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Concentrate and crude protein levels in diets for dairy Gyr lineage cows grazing elephant-grass during the rainy season[1]

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ABSTRACT - The objective of this work was to evaluate the effects of three levels of concentrate (2.0, 4.0 and 6.0 kg/cow/day) and two levels of crude protein (CP) (14 and 16% total dietary dry matter), in comparison to mineral mixture (control) on the intake, apparent digestibility, milk composition and yield and on feed efficiency and use of concentrates of cows grazing elephant grass (*Pennisetum purpureum*, Schum) in the rainy season. Twenty-one milking Gyr cows with average body weight of 426 kg and yield of 13.0 kg of milk/cow/day at 55 days of lactation were distributed in randomized blocks design, with seven diets (treatments) in a 3 × 2 + 1 factorial arrangement and three replications, in a period of 84 days. Forage dry matter intake was not influenced by the diets, but total dietary dry matter intake increased by 45% with the inclusion of concentrate in the diet. However, milk yield increased by only 17% (1.76 kg more milk per day) with the use of concentrate. For dairy Gyr cows grazing elephant-grass during the rainy season, 2 kg of concentrate/cow/day and 14% of CP in the total diet provided the best productive response without harming body weight.

Key Words: concentrate ration, elephant grass, intake, milk, production, protein

Níveis de concentrado e proteína bruta em dietas para vacas da raça Gir linhagem leiteira sob pastejo de capim-elefante durante a época das águas

RESUMO - Objetivou-se avaliar os efeitos de três níveis de concentrado (2,0; 4,0 e 6,0 kg/vaca/dia) e dois de proteína bruta (PB) (14 e 16% da matéria seca total da dieta) em comparação à mistura mineral (controle) no consumo, na digestibilidade aparente, na produção e composição do leite e na eficiência alimentar e de utilização de concentrados de vacas sob pastejo de capim-elefante (*Pennisetum purpureum*, Schum) na época das águas. Vinte e uma vacas Gir linhagem leiteira com média de 426 kg de peso vivo e produção de 13,0 kg de leite/vaca/dia, aos 55 dias de lactação, foram distribuídas em delineamento de blocos casualizados, com sete dietas (tratamentos), em arranjo fatorial 3 × 2 + 1 e três repetições, num período de 84 dias. O consumo de matéria seca de forragem não foi influenciado pelas dietas, mas o consumo de matéria seca total aumentou 45% com a inclusão de concentrado na dieta. Entretanto, a produção de leite aumentou apenas 17% (1,76 kg a mais de leite por dia) com o uso de concentrado. Para vacas Gir linhagem leiteira em pastagens de capim-elefante na época das águas, 2 kg de concentrado/vaca/dia e 14% de PB na dieta total proporcionam melhor resposta produtiva sem prejudicar o peso corporal.

Palavras-chave: capim-elefante, consumo, leite, produção, proteína, ração concentrada

Introduction

The knowledge on nutritional requirements of animals and feed composition allow the formulation of balanced diets for several levels of production, in order to improve the utilization of nutrients by the animals. In the nutritional point of view, the challenges are to monitor the pasture for quantity and quality of the forage, the nutritional status of the milking cow, and to balance the differences in order to optimize the bio-economical efficiency of the system. In these conditions, the animal performance depends on the production and quality of the pasture, on the amount and chemical composition of the supplements, on the voluntary intake of the diet and on the capacity of response of the milking cow to the nutritional level, sanitary and reproductive management (Lopes, 2008).

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Elephant grass (*Pennisetum purpureum*, Schum) is a tropical grass with great potential of dry matter production under pasture, if the climate, soil, topography and management are favorable. During the rainy season, in samples simulating pasture intake, daily rates of elephant grass dry matter accumulation up to 100 kg/ha (Deresz, 2001) can be found. In addition to the high production of dry matter, it can be observed in research works with this forage satisfactory milk production, in the order of 15,000 kg/ha/180 days during the rainy season in crossbred Holstein × Zebu cows submitted to rotation pasture system (Deresz & Mozzer, 1994).

Another point to be evaluated is the concentrate supplementation, specially the cost:benefit ratio. In this manner, by evaluating the level of concentrate in milk production of cows maintained in pasture of elephant grass during the rainy season, it has been observed productive responses from 0.5 to 1.0 kg of milk per each 1 kg of concentrate offered to crossbred Holstein × Zebu and Holstein cows (Vilela et al., 1996; Alvim et al., 1997).

In addition to the response in milk production with the use of concentrate, it was observed in other studies curvilinear response in milk production attributed to accelerated initial increase in milk production by increase in concentrate supply to pasture fed cows, in which the marginal productive response decreased with the increase in amount of concentrate with mean value of 0.8 kg of milk per additional kilogram of concentrate (Bargo et al., 2003; Sairanen et al., 2006). In some of these studies, the milk production response to the use of concentrate was only up to 2 to 4 kg of concentrate/animal/day (Fulkerson et al., 2006).

Most of nutritional systems in use calculate the dietary requirements of energy and protein of the animals to satisfy their demands of maintenance and determined level of production. However, in practice, the situation is different; there is no need for the farmer to meet the nutritional requirements of the animal if it is against the economical interest (Lana et al., 2007). Therefore, it is evident the need for studies on animal response to increasing levels of concentrates or specific nutrients, especially in dairy cattle, such as the milking Gyr cows.

The objective of this work was to evaluate the effect of three levels of concentrate and two levels of crude protein in the diets for milking Gyr cows in elephant grass pastures during the rainy season on intake of dry matter and nutrients, coefficients of apparent digestibility, milk production and composition and efficiency of feed and concentrate utilization.

Material and Methods

The experiment was carried out in Fazenda Experimental Getúlio Vargas (FEGT), located in the municipality of Uberaba, Minas Gerais state, Brazil, which belongs to Empresa de Pesquisa Agropecuária de Minas Gerais - EPAMIG. The experiment was conducted from November 30th, 2005 to February 22nd, 2006, when the mean temperature was 24.0 ± 0.5°C, relative humidity of air of 74.3 ± 4.2% and total average precipitation of 262 ± 37 mm.

Twenty-one milking Gyr cows with an average body weight of 426 kg of body weight, daily production of 13.0 kg of milk and at 55 days of lactation (minimum of 40 and maximum of 70 days) were allotted in random blocks design, with seven diets (treatments) and three replicates as a function of milk production in total period of 84 days. During the experimental period, the animals were kept in the normal management of the farm, together with other 39 cows, which were submitted to intermittent elephant grass (*Pennisetum purpureum*, Schum) grazing, with one day of occupation and, on average, 28 days of resting (considering the performance of the forage stand). Forage availability was estimated by using the square method and pasture sampling (total collection and pasture simulation) in the beginning and on 28, 56 and 84 days of experiment. The paddocks had on average 3,000 m^2 of area with mean production of green dry matter of available forage of 6,840.9 kg/ha.

The diets were formulated with three levels of concentrate (2.0; 4.0 and 6.0 kg/cow/day in as fed basis) and two of crude protein in total dry matter (DM) of the diet (12 and 15%), but, because of the high content of crude protein of the elephant grass pasture, the total crude protein content of the diets were 14 and 16%. The diets were evaluated in comparison with one untreated control, consisting of mineral mixture, in a 3 × 2 + 1 factorial arrangement of concentrates (Table 1) in pasture of elephant grass (Table 2). The concentrates were offered at two daily portions, after each milking, individually, and the mineral supplementation was added in separate feeder, *ad libitum*.

Weighing of animals and the evaluation of body condition score were performed in the beginning of the experiment and at every 28 days, in only one day, after the morning milking. The evaluation of concentrate intake was performed daily, with supply of the proposed amount of each diet (2, 4 and 6 kg as fed basis) and next day collection of the orts for determination of the observed intake. During the experimental period, it was collected samples of

Table 1 - Composition of concentrates, on dry matter basis (% of DM)

Item	Mineral mixture	Concentrate 2 kg/day 14% Crude protein	2 kg/day 16% Crude protein	4 kg/day 14% Crude protein	4 kg/day 16% Crude protein	6 kg/day 14% Crude protein	6 kg/day 16% Crude M protein[1]
Corn		63.7	12.9	83.6	58.2	89.7	72.8
Soybean meal		29.3	80.1	12.9	38.3	7.99	24.8
Urea		3.63	3.62	1.80	1.80	1.20	1.20
Ammonia sulfate		0.48	0.47	0.24	0.24	0.16	0.16
Sugarcane molass		2.89	2.88	1.43	1.43	0.95	0.95
Mineral salt[2]	100	0.00	0.00	0.00	0.00	0.00	0.00

[1] Estimated content in the total diet.
[2] Calcium – 15.6%; phosphorus – 5.1%; sulfur – 2.0%; magnesium – 3.3%; sodium – 9.3%; potassium – 2.82%; cobalt – 0.003%; copper – 0.040%; chromium – 0.001%; iron – 0.2%; iodine – 0.004%; manganese – 0.135%; selenium – 0.002%; fluoride – 0.051%; zinc – 0.170%; vit. A – 135,000.00 I.U.; vit. D3 – 68,000.00 I.U.; vit. E – 450.00 I.U. Solubility of phosphorus at 95%.

Table 2 - Chemical composition of elephant grass pasture (*Peninsetum purpureum*, Schum) and concentrates

Item	Forage	Concentrate 2 kg/day 14% CP	2 kg/day 16% CP	4 kg/day 14% CP	4 kg/day 16% CP	6 kg/day 14% CP	6 kg/day 16% CP
Dry matter[1]	20.7	87.3	87.4	87.3	87.3	87.3	87.3
Undigestible dry matter[1]	18.9	3.05	2.81	3.15	3.03	3.18	3.10
Organic matter[1]	90.8	84.4	82.0	85.5	84.3	85.8	85.0
Crude protein[1]	12.5	29.1	47.4	17.8	26.9	14.2	20.3
Non protein nitrogen[2]	27.3	28.7	18.3	29.9	24.7	30.2	26.7
Neutral detergent insoluble nitrogen[2]	38.2	7.52	7.25	7.85	7.72	7.96	7.87
Acid detergent insoluble nitrogen[2]	8.75	3.43	2.87	3.74	3.46	3.84	3.65
Ether extract[1]	1.61	2.33	1.13	2.82	2.22	2.97	2.58
Total carbohydrates[1]	77.3	61.6	42.2	69.6	59.9	72.0	65.6
Neutral detergent fiber (NDF)[1]	70.1	13.1	12.5	13.8	13.5	14.0	13.8
Neutral detergent fiber corrected for ash and protein[1]	64.5	10.8	8.94	11.8	10.9	12.2	11.6
indigestible NDF[1]	17.6	1.27	1.17	1.34	1.29	1.37	1.33
Non-fibrous carbohydrates[1]	7.24	48.5	29.6	55.8	46.4	58.1	51.8
Non-fibrous carbohydrates corrected for ash and protein[1]	12.9	50.8	33.2	57.7	48.9	59.9	54.0
Acid detergent fiber (ADF)[1]	47.2	5.37	8.78	4.40	6.10	4.11	5.24
Indigestible acid detergent fiber ADFi[1]	11.2	0.61	0.58	0.65	0.63	0.66	0.64
Lignin[1]	4.51	1.32	1.09	1.44	1.33	1.48	1.41
Lignin/NDF	6.43	10.1	8.69	10.5	9.86	10.6	10.2

[1] Values in percentage of dry matter.
[2] Values in percentage of total nitrogen.

concentrates and pasture, stored in plastic bags in a *freezer* at -10°C for posterior analyses. At the end each 28-day period, these samples were homogenized and a composed sample was obtained per animal.

Chromium oxide was used for pasture intake estimation, supplied to the animals in the amount of 20 g/day during eight days per experimental period. The marker was stored in paper bags and put in the mouth of the animals, always after the morning milking and before concentrate supply. Fecal collection was performed during four consecutive days per experimental period, obtaining a composed sample per animal per period, and beginning five days after the start of chromium oxide supply.

The analyses of dry matter (DM), organic matter (OM), total nitrogen compounds, neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, ether extract (EE), neutral detergent fiber insoluble nitrogen (NDFIN), acid detergent fiber insoluble nitrogen (ADFIN), and minerals were carried out according to procedures described by Silva & Queiroz (2002). The non-protein nitrogen compounds were determined according to Licitra et al. (1996), whereas the contents of total carbohydrates (TC) were calculated according to Sniffen et al. (1992): TC = 100 – (%CP + %EE + % ash), and the contents of non-fibrous carbohydrates (NFC) obtained by using the formula NFC = TC – NDF.

The analyses of chromium in samples of feces were performed according to the analytical procedures described by Kimura & Dyer (1959), by using the nitropercloric digestion of chrome, and the reading was done in an atomic absorption equipment.

The fecal dry matter excretion was estimated by using chromium oxide (Burns et al., 1994; Gomide et al., 1984), being calculated based on the ratio between the amount of the furnished marker and its concentration in feces.

The indigestible acid detergent fiber (ADFi) was used as internal marker to estimate the dry matter intake, adopting the methodology described by Cochran et al. (1986). Then, it was established the ratio between the daily ingestion of internal marker (ADFi) in the concentrate plus forage and its concentration in feces. The ADFi was obtained after ruminal incubation of the concentrates, samples of pasture (forage) and feces in ankon bags (*filter bag 57*) for a period of 264 hours, according to Casali et al. (2008). It was used the equation proposed by Detmann et al. (2001) for estimation of dry matter intake per animal.

The observed total digestible nutrients values (TDNobs) were calculated for the different diets by the equation: TDNobs = DCP + (DEE × 2.25) + DNDF + DNFC, in which DCP = digestible crude protein; DEE = digestible ether extract; DNDF = digestible neutral detergent fiber; and DNFC = digestible non-fibrous carbohydrates.

Milk production evaluation was performed in the beginning, middle and end of the experimental period, by the official milk control of Associação Brasileira dos Criadores de Zebu - ABCZ. Because the cows were milking Gyr, there was a need for the calf in the milking parlor to stimulate milk secretion. Therefore, the management for evaluation of milk production followed the following procedure: the calf was placed in the milk parlor and after the first contact with the cow udder, it was separated, not allowing milk ingestion and if there was some milk ingestion, the production was not considered by a possible error for all cows. Then, all four compartments of the udder were mechanically suckled in the morning and afternoon milking.

Samples were collected for analyses of protein, fat, lactose, total dry extract, non-fat dry extract and somatic cells count in milk according to methodology described by the International Dairy Federation (1996). These samples were collected by a component attached to the milking machine, collecting 300 mL on avarage, on the 28th day of each experimental period, in the morning and afternoon milking, making composed samples, which were conditioned in plastic flasks with preservative (Bronopol®), maintained at 2 to 6ºC, and sent to laboratory of milk quality analyses of Embrapa (dairy cattle research station), in Juiz de Fora, Minas Gerais state, Brazil. Milk production (CMP) corrected for 3.5% of fat was calculated according to Sklan et al. (1992), by using the following equation: CMP = (0.432 + 0.1625 × % fat in milk) × milk production in kg/day.

The results were submitted to analyses of variance in a random block design at 5% significance, by using the program SAEG, version 8.1 (UFV, 2000). In the analyses of variance, it was used orthogonal contrasts according to the following statistical model:

$$y_{ijkl} = \mu + r_i + p_j + c_k + pc_{jk} + b_l + e_{ijkl}$$

in which: y_{ijkl} = value of dependent variable; μ = general mean; r_i = effect of concentrate ration i (1 = presence and 0 = absence); p_j = effect of crude protein level j in the diet (14 and 16%); c_k = effect of concentrate level k in the diet (2, 4 and 6 kg/day; linear and quadratic); pc_{jk} = effect of interaction between the level of crude protein j and the level of concentrate k in the diet; b_l = effect of blocks l; e_{ijkl} = random error associated with each observation.

Results and Discussion

The pasture dry matter intake in kg/day and in percentage of body weight was not influenced by the levels of concentrate and crude protein of the total diet offered to the animals (Table 3). Generally, when it is used concentrate in pasture based diet there is a substitutive effect, mainly in high level concentrate, with decrease in pasture intake of 0.32 to 0.40 kg of dry matter per kg of consumed concentrate (Bargo et al., 2003; Santos et al., 2008). On the other hand, Peyraud & Delaby (2001) related that the amount of furnished concentrate has not presented consistent data related to replacement rate, even in great amount of concentrate.

The high dietary protein (16 versus 14% of CP), reached by the use of different crude protein contents in the concentrates, did not influence the pasture intake, following the same standard of some works with elephant grass. According to Pereira et al. (2009), the crude protein concentration in the supplements apparently has low influence in the pasture dry mater intake, especially when the pasture crude protein level is higher than 6%, in which the concentrate with low crude protein content can be preferentially used.

When evaluating the total dry matter intake by contrast with and without concentrate, it can be observed that the concentrate allowed increase in total dry matter intake of 4.29 kg or 0.8% in body weight, which means an increase of 45 and 33%, respectively. Bargo et al. (2003) in a review work, verified that the use of concentrate increased the total dry matter intake from 10 to 50%.

The inclusion of concentrate allowed increase of 34% in intake of total digestible nutrients (TDN) in relation to animals of the control diet (Table 3). The productive response of animals maintained in well managed pastures is by

Table 3 - Mean daily intake of nutrients in the forage and concentrate

Item	Concentrate		Concentrate level (kg/cow/day)			Dietary CP (% DM)		Significant Contrasts (P<0.05)[1]	CV	EQ[2]
	Without	With	2	4	6	14%	16%			
	kg/day									
Forage	9.5a	10.4a	11.0	9.9	10.4	10.2A	10.6A	-	16.5	ns
Concentrate	0.0b	3.35a	1.69	3.38	5.05	3.29A	3.41A	1 and 3	5.08	1
Dry matter	9.5b	13.8a	12.7	13.3	15.3	13.5A	14.0A	1 and 3	13.6	2
Crude protein	1.19b	2.06a	2.02	2.00	2.16	1.87B	2.25A	1 and 5	12.2	ns
Ether extract	0.15b	0.25a	0.21	0.24	0.30	0.26A	0.25A	1 and 3	12.4	3
Organic matter	8.6b	12.3a	11.4	11.9	13.6	12.1A	12.5A	1	13.7	ns
Neutral detergent fiber (NDF)	6.65a	7.76a	7.96	7.86	7.72	7.62A	7.90A	-	15.8	ns
Total carbohydrates	7.3b	10.2a	9.4	9.9	11.4	10.2A	10.2A	1 and 3	13.9	4
Non-fibrous carbohydrates	0.69b	2.45a	1.45	2.45	3.48	2.57A	2.35B	1, 2, 3 and 5	8.1	5
Total digestible nutrients	6.47b	9.44a	8.99	8.97	10.4	9.17A	9.71A	1 and 3	15.5	ns
	% BW									
Forage	2.40a	2.42a	2.60	2.34	2.32	2.37 A	2.47A	-	14.0	ns
Concentrate	0.0b	0.78a	0.41	0.81	1.13	0.77 A	0.79A	1 and 3	9.53	6
Dry matter	2.40b	3.20a	3.00	3.15	3.45	3.14 A	3.26A	1 and 3	11.6	7
Crude protein	0.30b	0.48a	0.48	0.47	0.48	0.43 B	0.53A	1 and 5	10.5	ns
Total digestible nutrients	1.63b	2.19a	2.13	2.12	2.33	2.13 A	2.26A	1	12.7	ns

Different lower case letters for concentrate and capital letters for protein in the same row differ at 5% significant level by F test.
[1] Contrasts: 1 - Conc (with vs. without concentrate); 2 - CL × CP (interaction between concentrate and crude protein levels in the diet); 3 - L (linear effect of concentrate level); 4 - Q (quadratic effect of concentrate level); 5 - CP (level of crude protein in the diet); CV = coefficient of variation; EQ = regression equation.
[2] Equations: 1 – $\ddot{Y}$ = 0.6578 + 0.7758CL (r^2 = 0.92); 2 – $\ddot{Y}$ = 11.4803 + 0.654753CL (r^2 = 0.92); 3 – $\ddot{Y}$ = 0.17546 + 0.0216963CL (r^2 = 0.99); 4 – $\ddot{Y}$ = 8.54799 + 0.477761CL (r^2 = 0.92); 5 – $\ddot{Y}$ = 0.902018 + 0.445112CL (r^2 = 1.0); 6 – $\ddot{Y}$ = 0.199 + 0.1662CL (r^2 = 0.87); 7 – $\ddot{Y}$ = 2.81154 + 0.111862CL (r^2 = 0.96).

satisfaction of protein plus energy requirements in relation to isolated protein requirement by using supplement based on corn, soybean meal and urea (high intake supplements) or supplements rich in urea and mineral salt (low intake supplements), respectively. However, the efficiency of use of energetic concentrate is lower inasmuch as it demands great amount of supplementation (Lana et al., 2007).

Reeves et al. (1996) conducted a study with the objective of determining if cows maintained in a well managed tropical pasture with quicuio grass (*Pennisetum clandestinum*) with 20.5% of crude protein, would be more responsive to supplementation with sources rich in energy or in non-degradable ruminal protein (NDRP). Four levels of isoenergetic concentrates were given: 0, 3, 6 and 9 kg/cow/day. The concentrate had barley grain and canola meal treated with formaldehyde (rich in NDRP). The results showed that the milk production (17 to 23 kg/cow/day) was influenced by supply of energy and not by increase in NDRP in the diet; however, the mean of kg of milk/kg of concentrate decreased.

The utilization of increased levels of concentrate and CP did not influence the apparent digestibility of dry matter (Table 4). It was observed quadratic effect of concentrate in the apparent digestibility of dry matter (P<0.05), estimating the minimum value in the level of 3.37 kg of concentrate per day. In experiment with milking Gyr cows in confinement, also evaluating levels of inclusion of concentrate ration in the diets, there was also quadratic effect in the digestibility coefficient of dry matter (Teixeira et al., 2010).

The mean digestibility of forage in the presence of concentrate ration was lower in relation to the absence of concentrate. The addition of concentrates in the diet of ruminants can cause reduction in ruminal digestibility of fiber due to increase in the proportion of highly fermentable carbohydrates and the consequent reduction in pH of the ruminal environment, that sensible decrease the activity of fibrolytic bacteria. Levels of pH between 6.5 and 6.8 are the most appropriate for the activity of most ruminal bacteria (Grant & Mertens, 1992).

The digestibility coefficient of crude protein of diets with 16% CP was greater than that with 14% CP (P<0.05). The concentrates given to animals in the 16% CP diets presented higher soybean meal content than the concentrates in the 14% CP diets. Because soybean meal is rich in ruminal degradable protein (RDP), the ingestion of this kind of protein by the animals fed diets with 16% CP was greater. Thus, the increase in the apparent digestibility of crude protein could be a result of the greater intake of RDP in the concentrate (Valadares Filho et al., 2000; Broderick, 2003).

Regardless to the increase in concentrate level given to the animals (2, 4 and 6 kg/day), the crude protein intake was the same (Table 3). It can be observed from the concentrate composition (Table 1), that the content of crude protein and soybean meal decreased as the amount of concentrate increased in the diets, which means that the intake of ruminal degradable protein also decreased with the increase of concentrate levels in the diets, which might be a possible explanation for the decrease in the coefficient of digestibility of crude protein.

The use of concentrate and the increase in crude protein content of the diet did not influence the neutral detergent fiber digestibility whereas the increase in the concentrate caused a quadratic response (P<0.05). Overall, the observed behavior for the total digestion of NDF in condition of increasing level of concentrate is decreasingly linear because of the depression of ruminal fiber digestibility by decrease in ruminal pH, mainly when there is substitution effect of forage by concentrate (Dias et al., 2000; Véras et al., 2008).

There was no effect of crude protein level on the content of observed total digestible nutrients (TDN) and TDN intake. This can be nutritionally positive due to diets with low crude protein level be less expensive. The level of concentrate still caused a quadratic effect (P<0.05) in the content of observed TDN, with minimum estimated value of 3.81 kg of concentrate per day. This result is also similar to the observed with milking Gyr cows in confinement with increasing levels of concentrate and crude protein in the total dietary dry matter (Teixeira et al., 2010).

By comparing milk production of animals which received concentrate with animals kept exclusively in pasture, it was observed that there was increase of 1.76 kg/day of milk production (P<0.05) with use of concentrate (Table 5). This was probable caused by supply of nutrients by concentrate inasmuch as a minimum of concentrate need to be used when working with animals raised in good pastures during the rainy season. The mean concentrate intake was 3.35 kg/day, which is in accordance with Mattos (1995), who stated that

Table 4 - Digestibility coefficients of dry matter and other constituents of the diets

	Concentrate		Concentrate level (kg/cow/day)			Dietary CP (% DM)		Significant Contrasts (P<0.05)[1]	CV	EQ[2]
Item	Without	With	2	4	6	14%	16%			
Dry matter	58.0a	60.5a	61.8	57.6	62.2	60.4A	60.7A	4	6.5	1
Forage	58.0a	47.3b	55.8	42.3	43.7	47.0A	47.6A	1 and 3	13.2	2
Crude protein	59.1a	59.9a	65.2	56.4	58.0	58.0B	61.8A	3 and 5	6.4	3
Ether extract	26.5a	33.7a	33.2	23.9	43.9	41.9A	25.4B	4 and 5	27.2	4
Neutral detergent fiber	63.2a	63.1a	65.1	59.7	64.3	62.8A	63.3A	4	6.2	5
Total carbohydrates	62.2a	63.5a	65.1	60.7	64.6	63.3A	63.7A	4	5.8	6
Non-fibrous carbohydrates	52.9b	64.6a	65.1	63.5	65.3	65.2A	64.0A	1	12.9	ns
Organic matter	60.9a	61.6a	63.9	58.5	62.5	61.4A	61.9A	4	6.0	7
Observed total digestible nutrients	56.5a	57.5a	59.8	54.5	58.3	57.5A	57.5A	4	5.9	8

Different small letters for concentrate and capital letters for protein in the same row differ at 5% significant level by F test.

[1] Contrasts: 1 – Conc (with vs. without concentrate); 2 – CL × CP (interaction between concentrate and crude protein levels in the diet); 3 – L (linear effect of concentrate level); 4 – Q (quadratic effect of concentrate level); 5 – CP (level of crude protein in the diet); CV = coefficient of variation; RE = regression equation.

[2] Equations: 1– $\ddot{Y} = 64.7848 – 3.38879CL + 0.502458CL^2$ ($R^2 = 1.0$); 2 – $\ddot{Y} = 56.8185 – 2.72264CL$ ($r^2 = 0.67$); 3 – $\ddot{Y} = 64.5253 – 1.33051CL$ ($r^2 = 0.60$); 4 – $\ddot{Y} = 58.5029 – 20.2875CL + 3.04360CL^2$ ($R^2 = 1.0$); 5 – $\ddot{Y} = 68.8953 – 4.16604CL + 0.576502CL^2$ ($R^2 = 1.0$); 6 – $\ddot{Y} = 68.6169 – 3.72939CL + 0.521583CL^2$ ($R^2 = 1.0$); 7 – $\ddot{Y} = 67.7774 – 4.15456CL + 0.554187CL^2$ ($R^2 = 1,0$); 8 - $\ddot{Y} = 63.9639 – 4.27612CL + 0.561720CL^2$ ($R^2 = 1.0$).

Table 5 - Milk production and composition, body weight, daily variation in body weight and body score of milking cows in pasture of elephant grass during the rainy season

	Concentrate		Concentrate level (kg/cow/day)			Dietary CP (% DM)		Significant Contrasts (P<0.05)[1]	CV	RE
Item	Without	With	2	4	6	14%	16%			
Milk production (kg/day)	10.3b	12.0a	11.6	11.8	12.6	11.6A	12.4A	1	9.9	ns
Corrected milk production (kg/day)	11.3a	12.5a	12.6	12.1	12.8	12.7A	12.3A	-	11.2	ns
Fat (%)	4.12a	3.81a	4.10	3.64	3.68	4.13A	3.49A	-	15.6	ns
Protein (%)	3.39a	3.38a	3.36	3.37	3.41	3.33A	3.43A	-	6.1	ns
Lactose (%)	4.50a	4.54a	4.60	4.53	4.51	4.54A	4.56A	-	3.1	ns
Dry extract (%)	13.2a	12.8a	13.2	12.6	12.7	13.1A	12.6A	-	6.0	ns
Non fatty dry extract (%)	9.09a	9.04a	9.09	9.00	9.03	9.01A	9.07A	-	2.8	ns
Plasma urea nitrogen (mg/dL)	6.7b	14.5a	14.9	14.2	14.5	13.6A	15.4A	-	21.8	ns
Initial body weight - BW (kg)	398a	430a	426	423	443	432A	429A	-	9.9	ns
Final BW (kg)	393a	429a	422	420	446	432A	427A	-	9.5	ns
Daily variation in BW (kg)	-0.14a	0.00a	-0.06	0.01	0.05	-0.01A	0.01A	-	183.0	ns
Initial body condition score	4.00a	4.64a	4.66	4.25	5.00	4.44A	4.83A	-	30.4	ns
Final body condition score	3.87a	4.81a	4.65	4.33	5.46	4.67A	4.95A	-	24.2	ns

Different small letters for concentrate and capital letters for protein in the same row differ at 5% significant level by F test.

[1] Contrasts: 1 – Conc (with *vs.* without concentrate); 2 – CL × CP (interaction between concentrate and crude protein levels in the diet); 3 – L (linear effect of concentrate level); 4 – Q (quadratic effect of concentrate level); 5 – CP (level of crude protein in the diet); CV = coefficient of variation; RE = regression equation.

the amount of concentrate used for forage supplementation has to be attentivaly observed because of its high cost, and because only the first 2.0 to 4.0 kg allow great response in milk production.

There was no effect of crude protein content in the diet (14 × 16%) nor concentrate level on milk production (P>0.05). With this result, it is recommended for milk production the utilization that allow 14% CP in the diet, and in low amount, for dairy Gyr cows maintained in elephant grass pasture during the rainy season. The increase in crude protein content in the total diet above the requirements of animals is not a good strategy for increasing milk production in animals kept in well managed tropical pastures. In this case, the ingestion of metabolizable energy is a greater limitation for milking cows in pastures (Davison & Elliott, 1993; Reeves et al., 1996; Santos et al., 2008).

Voltolini (2010) evaluated the protein adjustment in rations of cows in pasture or receiving sugarcane and observed that cows in elephant grass pasture with 12% CP in DM, with daily mean production of 18.5 kg of milk did not respond to as fed crude protein content in the concentrate above 15.8%. Pereira et al. (2009) maintained milking Holstein × Zebu cows in elephant grass pasture and supplemented with 6.0 kg/cow/day of concentrate with 15.2; 18.2 and 21.1% of CP and did not find difference in the intakes of pasture and total dry matter, nor in milk production and composition and concluded that concentrate with the lowest crude protein content could be preferable used.

When milk production was evaluated by comparing the increasing levels of concentrate, a quadratic effect was observed (Figure 1). The saturation kinetics model of Michaelis-Menten indicates that by increasing supply of nutrients, there is an effective reduction in nutrients utilization in a hyperbolic curve and this allow explain the curvilinear response in body weight and milk production by increasing the amount of concentrate (Lana et al., 2005).

According to the data presented in Table 5, there was no significant difference for the contents of fat, protein, lactose, dry extract and non fatty dry extract as a function of increasing levels of concentrate and crude protein in the diet. Increases in milk solids can occur primarily when the animals are not receiving balanced diets in fibrous carbohydrates and crude protein, as example, and then start receiving a balanced diet (Clark et al., 1992; Pereira et al., 2001). Thus, it can be inferred that elephant grass pasture already supplied nutrients to meet certain amounts of solids composition in milk and, because of that, the utilization of concentrates and increasing crude protein levels in the diet were not able to cause changes in milk composition. Genetic factors also have to be considered in milk solids composition because each breed has its peculiarity in milk solids.

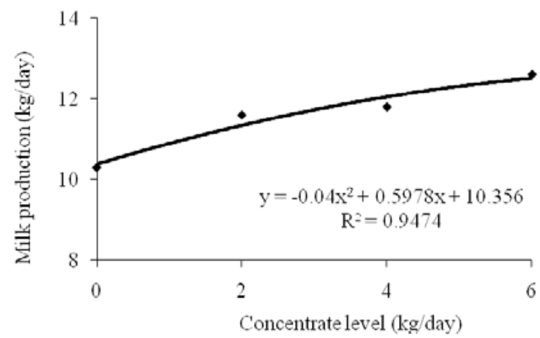

Figure 1 - Productive response of dairy Gyr cows in elephant grass pasture during the rainy season.

The use of concentrate increased plasma urea nitrogen (PUN; P<0.05; Table 5) from 6.69 mg/dL in animals maintained only on pasture to 14.5 mg/dL in concentrate fed animals. PUN concentration presents elevated positive correlation with the dietary crude protein level (Broderick & Clayton, 1997) and ruminal degradable protein (Oliveira et al., 2001). Increase in concentrate intake caused increase in crude protein intake and consequently in plasma urea nitrogen. However, PUN level greater than 19 to 20 mg/dL causes losses of dietary nitrogen in dairy cows (Oliveira et al., 2001). Valadares et al. (1997a) found values from 14 to 16 mg/dL of PUN for Zebu steers, representing limits out of which could occur losses of dietary protein. These values correspond to maximum microbial efficiency. Therefore, it can be inferred that the total dietary crude protein level (14 and 16%) allowed values of PUN of 13.64 and 15.40 mg/dL, respectively, which is within the acceptable limit for increasing microbial protein production.

The use of concentrate with excessive CP content results in high urea nitrogen in plasma and milk. This can impair the reproductive performance of cows and increase the energy requirements inasmuch as it is necessary 13.3 kcal of digestible energy to excrete one gram of nitrogen. Generally, protein rich concentrates are expensive and a great amount of excreted nitrogen can generate negative environmental impacts (Santos et al., 2008).

The efficiency of milk production and corrected milk production presented significant reduction (P<0.05) as a function of dry matter intake (Table 6). In the first case, the efficiency changed from 1.09 kg of milk/kg of dry matter in the control diet (zero concentrate) to 0.88 kg of milk/kg of dry matter with utilization of concentrate, without effect of concentrate and protein levels. The use of concentrate allowed increase in dry matter intake of 45% (Table 3) and increase in milk production (Table 5) of 17%, explaining

Table 6 - Efficiency of milk production and productive response of milking cows to use of concentrate in the diet

Item	Concentrate		Concentrate level (kg/cow/day)			Dietary CP (% DM)		Significant contrasts (P<0.05)[1]	CV	RE[2]
	Without	With	2	4	6	12%	15%			
Milk production/dry matter intake	1.09a	0.88b	0.91	0.91	0.81	0.86A	0.90A	1	15.2	ns
Corrected milk production/ dry matter intake	1.20a	0.92b	0.99	0.93	0.83	0.95A	0.89A	1	17.0	ns
Milk production/forage intake	1.09a	1.17a	1.05	1.24	1.21	1.14A	1.19A	-	18.6	ns
Milk production/concentrate intake	10.27a	4.32b	6.90	3.52	2.53	4.32A	4.31A	1 and 3	11.5	1
Productive response[3]	-	-	0.82	0.47	0.46	-	-			

Different small letters for concentrate and capital letters for protein in the same row differ at 5% significant level by F test.
[1] Contrasts: 1 – Conc (with *vs.* without concentrate); 2 – CL × CP (interaction between concentrate and crude protein levels in the diet); 3 – L (linear effect of concentrate level); 4 – Q (quadratic effect of concentrate level); 5 – CP (level of crude protein in the diet); VC = coefficient of variation; RE = regression equation.
[2] Equation: 1 = $\hat{Y} = 7.81539 - 0.999507NC$ ($r^2 = 0.91$).
[3] Differential of milk production/differential of concentrate intake in dry matter for the levels 2, 4 and 6 kg/day in relation to the control diet (zero concentrate).

this result. This show that, for milking Gyr cows kept in elephant grass pasture during the rainy season, the concentrate utilization has to be done carefully, both in protein level and in the amount used.

The relationship between milk production and forage intake was not affected by the diets, but the milk/concentrate ratio decreased by 0.9995 kg/kg with increase in concentrate level (P<0.05; Equation 1; Table 6). This result can be explained by the productive response of milk as a function of additional concentrate intake above the control diet, which decreased as the concentrate level increased. This observance has been found in several publications (Gomide, 1993; Bargo et al., 2003; Oliveira et al., 2007), which observed a decreasing increment in milk production as response to increase in concentrate intake. These data showed low efficiency of bovines in producing milk when replacing forage by concentrate, especially in high concentrate levels (Lana, 2005). In this study, there was no substitution of forage by concentrate, being evident that the decreasing response in milk production by increase in concentrate ration in the diets of milking Gyr cows was not caused by the replacement effect reported by Moore et al. (1997).

Conclusions

Use of concentrate up to 6 kg/cow/day in diet for dairy Gyr cows kept in elephant grass pasture during the rainy season has no substitution effect in forage intake, increasing the total dry matter intake in an addictive manner. Milking Gyr cows kept in pasture of elephant grass during the rainy season have better productive response when submitted to supplementation with concentrate at the level of 2 kg/day in diets with 14% CP.

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