METABOLIZABLE PROTEIN SUPPLY ACCORDING TO THE NRC (2001) FOR DAIRY COWS GRAZING ELEPHANT GRASS

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ABSTRACT: Tropical pastures fertilized with nitrogen may have high crude protein (CP) contents with high rumen degradability. High crude protein concentrates offered to cows grazing these pastures may increase feed costs without positive effects on their performance. The objectives of this trial were to evaluate the effects of increasing metabolizable protein (MP) supply beyond the NRC (2001) recommendations for mid lactating dairy cows grazing elephant grass pasture (*Pennisetum purpureum* Schum. cv. Napier) managed with high stocking rates. Three concentrates (6.3 kg DM^{-1} cow^{-1} day^{-1}) were evaluated: control (17%CP) was adjusted in relation to MP according to the NRC (2001); the other two contained extra soybean meal, to increase the CP content to 21.2% (CP) and 25.0% (CP). Twelve multiparous Holstein cows, averaging 150 days of milk production at a rate of 19.5 kg of milk day^{-1}, were used in a 3 × 3 latin square design, replicated four times. Forage consumption was 11,270 kg DM ha^{-1} with 34% of green leaves and 12% CP. Milk production, corrected to 3.5% fat, milk fat, protein, lactose and total solids contents were not affected by treatments ($P > 0.05$). Milk urea nitrogen and plasma urea nitrogen increased linearly ($P < 0.05$) as the MP supply increased. Treatments did not affect ($P > 0.05$) body weight gain, body condition score, grazing time, ruminating time, resting time, rectal temperature and respiratory rate. Crude protein content in the concentrate formulated according to NRC (2001) is adequate for mid lactating cows grazing tropical pastures.
Key words: concentrates, milk production, protein content, tropical pastures

SUPRIMENTO DE PROTEÍNA METABOLIZÁVEL DE ACORDO COM O NRC (2001) PARA VACAS LEITEIRAS PASTEJANDO CAPIM ELEFANTE

RESUMO: Pastagens tropicais fertilizadas com nitrogênio podem ter alto teor de proteína bruta (PB) com alta degradabilidade ruminal. O fornecimento de concentrado com alto teor de PB para vacas mantidas nestas pastagens pode aumentar os custos sem efeitos positivos no desempenho. Objetivou-se avaliar os efeitos do suprimento de proteína metabolizável acima das recomendações do NRC (2001) para vacas em pastejo. Quatorze piquetes de capim elefante (*Pennisetum purpureum* Schum. cv. Napier) com 0,2 ha cada, foram usados em sistema de pastejo rotacionado. Três concentrados (6,3 kg MS^{-1} vaca^{-1} dia^{-1}) foram avaliados. O concentrado controle com 17% PB na MS (17% PB) foi ajustado em proteína metabolizável de acordo com o NRC (2001). Os outros dois concentrados foram formulados inclusão extra de farelo de soja para conterem 21,2 (CP) e 25% (CP) de PB na MS. Foram usadas 12 vacas holandesas multíparas com 150 dias em lactação e produção de leite de 19,5 kg dia^{-1}, arranjadas em QL 3 × 3 com quatro replicatas. A massa de forragem disponível era de 11.270 kg MS dia^{-1} com 34% de folhas verdes e 12% de PB. A produção de leite, produção de leite corrigida para 3,5%, teores de gordura, proteína, lactose e sólidos totais não foram afetados ($P > 0,05$) pelos tratamentos. O nitrogênio uréico do leite e o nitrogênio uréico do plasma aumentaram linearmente ($P < 0,05$) com o aumento do suprimento de proteína metabolizável. Os tratamentos não afetaram ($P > 0,05$) o ganho de peso, condição corporal, tempo de pastejo, tempo de ruminação, tempo em ócio, temperatura retal e a freqüência respiratória dos animais. O teor de PB no concentrado para suprir proteína metabolizável de acordo com o NRC (2001), é adequado para vacas em terço médio de lactação, mantidas em pastagens tropicais.
Palavras-chave: concentrados, produção de leite, teor de proteína, pastagens tropicais

INTRODUCTION

Milk production in Brazil is based primarily on pasture systems. These pastures when intensively managed, present high forage dry matter production, allowing high stocking rates during the hot rainy season (around 200 days $year^{-1}$ in most parts of the country). Cows fed exclusively tropical pastures produce only 2,500 to 3,500 kg of milk $year^{-1}$. Supplementing concentrate for good genetic merit dairy cows raised on tropical pastures has allowed milk productions to reach 6,000 to 7,000 kg $year^{-1}$ which more than doubled the milk production ha^{-1} (Santos & Juchem, 2000; Santos et al., 2003a, 2003b). Criteria for the amount of concentrate to feeds is dependent on the genetic merit of the animals, cost of the concentrate and milk price. In most important dairy areas in the country, 1 kg of 18% crude protein (CP) concentrate costs less than 1 L of milk (Santos et al., 2003a, 2003b).

The new metabolizable protein system (NRC, 2001) opens the possibility for fine-tuning on protein nutrition of dairy cows as compared to the old crude protein (NRC, 1978) or absorbed protein model (NRC, 1989). Diets deficient in protein affect cow performance, however, excess of protein is a waste of money and can be deleterious for cow reproductive performance and environment (NRC, 2001). Intensively managed tropical pastures contain 12% to 22% CP (dry basis) and 60 to 65% total digestible nutrients (TDN) (Santos et al, 2003a, 2003b). Most commercial concentrates for lactating cows in Brazil, contain 18 to 24% CP (as fed). According to NRC (2001), for cows milking 18 to 24 kg day^{-1}, fed 5 to 7 kg day^{-1} of concentrate and grazing tropical pastures with 12 to 22% CP, these concentrates would supply excesses of RDP (Rumen Degradable Protein) and MP (Metabolizable Protein).

The objectives of this trial were to evaluate the effects of increasing metabolizable protein supply beyond NRC (2001) recommendations for mid lactating dairy cows grazing elephant grass pastures (*Pennisetum purpureum* Schum. cv. Napier).

MATERIAL AND METHODS

Location

The trial was carried out in Piracicaba, state of São Paulo, Brazil (22º43'S, 47º25'W and altitude of 580 m), for a period of 60 days during summer, from December 2001 through February 2002. An elephant grass pasture area of 8.0 ha was divided in 40 paddocks of 0.2 ha each. Pastures were 33 years old and since their introduction they have been managed under high stocking rates (7 to 10 cows ha^{-1} during the hot rainy season). During the experimental period, the pasture was fertilized with 80 kg ha^{-1} N every grazing cycle (40 days). Every paddock was grazed for three days and rested for 37 days. Experimental cows and other herd lactating cows grazed a new paddock every day as a first grazing group. Dry cows and heifers, grazed the paddock for the next two days as a second grazing group.

Animals

Twelve multiparous lactating Holstein and Jersey (150 DIM and 511 kg LBW) cows were used as experimental cows and grazed together with other 24 herd lactating cows. Stocking rate for the first grazing group was of 4.6 cows ha^{-1}. Average LBW for the 36 cows (12 experimental + 24 herd cows) was 430 kg.

Treatments

Three treatments were compared (Table 1): control concentrate (17.3% CP as % of DM) was formulated to supply adequate RDP and MP according

Table 1 - Chemical composition of experimental concentrates (% Dry Matter).

Feeds	Treatments		
	17%CP	21%CP	25%CP
Ground dry corn	39.41	34.55	29.80
Citrus pulp	39.41	34.55	29.80
Urea	1.27	1.27	1.27
Soybean meal	15.08	24.80	34.30
Mineral mix[1]	4.83	4.83	4.83
Chemical composition			
Crude protein	17.3	21.2	25.0
Metabolizable energy, (Mcal kg^{-1})	**3.06**	3.10	3.14

CP = crude protein. 1- Mix Qualimix A (g kg^{-1}) = P – 55; Ca – 220; Cl – 105.5; Na – 70,0; Mg – 35; S – 22; (mg kg^{-1}) = Mn – 1.500; Fe – 500 ; Zn – 1550; Cu – 450; Co – 50; I – 40; Se – 20; Fluoride – 550; (UI kg^{-1}) = Vit A – 90000; Vit D3 – 75000; Vit E – 1000 . Solubility of P in citric acid at 2% - 90%

to NRC (2001) for the average cow (19.5 kg of milk/d); other two treatments consisted of concentrates with 21.2 and 25.0% CP were formulated to supply MP beyond NRC (2001) recommendations. To increase MP contents of the diets, soybean meal content was increased in the concentrates. For diet formulation, pasture dry matter intake (DMI) was assumed to be equal to the NRC (2001) total predicted DMI minus the concentrate DMI. Cows were fed 6.3 kg of concentrate DM, partitioned in two equal daily meals. The ten days pre-trial milk yield was 19.5 kg cow^{-1} day^{-1}, resulting in initial milk:concentrate DM ratio of 3.1:1.

Statistics

Statistical design was a 3 × 3 Latin Square, replicated four times. Cows were grouped in squares based on breed, milk yield and parity. Data were analyzed using the PROC GLM of SAS (1999). A contrast analysis was performed to detect a linear or quadratic effect of treatments (orthogonal contrast).

The statistical model was: $Y_{ijkl} = \mu + P_i + A_j + T_k + R_l + e_{ijkl}$,

where, Y_{ijkl} = observed variables; μ = general mean; P_i = effect of period $_i$, (where $_i$ = 1, 2 e 3); A_j = effect of animal $_j$, (where $_j$ = 1, 2...12); T_k = effect of treatment $_k$, (where $_k$ = 1, 2 e 3); R_l = effect of replication $_l$, (where $_l$ = 1, 2, 3 e 4); e_{ijkl} = experimental error of the observations Y_{ijkl}.

Cow Data Collection and Analysis

The 60 days of the experimental period were divided into three periods of 20 days each. The first 15 days were used for cow adaptation to the treatments and the last five days were used for data collection. Cows were milked twice a day, at 7h00 and 16h00. Milk production was recorded for every milking during the five days of the collection period. Milk samples were taken for every milking during the collection period and made composite per day for every cow. Milk samples were preserved with 2-bromo-2-nitropropane-1-3-diol, under refrigeration for the five days of collection and then used for infra-red analysis of fat, protein, lactose, total solids, somatic cell count and milk urea N.

Cows had access to pasture after the afternoon milking to the morning milking and from the morning milking to 12h00. From 12h00 to the time of the afternoon milking cows had access to a tree shaded area. Concentrate was individually fed twice a day, after and before the morning and afternoon milkings.

Blood samples from the coccigea vein or artery were taken from every cow four hours after the concentrate feeding, in the morning (around 11h00 – 12h00) of the last day of each collection period. Samples were taken on vacuumtainers with five mg potassium oxalate as anticoagulant. After collection, samples were centrifuged for 20 minutes and the plasma was stored in 2-mL ependorf flasks at – 10°C and analyzed for urea-N (Kit N 535, Sigma Chemical Co.).

Cow body weights (BW) and body condition scores (BCS), 1 to 5 scale (Wildman et al., 1982) were taken for two consecutive days at the beginning and end of each experimental period. The lowest BW and the mean BCS values were used. Concentration of chromium in feces was estimated using chromium oxide (Cr_2O_3) as external marker. A daily dose of 30 g of chromium oxide was orally fed twice a day (15 g $feeding^{-1}$), enveloped in a 100% cellulose paper during 12 days (seven days for adaptation and five collection days) according to Aroeira et al. (1999). Grab fecal samples (100 g $sample^{-1}$) were taken from every cow twice a day during the five collection days. Samples were combined by cow and period and stored at – 10ºC. Fecal chromium concentration was determined by atomic absorption analyses (Nascimento Filho, 1999).

Behavior measures of the 12 experimental cows (grazing time, ruminating time and resting time) were taken during a 24 h period, within five min intervals, during the last day of every collection period. Cow activities in the way to the milking parlor, during milking and concentrate feeding period, were not computed. Rectal temperature and respiratory frequency measures were taken on the same day of the behavior measures, at 8h00 and 15h00.

Feed Data Collection and Analysis

The quantitative measures made on pastures were: pasture forage mass (FM) pre and post first grazing group (kg DM ha^{-1}), pasture morphological composition of forage mass pre and post first grazing group, sward height (m) pre and post first grazing group and sward volumetric density (kg DM $ha^{-1}cm^{-1}$).

Forage mass pre and post grazing was measured at four randomly chosen points in the paddock, using areas of 1 m^2 sampled according to Penati et al. (2001). During every experimental period, samples were collected from the three paddocks grazed just before the five-day collection period and from the five paddocks grazed during the collection period. Forage was cut to a stubble height of 20 cm. Two 300 g sub samples were taken, the first was dried at 55ºC for 48 hours in a forced air oven, for FM calculation, and the second was used for pasture morphological composition measures. Leaves, steam and dead material from the fresh sub-samples were separated, weighted and dried at 55ºC for 48 hours in a forced air oven, to determine DM content. This was made for the subsamples taken every period, for pre and post-grazing paddocks.

Sward height was determined in 20 randomly points of the eight paddocks sampled every period. The tillers were measured from the ground to the inflection points of the leaves. Sward volumetric density (kg DM $ha^{-1}cm^{-1}$) was calculated dividing the FM before grazing by the sward height (discounting the 20-cm stubble height) according to Balsalobre (1996).

The forage samples used for chemical composition analysis were taken as hand plucked samples from eight paddocks every period. They were dried at 55ºC for 48 hours in forced air oven, ground in a Willey mill, first trough a 5 mm and then trough a 1 mm screen size. Composite samples of dry matter, organic matter (OM) and ether extract (EE) were determined according to AOAC (1975). The Neutral Detergent Fiber (NDF) and the acid detergent fiber (ADF) were determined using ANKOM 200 analyzer (*ANKOM Technology Fairport, NY*), using sodium sulphite and alfa amilase (Soest, 1994). Lignin analyses were made with addition of sulphuric acid 72% in acid detergent fiber residue according to Soest (1994).

Crude protein (CP) was determined in an N analyzer (LECO FP-2000 *nitrogen analyzer, Leco Instruments, Inc. St. Joseph, MI*). The *in vitro* DM digestibility (IVDMD) was determined using a *DAISY ANKOM* (*ANKOM Technology Fairport, NY*) (Goering & Soest, 1970). Forage TDN was calculated according to NRC (2001). Samples of concentrates were taken for every batch and checked for CP (LECO FP-2000 *nitrogen analyzer, Leco Instruments, Inc. St. Joseph, MI*) before used in the trial. Batches that did not mach the expected CP content were discharged. Concentrate samples were composite for period, dried and analyzed for DM, EE, NDF, ADF and CP determinations.

RESULTS AND DISCUSSION

Average pre grazing FM was 11,270 kg DM^{-1} ha^{-1} (Table 2), indicating the high DM production potential of tropical forages, particularly elephant grass. High DM productions (5,400 a 14,970 kg ha^{-1} per grazing cycle) for elephant grass grazed every 19 to 40 days, have been reported by several authors (Balsalobre, 1996; Rosseto, 2000; Martinez, 2004; Voltolini, 2006). Brazilian dairy farmers who intensified their grazing systems, have reached stocking rates around 6 to 12 cow ha^{-1} during 200 days $year^{-1}$ (Corsi et al., 2001; Santos et al., 2003a, 2003b).

Despite this high production, elephant grass growing in high fertility soil, during the hot rainy summer in Brazil, presented a high proportion of stems (63.55%) and low proportion of leaves (33.93%) when grazed every 37 days (Table 2). Dry matter intake may be compromised by high stem content in the forage due to the lower digestibility and sward structure, making more difficult for the cow to have access to the high quality green leaves.

Based on the amount of forage “disappearing” from the sward (an average of 2,951 kg of leave DM and 3,037 kg of stem DM), the selection capacity of the cows becomes clear, which prefer leaves instead of stems. The low proportion of leaves in the pasture, makes it necessary to offer at least 30 kg cow^{-1} of pasture DM, so the cows could have around 10 kg of available DM of high quality leaves, characterizing a low grazing efficiency.

Pre-grazing sward height (Table 3) was 1.72 m. This sward height may have negative effects on pasture DMI and grazing efficiency. For intensively managed elephant grass, as sward height increased, the grazing efficiency decreased do to the increased forage consumption during the grazing activity (Hillesheim, 1987). Higher swards are also correlated with older forage, higher stem:leafe ratio, lower pas-

Table 2 - Forage mass (FM) and morphological composition of elephant grass pasture.

Components	Periods of collection			Mean	SD[1]
	December	January	February		
FM pre-grazing, (kg DM^{-1} ha^{-1})	10,707	11,207	11,442	11,270	4,900
Leaves, (%)	34.71	32.25	34.83	33.93	6.58
Stems, (%)	62.39	64.88	63.37	63.55	6.76
Dead material, (%)	2.90	2.88	1.80	2.53	1.42
FM post-grazing, (kg DM^{-1} ha^{-1})	3,662	8,122	4,660	5,481	2,538
Leaves, (%)	12.70	19.28	15.80	15.93	4.92
Stems, (%)	83.8	77.11	64.83	75.25	4.80
Dead material, (%)	3.51	3.61	2.70	3.27	1.67

[1] = Standard deviation.

ture density and more limited animal access to high quality green leaves.

To improve the grazing efficiency and to allow the cows to have better DMI of higher quality forage the well fertilized summer elephant grass should be grazed more frequently than every 37 days. More recent data obtained in the same area suggests 1.00 m sward height as adequate for grazing elephant grass. This sward height was determined as the height when 95% of the light is intercepted by the forage. Silva (2005) has suggested the criteria based on 95% of light interception as being more adequate to graze tropical pastures than the criteria based on fixed resting periods. This resulted in shorter resting periods, that varied from 17 to 21 days during summer (Voltolini, 2006) and FM with better morphological composition (around 54% leaves and 42% stems) as compared with this trial (Voltolini, 2006; Carareto, 2007). Milk production per cow and stocking rate was higher for pastures grazed with 1.00 m height than for pastures grazed with a fixed resting period of 27 days (Voltolini, 2006; Carareto, 2007).

Pasture volumetric density is correlated with forage intake. Classical studies by Stobbs (1977) indicate that one of the major morphological aspects of the sward that could affect forage DMI is its volumetric density. In pastures with low volumetric density, animals have more difficulty to harvest the forage. This increases grazing time and may decrease DMI.

The volumetric density observed in this study was on average 74 kg DM $ha^{-1}cm^{-1}$, and is within the range reported by Balsalobre (1996), from 34.97 to 74.97 kg DM $ha^{-1}cm^{-1}$. Martinez (2004) reported for the same pasture area, a higher value during the autumn period, 98.00 kg DM $ha^{-1}cm^{-1}$. These volumetric densities are considered low, representing a possible limiting factor for pasture DMI (Stobbs, 1977).

The post-grazing FM values were high (5,481 kg ha^{-1} DM), with high proportion of stems (75.25%) and low proportion of leaves (15.93%). This means that a second group of animals grazing this area would have difficulty to reach an adequate DMI, with a consequent low performance. Again, these data suggest that this pasture should be grazed more frequently during the hot and rainy tropical summer in Brazil, as reported by Voltolini (2006).

The CP, NDF, ADF, lignin, EE and ashes (Table 4) are within the range reported by Balsalobre (1996; 2002) for tropical forages. Teixeira et al. (1999) worked with *Panicum Maximum*, cv. Tobiatã, intensively managed during the hot rainy season (10 to 12 cows/ha), grazed every 33 days, and reported a higher CP (14.6%), a lower NDF (61.6 %) and a higher *in vitro* DM digestibility (IVDMD) (77%) than the ones observed into the present study. Santos et al. (2003a, 2003b), summarized several data of nutrient composition for different tropical forage species managed intensively in south and southeast of Brazil. The authors

Table 3 - Sward height pre and post-grazing and sward density of elephant grass pasture grazing by lactating dairy cows.

Components	Periods of collection			Mean	SD[1]
	December	January	February		
Sward height pre-grazing (m)	1.62	1.72	1.77	1.72	0.09
Sward height post-grazing (m)	0.97	1.34	1.25	1.19	0.21
Density (kg DM ha^{-1} cm^{-1})	75.40	73.70	72.90	74.00	19.5

[1] = Standard Deviation.

Table 4 - Chemical composition of hand plucked samples of 37 days rest elephant grass pasture.

Components	Periods of collection			Mean	SD[1]
	December	January	February		
Dry matter	14.27	17.06	14.56	15.30	1.53
Organic matter	82.22	84.95	85.73	84.30	11.05
Crude Protein	11.53	11.88	13.01	12.14	0.77
Neutral detergent fiber	64.75	65.00	65.20	64.98	0.23
Acid detergent fiber	36.92	37.00	37.20	37.04	0.14
Lignin	3.08	2.92	2.03	2.67	**0.36**
Ether extract	2.66	2.71	2.07	2.48	0.35
Ash	17.78	15.05	14.27	15.70	1.84
IVDMD	75.43	77.30	78.14	76.96	8.00

[1] = standard deviation.

reported CP values around 14 to 18.5% and TDN values from 60 to 65% TDN. In a recent study, Fontaneli (2005) reported 19 to 22% CP for elephant grass pasture fertilized with 500 kg ha^{-1} of Nitrogen, grazed every 21-27 days.

The low TDN calculated according to NRC (2001) and those of Balsalobre (2002) do not agree with the high IVDMD reported in this trial and by Teixeira et al. (1999). The estimated TDN of 50%, would not support an average milk production of 10 kg cow^{-1}, reported by Santos et al. (2003a, 2003b) for cows grazing tropical forage (pasture DMI of 2.37% of LBW) without concentrate. Cows with this pasture DMI, would require forage TDN of at least 60% to produce around 10 kg of milk day^{-1}, according to NRC (2001).

The ash values reported in this trial (15.7% kg DM) were extremely high. Balsalobre (1996; 2002) also reported high ash values for tropical forages managed intensively, but not as much as those found here. Forage ash content has a great negative impact on forage TDN calculated using the equation incorporated by NRC (2001).

The pasture estimated TDN of 50.8% appears to be underestimated. On the other hand, the IVDMD of 76.9% kg DM may overestimate the pasture energy.

Average milk production of 18.3 kg of cows fed 6.3 kg d^{-1} of concentrate DM in this trial was lower than that reported in Santos et al. (2003a, 2003b), in which cows grazing tropical pastures milked 22.8 kg d^{-1} with 6.45 of by concentrate DM. Milk (17.88; 18.37; 18.43 kg d^{-1}) and FCM (17.37; 17.58; 17.75 kg d^{-1}) yields were not affected ($P > 0.05$) by concentrate protein content (Table 5). This indicates that the NRC (2001) was a good tool to balance protein in the concentrate for mid lactating dairy cows grazing tropical forages. According to NRC (2001), 17%CP e 21%CP concentrates supplied excessive RDP and MP to these cows.

In Brazil, most of the farmers who manage well tropical pastures, feed concentrate with 20 to 24% CP (% DM) for lactating dairy cows producing 15 to 25 kg of milk day^{-1}. Santos et al. (2003a) reported CP contents in tropical pastures higher than the 12% observed in this study. Therefore, there is a good possibility to reduce feed costs when improving the diet formulation using the NRC (2001) in the Brazilian dairy farms.

Fontaneli (2005) fed only ground dry corn and mineral mix for lactating Holstein cows, grazing elephant grass, tifton 68 or kikuiu pastures with 19 to 22% CP (% DM) during two consecutive summers. Cows produced 20 and 25 kg of milk d^{-1}, respectively. Milk fat (3.37; 3.27, 3.30%), protein (3.07; 3.12; 3.08%) lactose (4.39; 4.40; 4.41%) and total solids (11.73; 11.66; 11.70%) contents and yields were not affected ($P > 0.05$) by treatments (Table 5). Milk protein yield in high producing dairy cows (> 30 kg day^{-1}) is affected by the MP supply and by the MP amino acid profile (Schwab et al., 1976; Santos et al., 1998). Data from this trial indicate that the 17.3% CP concentrate supplied enough RDP and MP for cows milking around 18.3 kg d^{-1}, grazing tropical pastures.

Table 5 - Milk production (MP), milk composition, milk urea nitrogen, plasma urea nitrogen and chromium fecal concentration of lactating dairy cows grazing elephant grass pasture with three protein contents in the concentrate.

Component	Treatments					
	17%CP	21%CP	25%CP	SEM[4]	L[2]	Q[3]
Milk, (kg cow^{-1} day^{-1})	17.88	18.37	18.43	0.23	0.09	0.46
FCM-3.5%, (kg cow^{-1} day^{-1})	17.37	17.58	17.75	0.31	0.38	0.92
Fat, %	3.37	3.27	3.30	0.01	0.38	0.38
Fat, kg day^{-1}	0.59	0.59	0.60	0.01	0.58	0.77
Protein, %	3.07	3.12	3.08	0.03	0.72	0.29
Protein, kg day^{-1}	0.54	0.56	0.55	0.01	0.13	0.26
Lactose, %	4.39	4.40	4.41	0.06	0.44	0.95
Lactose, kg day^{-1}	0.79	0.81	0.81	0.01	0.09	0.42
Total solids, %	11.73	11.66	11.70	0.05	0.43	0.60
Total solids, kg day^{-1}	2.08	2.13	2.14	0.03	0.18	0.65
MUN, mg dL^{-1}	11.17	13.93	15.63	0.38	0.01	0.28
PUN, mg dL^{-1}	18.57	19.93	21.99	1.58	0.004	0.64
Chromium concentration, mg kg^{-1}	1.90	1.87	1.85	0.05	0.26	0.60

[1] - probability, [2] - linear effect, [3] - quadratic effect, [4] - standard error of de mean. CP = Crude Protein

Increasing concentrate CP by feeding extra soybean meal beyond the NRC (2001) recommendation increased milk urea nitrogen (MUN) linearly ($P < 0.05$) (11.17; 13.9; 15.63 mg dL^{-1}). This increase in MUN without an increase in milk yield and composition, indicates a poor utilization of this extra protein in the concentrate. The MUN observed for the control diet is in the range considered adequate for cows milking around 18 kg of milk day^{-1}. Excessive MUN concentrations indicate that nitrogen is being wasted. Reproductive efficiency can be compromised and negative environmental impacts may occur when excess protein is offered (Fergusson et al., 1988).

Plasma urea N (PUN) concentrations (18.57; 19.93; 21.99) increased linearly ($P < 0.05$) as concentrate CP increased. This is in agreement with MUN concentrations trends and confirms that feeding MP and RDP beyond NRC (2001) recommendations was excessive for cows milking around 18.3 kg of milk day^{-1}, grazing elephant grass. The PUN values for the 17%CP and 21%CP concentrates were around the critical ones for negative effects on reproductive performance (Fergusson et al., 1988).

There were no effects of treatments ($P > 0.05$) on fecal chromium concentration in feces. This suggests that DMI was not affected by treatments. Body condition score and live weight variations were not affected by treatments ($P > 0.05$) (Table 6). Cows gained 0.28 BCS (2.54 to 2.82) and 35 kg of LBW (511 to 546 kg) during the 60 days experimental period. Is was observed that cows producing around 18.3 kg of milk day^{-1}, grazing elephant grass and fed 6.3 kg of concentrate DM according to NRC (2001), were able to gain BCS during mid lactation.

The performance data observed in this trial indicate that energy and not protein is the major limiting nutrient in high production elephant grass for lactating dairy cows.

Treatments did not affect ingestive behavior of lactating cows grazing tropical pastures with 12% CP (Table 7). Grazing time, rumination and idle time were not different for the treatments ($P > 0.05$). The values presented on Table 8 are in agreement with other data for lactating cows grazing elephant grass (Lucci et al., 1972; Lima, 2002).

Cows presented three peaks for grazing, from 9h30 to 12h00, from 18h30 to 21h00 and from 2h00 to 4h00 (Figure 1). Most of the grazing activity occurred at night and early morning, when environmental temperatures were lower. Grazing activity from 9h30 to 12h00 is not common for dairy cows in tropical areas. Morning milking was initiated late, at 07h00 and delayed the cows to graze after milking.

Treatments did not affect ($P > 0.05$) cow rectal temperatures and respiratory rates (Table 8). By 8:00h cows were not under heat stress conditions, presenting a rectal temperature of 37.56ºC and a respiratory frequency of 48.53 (Table 8). These values raised dramatically at 15h00. The rectal temperature (39.3ºC) and respiratory rate (72.4) indicate that cows were heat stressed. Grazing activity from 9h30 up to 12h00 in the morning and shaded area with young trees, contributed to increase the heat stress of the cows.

Table 6 - Initial live weight (LWi), live weight variation (LWv) in periods of 20 days and initial body condition score (BCSi) and body condition score variation (BCSv) of dairy cows receiving three levels of protein in concentrate grazing elephant grass.

Component	Treatments					
	17%CP	21%CP	25%CP	SEM[4]	L[2]	Q[3]
LWi, kg	511	502	521	-	-	-
LWv, kg	13.50	8.60	14.67	5.87	0.88	0.47
BCSi, points	2.56	2.69	2.38	-	-	-
BCSv, points	0.04	0.14	0.08	0.06	0.65	0.38

[1] - probability, [2] - linear effect, [3] - quadratic effect, [4] - standard error of the mean. CP = Crude Pretein

Table 7 - Grazing, ruminating and resting time of lactating dairy cows in elephant grass pasture fed concentrates with increasing CP contents.

Component	Treatments					
	17%CP	21%CP	25%CP	SEM[4]	L[2]	Q[3]
Grazing time (hours day^{-1})	7.24	7.17	7.27	0.32	0.95	0.84
Ruminating time (hours day^{-1})	8.33	7.80	8.06	0.27	0.47	0.26
Resting time (hours day^{-1})	3.25	3.85	3.49	0.27	0.53	0.18

[1] - probability, [2] - linear effect, [3] - quadratic effect, [4] - standard error of the mean. CP = Crude Protein

Table 8 - Rectal temperature (RT) and respiratory frequency (RF) of lactating dairy cows in elephant grass pasture receiving three protein supplementations, during summer.

Component	Treatments					
	17%CP	21%CP	25%CP	SEM[4]	L[2]	Q[3]
RT (ºC)[5]	37.54	37.50	37.63	0.08	0.47	0.44
RT (ºC)[6]	39.29	39.47	39.16	0.12	0.46	0.12
RF (resp/min)[5]	49.0	50.4	46.2	2.3	0.38	0.34
RF (resp/min)[6]	71.8	72.1	73.3	2.0	0.60	0.86

[1] – probability, [2] – linear effect, [3] – quadratic effect, [4] – standard error of the mean, [5] – measured at 8h00, [6] – measured at 15h00. CP = Crude Protein

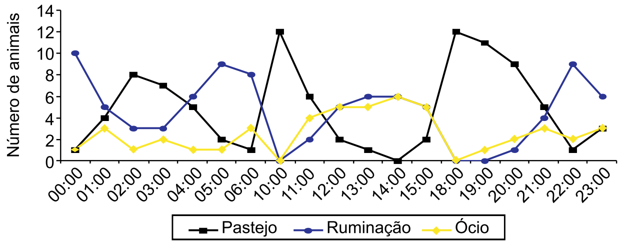

Figure 1 - Grazing, ruminating and resting times in a 24 hours period for lactating dairy cows grazing elephant grass pasture, during summer.

CONCLUSIONS

Balancing ration MP and RDP according to NRC (2001) is adequate for mid lactating cows grazing elephant grass containing 11 to 13% CP.

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