the absence of difference in responses to supplements, probably adding NFC to the rumen of animals kept in this condition promoted a stimulation of the growth of ruminal microflora, promoted by the synchronism between nutrients, resulting in increased nutrition supply to the animal.

The use of supplementation promoted average increases of 19.51% and 14.36% for the production of milk and fat-corrected milk and marginal responses of approximately 0.64 kg and 0.52 kg of milk for each kg of supplied supplement. Using the equation proposed by Oliveira et al. (2010) obtained by analysis of 31 experiments, a calculated marginally productive response of 1.38 kg of milk per kg of concentrate was obtained. The discrepancy between the marginal productive responses observed and calculated shows the low productive potential of the cows in late lactation is associated with the reduced nutritional value of forage during the experiment and justified, in part, the absence of differences between supplements.

Similarly, the supplementation reduced milk fat content compared to using only the mineral mixture. This is most likely to be related to the formation of fatty acid biohydrogenation intermediates in the rumen and the intestinal absorption of compounds with the double bond of trans isomerism at the carbon 10 and its effect on fat synthesis in the mammary gland, due to supplementation. Therefore, it is necessary to set certain conditions in the rumen, such as the presence of specific bacterial species and communities and reducing the pH of the rumen fluid (BAUMAN et al., 2006). However, it should be noted that the milk fat, even for the supplemented animals, was much higher than 3.2%, seen as a limit below which it is considered as milk fat depression in Holstein cattle (OETZEL, 2007).

Higher milk protein levels were observed in animals receiving multiple supplementation (protein and energy) compared to energy supplementation ($P<0.05$), and a tendency was observed ($P=0.079$) of higher protein in milk in animals receiving protein supplement compared to those receiving energy supplementation. However, no differences were observed in the content of milk protein from either supplemented or non-supplemented animals ($P>0.05$).

The increase in milk protein content is related to increased availability of amino acids for use by the mammary gland, mostly from the increased flow of microbial protein and/or non-degraded protein in the rumen, and thus the increased availability of amino acids for absorption into the small intestine (KALSCHEUR et al., 2006). As protein feeds used in the protein and protein-energy supplements present a large proportion of rumen degradable protein, their inclusion in the diet allows the increase in amino acid and ruminal ammonia nitrogen content (CHANDLER, 1989).

Based on the above, a higher protein content in the milk of animals subjected to protein supplement should be expected, however when observing the urea nitrogen values in the milk, it is noted that the value observed for the milk of animals that received this supplement was greater than the value of 18 mg dL^{-1}, which is considered as the upper limit, from which excess is associated with degradable protein or energy deficiency in the rumen (HARRIS JÚNIOR, 1996).

Regarding urea nitrogen values in milk, it is observed that the supplemented animals showed higher values than the non-supplemented ones ($P<0.05$), while animals that received the energy supplement had lower urea nitrogen values compared to those supplemented with multiple and protein concentrate, with higher concentration observed in animals subjected to protein supplementation.

Given that the CP content in the forage was already considered well above what is the expected average and commonly observed in the dry season for tropical forages, the combination of this forage CP with the protein supplement certainly increased the degradation of the CP in the rumen and the fermentation of amino acids, thus promoting an increased NH_3 concentration in this environment and its dissemination by the rumen epithelium. In order to prevent the elevation of circulating NH_3 concentration (plasma), the hepatocytes convert NH_3 to urea, which can be recycled to the rumen and excreted from the body via the renal or can be excreted in the milk. Thus, the increase in milk urea concentration indicates an imbalance in availability of energy and N in the rumen, as evidenced in this work.

Under conditions of N excess and rapid fermentation energy deficiency, a higher NFC intake is required to provide substrates for the appropriate ruminal fermentation, an increase in microbial protein synthesis and an increase in amino acid availability in the gut for the absorption and synthesis of milk components. Accordingly, in an experiment conducted by Reis and Combs (2000), the authors observed a linear decrease in the urea nitrogen values in milk with increased supply of concentrate based on maize, similar to that observed for the energy supplement used in this study.

Higher values of NE_L per kg of milk was observed for animals with multiple supplementation compared to animals that received energy supplementation, probably due to the higher protein content in the milk of the first group. Despite the increase in the excretion of NE_L by increasing the production of milk and corrected milk using the supplementation, no increase in feed efficiency or reduction in energy efficiency was observed, regardless of the supplement type.

The time of year and the reduction in the nutritional value of forage during the trial period, added to the low production potential of animals at the end of lactation, showed a lower marginal productive response and correlated with the lower energy efficiency in milk production. In this case, no supplementation is of interest in maximizing the use of productive resources. However, as the energy balance was positive and different from zero by the supplemented animals, especially in the use of a multiple supplement, even with low productive responses, these strategies would be of interest in promoting an increased body score, if necessary, by animals in late lactation.

There was a higher nitrogen intake for animals supplemented compared to those without supplementation (Table 7). Among the supplements, the use of the protein one resulted in an increased intake of N compared to the other supplements and mineral mixture. It is noted that there is need for further adjustments in protein and energy content when using this type of supplement, to reduce nitrogen excretion, since an increase in nitrogen excretion by the urinary tract was observed compared to that of the mineral mixture (90.67%) and multiple supplement (51.70%).

Table 7. Least squares means for nitrogen balance and synthesis efficiency of microbial protein of dairy cows kept in pasture Tanzania grass.

Variables[1]	Mineral Mixture	Supplements			SEM[2]	*p* value of contrasts[3]			
		Energy	Protein	Multiple		A	B	C	D
Intake N, g day^{-1}	180.48	210.61	335.73	296.61	38.89	<0.01	<0.01	<0.01	0.09
Excretion N, g day^{-1}	193.44	204.89	326.32	294.31	29.48	<0.01	<0.01	<0.01	0.11
Balance N, g day^{-1}	-12.96	5.72	9.41	2.29	32.64	0.40	0.89	0.90	0.79
N milk, g day^{-1}	31.01	36.34	39.43	37.15	4.24	<0.01	0.10	0.65	0.21
% total excreted	16.50	18.10	12.42	12.67	0.94	0.06	<0.01	<0.01	0.85
% intake	19.94	18.29	12.16	13.40	2.44	<0.01	<0.01	0.02	0.54
N fecal, g day^{-1}	45.59	62.64	64.10	69.36	5.88	<0.01	0.83	0.33	0.44
% total excreted	24.56	32.56	19.72	23.78	2.91	0.76	<0.01	0.01	0.21
% intake	28.89	32.84	19.22	24.64	4.94	0.11	<0.01	<0.01	0.03
N urine, g day^{-1}	116.84	105.91	222.79	187.8	23.08	0.00	<0.01	<0.01	0.08
% total excreted	58.93	49.34	67.85	63.55	3.40	0.67	<0.01	<0.01	0.26
% intake	69.21	51.67	67.69	68.70	7.94	0.35	0.07	0.05	0.90
MPA, mmol day^{-1}	187.75	200.97	231.73	224.76	28.94	0.28	0.39	0.50	0.84
Min, g N day^{-1}	136.50	146.11	168.47	163.41	21.04	0.28	0.39	0.50	0.84
ME, g CPmic kg^{-1} TDN	165.26	133.58	145.14	134.03	28.05	0.20	0.65	0.99	0.67

[1]N= nitrogen, MPA= microbial purines absorbed, MIN= microbial nitrogen, ME= microbial efficiency. [2]SEM= standard error of the mean. [3]Mineral mixture versus supplement (A), energy supplement versus protein (B), energy supplement versus multiple (C), protein supplement versus multiple (D).

Regarding N secreted in milk, as a proportion of ingested N, it is observed that the supplemented animals showed lower values than the non-supplemented, and that animals subjected to protein and multiple treatments had lower values (P<0.05) than animals subjected to the energy supplement.

Tamminga (1992) reported that in the conditions of the Netherlands, where the temperate pastures with a high content of N are routinely used as a dietary ingredient, the proportion of ingested N lost in the urine would be around 50%, and the N incorporated into the milk around 19%, a value similar to that observed in this study for the animals not treated or receiving the energy supplement, but it is higher than the values observed for animals that received the protein and multiple supplements.

The use of energy supplementation provided less excretion (P<0.05) of urinary N compared to the animals subjected to multiple and protein supplements, but there was no difference between supplemented and non-supplemented animals for this variable, certainly due to the high values observed in animals who received the protein and multiple supplements. It should be noted that a lower urinary N excretion can result in lower energy expenditure for the production of hepatic urea from NH_3 originating from the rumen, thereby allowing a greater production efficiency of animals.

There were no differences (P>0.05) between any supplementation strategy regarding the total absorbed purines, rumen microbial N production and microbial efficiency. Several factors should be highlighted regarding this, such as the high standard error of the mean for all the above variables due to individual variation between cows as regards to the urinary excretion and metabolism of microbial purine.

In the present study, spot urine collection was only carried out on one day per trial period to estimate urinary volume and consequently evaluate the purine derivatives and estimate microbial production. Thus, it is suggested, based on experiments conducted by the same research group (unpublished data), that there should be a spot urine collection on at least three days of each period to obtain more representative samples and better estimates of urinary volume, purine derivatives excretion and microbial production.

The response of dairy cows to the different types of supplements evaluated depends on the genetic potential of the animals as well as the interaction between pasture and supplement. In the dry season, usually tropical grasses have low levels of CP, causing in most cases the recommendation to provide supplemental nutrients mainly composed of nitrogen compounds (NPN or true protein). However, in more intensive milk production under grazing condition systems, in which more productive forage and pasture management strategies are used, which provide the best-quality forage to the animals, forage CP content can be above 10% in DM. In this case, there are doubts about the type of supplement to be used. In similar conditions of forage CP content as maintained in this study, supplementation with energy, protein, or protein/energy, promote similar effects on intake, production and milk composition and the cost of the supplement can be the main determining factor of which one to choose. Probably, in pastures with medium or low CP content (<7% DM), the use of protein or multiple supplements promotes better responses than the energy supplement.

Conclusion

The supply of moderate amounts of concentrated supplements (0.4% of BW), independent of crude protein content, promotes increased production performance without affecting forage intake and improves the digestibility of dry matter and production of fat, protein and lactose (kg day^{-1}) in low production dairy cows maintained in tropical grass pastures. The use of an energy supplement (6% CP, DM basis) is recommended, due to its lower cost, over the use of protein (47% CP, DM basis) and multiple (22.9%) supplements, for dairy cows in late lactation (with production under 8 kg of milk per cow day^{-1}) kept in tropical grass pastures similar to those used in this study.

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