



Effects of supplementary concentrates and voluntary forage intake on grazing cattle in pastures of *Cynodon plectostachyus* in a semi-tropical region of Mexico

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ABSTRACT

The objective of the present study was to evaluate the effects of different levels of concentrate on the productive response of grazing dairy cattle and to determine voluntary forage intake. The experiment was carried out in a 4×4 Latin square design repeated three times, in which different inclusion levels of experimental concentrate were evaluated (EC) vs commercial concentrate (CC). The treatments were as follows: EC1= 7.12 kg DM of EC + grazing, EC2=6.23 kg DM of EC+ grazing, EC3=5.34 kg DM of EC+ grazing, CC=7.12 DM kg of a commonly used commercial concentrate (CC), and free-access grazing. The voluntary intake was determined through n-alkanes technique. The variables evaluated in cattle were milk output, live weight, and body condition; milk samples were also taken to determine protein and fat contents. Net herbage accumulation (NHA), forage height, neutral detergent fiber (NDF), acid detergent fiber (ADF), digestibility of organic matter (DIVMO), and metabolizable energy (ME) of pasture grasses were evaluated in addition to voluntary intake and production costs. Significant differences in crude protein content were found between the evaluation periods but were not found for NDF, ADF, DIVMO, NHA, and ME. Significant differences were not found in voluntary intake but were present in total intake. The evaluated treatments did not differ with respect to animal response. Finally, significant differences were found in milk output. Greater milk production was obtained in treatments 1 and 2 (14.92 and 14.50 kg/day/animal, respectively).

Keywords: Concentrates, Dairy cows, Grazing, Intake, N-alkanes

The semi-tropical region of Mexico is characterized by dual-purpose cattle production systems wherein the sale of milk is the main source of income (Albarrán-Portillo *et al.* 2015). As in other regions with semi-tropical climate, the feeding regime is based on grazing on pastures of African stargrass (*Cynodon plectostachyus*), (Mislevy and Martin, 2006), and high levels of supplementary commercial concentrates (6 to 9 kg/cow/day) are added to cattle diets to maintain milk production (Hernández *et al.* 2013), especially in the dry season, when the production and quality of the grass decrease (Absalón-Medina *et al.* 2012). However, these concentrates increase production costs and threaten the long-term sustainability of this production system.

In addition, grass quality is low most of the year in these

production systems (Grimaud *et al.* 2006). One of the related challenges of raising cattle in these systems is therefore determining optimal forage intake. Different methods have been used to estimate voluntary intake of animals. One of these methods involves the use of n-alkanes and is based on the relationship between fecal concentration of an n-alkane naturally present in the diet (internal marker) and another orally administered n-alkane (external marker) (Dove and Mayes 2005, Pedraza-Beltrán *et al.* 2012, Estrada-López *et al.* 2014). Few reports on adequate levels of supplementation and voluntary cattle intake are available despite the importance of these production systems, their contribution toward national milk production (28%), and the number of jobs generated (SIAP 2017). The use of commercial concentrates increases feed costs in small-scale dairy systems. For this reason, using inputs produced in the same farm (such as maize and molasses), and others that can be obtained in the same region, allow that the producer make their own concentrates at a lower price (Pedraza-Beltrán *et al.* 2012, Estrada-Flores *et al.* 2014). One contribution of this work was the decrease in the use of concentrates by producers, who were using before work up to 10 kg of concentrate. Therefore, the objective of the present study was to evaluate the productive response of

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grazing cattle supplemented with different levels of an experimental concentrate and to determine the voluntary intake of animals in a pasture of African stargrass within a rural milk production system in a semi-tropical region.

MATERIALS AND METHODS

The study was carried out in the municipality of Tejupilco in the semi-tropical southwestern region of the State of Mexico (18° 45' 30" and 99° 59' 07") at an altitude of 1,340 masl.

Treatments: An experimental concentrate (EC) was formulated according to the AFRC (1993), to cover the needs that the grass may not cover taking in account the weight of the cows and the nutritional quality of this type of tropical grass, was composed of 60% maize, 27% soy, 10% molasses, and 3% urea, two of the ingredients are produced in the same farm (maize and molasses), the other two are easily achieved in the study region. The commercial concentrate was composed of ground rolled grains and their by-products, cotton seed, oilseed pastes, surplus fat. NNP, molasses, citrus pulp, common salt, ionophore; minerals: calcium, phosphorus, iodine of citrus, zinc, selenium. and cobalt; vitamins A, D and E.

The treatments were as follows: EC1= 7.12 kg DM of EC, EC2= 6.23 kg DM of EC, EC3= 5.34 kg DM of EC, CC= 7.12 DM kg of a commonly used commercial concentrate (CC), and free-access grazing. The treatments were formulated to cover most of the needs of the animals that the grass does not cover (AFRC, 1993). Half of the concentrate of each treatment was provided in the milking in the morning and the other half in the afternoon milking.

Experimental design: The experiment was carried out under a rural participatory framework (Conroy 2005) in three dual-purpose dairy production units. A 4×4 Latin square experimental design was used and repeated three times; the factors were 12 crossbred ZebuxHolstein cattle of second parity in the first third of lactation. Animals had an initial weight of 490±13 kg and an initial milk production of 12±1.4 kg.

The study lasted for 84 days that were divided into four experimental periods of 21 days each. Animals adapted to the experimental diet during the first 14 days, and samples were collected during the final seven days. In the experimental design the columns were the periods, the rows the cows, the sequence of the treatments in the squares and the assignment of cattle to treatments were decided at random.

The evaluated variables were milk production and initial cattle weight in addition to body condition and voluntary intake at the end of each experimental period. Milk samples were taken weekly to determine protein and fat contents.

The cattle continuously grazed and had free access to water. Each field had an extension of two hectares. Milking was performed manually two times per day (4:00 and 16:00 h); at this time, animals were fed supplementary concentrates. Cattle were weighed at the beginning and end of each experimental period, and body condition was

recorded according to the technique described by Rodenburg (2000).

Chemical analysis of African stargrass and concentrates: African stargrass was sampled using a simulated grazing technique during the seven measurement days of each experimental period. This involves hand plucking the forage to a similar height to that produced by grazing cattle. These samples were dried in an oven at 60°C until obtaining a constant dry weight and were ground. The digestibility dry matter (DIVMO) was determined according to the Daisy method (Ankom Technology 2004). Protein content was determined via the Kjeldahl method (AOAC 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using the ANKOM micro-bag method (Ankom Technology) by following the procedure of Van Soest *et al.* (1991). The metabolizable energy (ME) of the concentrate and African stargrass was estimated using the formula proposed by the AFRC (1993), as shown at following equation (1):

$$\text{ME (MJ/kg DM)} = (0.0157) (\text{DIVMO}) \dots(1)$$

The net herbage accumulation (NHA) was determined according to the method proposed by López-González *et al.* (2015), which is outlined by the following equation (2):

$$\text{NHA} = \text{DMf} - \text{DMi} \dots(2)$$

where NHA, net herbage accumulation (kg DM/ha); DMi, initial average weight of herbage outside of a cage at day 0 (kg DM/ha); DMf, final average weight of herbage inside of the cage at day 28 (kg DM/ha).

Estimation of voluntary intake: To determine voluntary intake, each animal was administered one controlled-release capsule (Captec Ltd, Auckland, New Zealand) at the beginning of each of the four experimental periods. The capsules contained alkanes C32 (n-Dotriacontane) and C36 (n-Hexatriacontane). A capsule was introduced with a gun (Lance bolos) within the rumen for each cow, they have 8 g of dotriacontane (C32) and 8 g of hexatriacontane (C36), and are designed to release 400 mg of both markers for a period of 20 days. During the seven evaluation days, a fecal sample of each animal was taken in the morning and afternoon directly from the rectum to determine the rate of alkane excretion with respect to levels normally present in the diet and the administered dose. Once milled, the samples of the morning and afternoon were mixed. Daily samples of African stargrass were also taken from the area where cattle were feeding.

To determine the type and concentration of n-alkane, gas chromatography was used according to the technique of Dove and Mayes (2005). For the extraction of n-alkanes 0.1 g of feces and 0.2 g of forage were weighed in duplicate into a threaded test tube and 0.11 g was added a solution composed of 0.3 mg/g n-docosane (Alltech) and 0.3 mg/g of n-tetatriacontane (Alltech) in heptane, and were used as internal standards. To each tube was added 1.5 ml of a 1M solution of potassium hydroxide in ethanol, the tubes were capped and heated at 90°C for 4.5 h in a Maria bath

with vigorous stirring every 20 minutes, after a partial cooling of 50–60°C, 1.5 ml of n-heptane and 0.4 ml of deionized water were added to each tube, centrifuged at 3,000 rpm for 10 minutes, the organic phase was subsequently collected and deposited in another clean tube, repeating the extraction again. Finally combining both fractions and evaporating the n-heptane by direct current of N₂. To the pellet was added 3.3 ml of n-heptane and filtered through a 5 cm column of silica gel. The gas chromatograph conditions were taken from Dove and Mayes (2003), using a capillary column (Elite 30 m × 0.53 mm internal diameter and 0.53 mm film thickness), adapted to a gas chromatograph model Claurus 500, manufactured by perkin Elmer (Connecticut, USA) with automatic injector and flame detector Hydrogen gas was used as a carrier gas at a flow of 1.8 ml/min split of 1:3, the injection conditions were: initial temperature 280°C sustained for 1 minute, 30°C/min. up to 320°C for 1 minute, Oven: 280°C for 3 min, 5°C/min up to 320°C for 9 min, detector: 350°C the injection volume was 0.5 µl, total run time of 23 minutes. Quantification of n-alkanes was performed using the internal standard technique (C34) and reference standard (C22) DMI was calculated according to the Dove and Mayes (2005) equations.

$$I \text{ (Kg/day)} = \frac{\text{Dose rate}_j}{\frac{F_i}{F_j} \times H_i - H_j}$$

where, F_i y H_j (mg/kg MS), Concentrations of odd alkane in faeces and forages F_j y H_j (mg/kg MS), Alkane concentrations in feces and forage par respectively.

Statistical analysis: To assess the productive response, a statistical design of 4×4 Latin squares repeated three times was used, and the analysis was based on a general linear model approach, as follows:

$$Y_{ijkl} = \mu + S_i + C_{(j)} + P_k + T_l + e_{ijkl}$$

where μ , overall average, S, effect due to square; i, 1, 2, 3; C, effect due to cows within squares; j, 1, 2, 3, 4; P, effect due to experimental period k, 1, 2, 3, 4; T, effect due to treatment l, 1, 2, 3, 4; and e, residual error term.

The variables in the field were analyzed based on a

Table 1. Chemical analysis, energy content, and digestibility of organic matter of concentrates used in the experiment

Variable	Experimental concentrate	Commercial concentrate
Chemical composition		
CP (g/kgDM)	200.80	180.00
NDF (g/kgDM)	213.56	315.92
ADF (g/kgDM)	90.15	131.24
DOM (g/kgDM)	930.50	825.10
ME (MJ/kgDM)	14.60	12.90

CP, crude protein content; NDF, neutral detergent fiber; ADF, acid detergent fiber; DOM, digestibility of organic matter; ME, metabolizable energy.

completely randomized design defined by the following mathematical model:

$$Y_{ij} = \mu + t_i + e_{ij}$$

where Y_{ij} , response variable; μ , overall average, t_i , effect of the experimental period (1, 2, 3, 4), and e_{ij} , residual error term.

RESULTS AND DISCUSSION

The results of the laboratory analysis performed on the two concentrates used in the experiments are shown in Table 1.

The results for chemical composition, DOM, and ME of *Cynodon plectostachyus* are presented in Table 2. Only the content of crude protein showed significant differences between the experimental periods ($P < 0.05$), decreasing in periods 3 and 4.

Overall, the protein content decreased over the course of the evaluation periods (Table 2). As grass enters into a mature stage, crude protein content decreases while structural carbohydrate content increases (Jarillo-Rodriguez *et al.* 2011), was a decrease in crude protein was observed as structural carbohydrates increased in tropical native grassland. Dean *et al.* (2008) similarly mentioned that crude protein content decreases and structural carbohydrate content increases as grasses mature, although without significant differences between the evaluation periods. Furusawa *et al.* (2013) found that the nutritional quality of

Table 2. Chemical composition, metabolizable energy, and digestibility of organic matter of African stargrass (*Cynodon plectostachyus*) per experimental period

Variable	1	2	3	4	Mean	SEM
CP (g/kg DM)	105.87 ^a	94.74 ^b	81.53 ^c	81.09 ^c	90.80	1.83
NDF (g/kg DM)	750.52	771.86	778.00	775.83	769.05	9.84
ADF (g/kg DM)	341.09	350.56	351.87	369.79	353.32	10.94
DOM (g/kg DM)	562.57	520.94	501.67	506.24	524.10	4.20
NHA (kg DM/ha)	1363.63	1224.70	1057.09	978.33	1155.93	14.30
ME (MJ/kg DM)	8.83	8.18	7.87	7.94	8.20	0.24

Means with different letters (a, b and c) within each column differ significantly ($P < 0.05$) 1, 2, 3, 4=evaluation periods; SEM, standard error of the mean; CP, crude protein content; NDF, neutral detergent fiber; ADF, acid detergent fiber; DOM, digestibility of organic matter; NHA, net herbage accumulation; ME, metabolizable energy.

grasses decreases over time. The results of the present study are different to those reported by López-González *et al.* (2015), who reported a crude protein content of 111.92 g/kg DM in pasture grasses, these authors evaluated three types of tropical grasses (*Cynodon plactostachyus*, *Paspalum notatum* and *Brachiaria decumbens*) and found that on an average tropical grasses had a content of crude protein of 111.92 g/kg DM.

The mean values for NDF was 769.05 g/kg DM, while the content of ADF in the pasture was 353.32 g/kg DM, the DOM was on mean 524.10 g/kg DM and an ANF of 1155.93 kg DM/ha. Although the NHA of forage decreased over time in which a forage decrease of 28% is observed in period 4 with respect to period 1. This can be explained by increasing grazing pressure. The experiment was carried out at the end of the rainy season when grasses had stopped growing or re-sprouting (Okello *et al.* 2005), even though the climate conditions, the animal load remained the same throughout the experiment.

The results for animal intake are presented in Table 3, significant differences in voluntary intake were not found ($P>0.05$) in treatments. Each animal consumed an average of 5.28 kg of dry material of forage. However, total intake did differ significantly between the evaluated treatments ($P<0.05$); a higher intake was observed in treatment 1, which differed with respect to the other treatments.

Total animal intake was significantly greater in treatment 1 with respect to the other treatments because of the higher level of supplementation and the improved characteristics of the EC (versus the CC). Although there were no differences in forage intake, the inclusion level of each treatment has an effect on total intake. Better nutritive characteristics of the concentrate, cause the animal to have a greater forage intake, as it is observed in treatment 1 (Table 3). Mayne *et al.* (2000) mention that quantity and quality of feed are factors that determine diet intake. Forage intake was not affected by the nutritive quality of the forage, as shown in Table 3, since the intake in all treatments were similar. Pedraza-Beltrán *et al.* (2012) provided 6 kg DM of experimental concentrate with four inclusion levels of coffee pulp (0, 10, 15 and 20%), obtaining total intake of

9.1 kg DM, lower than those obtained in this work. On the other hand, Pérez-Prieto *et al.* (2011), worked with different grazing assignments (low, medium and high) and determined the voluntary intake in grazing cows, these authors found intakes in average of 7.9 kg DM/cow, it is noteworthy that these intakes were made in temperate climate meadows, which in comparison with tropical grasses are more digestible.

The present results for total intake are distinct from those reported by Pedraza-Beltrán *et al.* (2012) and Pérez-Prieto *et al.* (2011), who reported total intakes of 9 kg DM/animal and 7.4 kg DM/animal, respectively.

The results for milk production are presented in Table 4. Significant differences ($P<0.05$) between the evaluated treatments were present; milk production was higher in treatments 1 and 2, which differed from treatments 3 and 4. Higher milk output was found in treatments 1 with respect to treatments 2, 3 and 4 (Table 4). Such an increase in milk production can be attributed higher protein content of the EC (Table 2), as well as higher dry matter consumed by animals, as shown in Table 3. Overall, the EC presented better chemical and nutritional characteristics in comparison to the CC commonly used by producers. The concentration of ME was also higher in EC treatments. Furthermore, the DOM was higher and associated with lower concentrations of NDF and ADF in the EC (Table 1). The quality of the forage was similar over time and contributed equally during the four periods, except for the crude protein content. However, the forage did not affect in a decisive way in milk production. The results of the present study are similar to those of Mohammed *et al.* (2016) and Granzin and Mc Dryden (2005), who reported milk production in the dry season of 14.2 kg/animal/day and 12.4 kg/animal/day, respectively, among cattle grazing on tropical grasses.

Live weight of animals did not significantly differ between the evaluated treatments ($P>0.05$), nor were significant differences in the body condition of animals observed ($P>0.05$). This latter variable was consistent across

Table 3. Feed intake of cattle during the experiment

Treatment	Forage intake (kg DM)	Concentrate intake (kg DM)	Total intake (kg DM)
EC1	5.33	7.12	12.45 ^a
EC2	5.03	6.23	11.26 ^b
EC3	5.95	5.34	11.29 ^b
CC	4.82	7.12	11.94 ^b
Mean	5.28	6.45	11.73
SEM	0.78NS	0.00NS	0.18*

Different letters in the same row indicate significant differences ($P<0.05$). EC1, 7.12 kg of experimental concentrate; EC2, 6.23 kg of experimental concentrate; EC3, 5.34 kg of experimental concentrate; CC, 7.12 kg of commercial concentrate. SEM=standard error of the mean.

Table 4. Productive response of grazing cattle to experimental supplementation of concentrates

Treatment	Milk output (kg)	Live weight (kg)	Body condition (1–5)	Fat content in milk (g/kg)	Protein content in milk (g/kg)
EC1	14.92 ^a	476.00	2.5	33.34	30.23
EC2	14.50 ^a	465.00	2.5	33.35	30.61
EC3	12.90 ^b	474.00	2.5	33.12	30.92
CC	12.79 ^b	468.00	2.5	33.31	30.78
Mean	13.77	470.00	2.5	33.28	30.63
SEM	0.53	6.02.00	0.0	0.30	0.86

Different letters in the same row indicate significant differences ($P<0.05$); ns, No significant differences are present ($P>0.05$) between the treatments. EC1, 7.12 kg of experimental concentrate; EC2, 6.23 kg of experimental concentrate; EC3, 5.34 kg of experimental concentrate; CC, 7.12 kg of commercial concentrate. SEM, standard error of the mean.

the experimental periods and had an average score of 2.5.

The live weight of animals and body condition were maintained constant across the three types of experimental treatments. The results suggesting that the quantity of concentrates and forage intake fulfilled the metabolizable protein and energy requirements of animals at the observed milk production level, even at the lowest level of supplementation (Tables 3 and 4). Urdaneta (2004), Pulido *et al.* (2007), and Razz *et al.* (2004) report this same tendency.

Fat and protein contents of milk did not differ significantly between the evaluated treatments ($P>0.05$). Average milk production was 13.77 kg/day/animal.

The intake of forage like stargrass, influenced the fat content in milk (Wanapat *et al.* 2018), Due to similar intakes of forage presented in this study, the fat production in milk were similar. Fat content in milk in the present study was less than that obtained in Pedraza-Beltrán *et al.* (2012) that provide 6 kg DM of experimental concentrate to which added four inclusion levels of coffee pulp (0, 10, 15 and 20%) for animals grazing on *Paspalum notatum* and report 43.3 g/kg. This latter study reported a fat content of 43.3 g/kg.

Meanwhile, protein content in milk is related to the proportion of undegradable protein in the diets and the provision of amino acids at intestinal level (Wanapat *et al.* 2018). In this study, the crude protein content of the experimental concentrate is higher than the commercial concentrate. However, it did not influence the protein results in milk; this is due to the fact that the proportion of protein in both concentrates covered the requirements of the cows. The present study was similar to that reported in Pedraza-Beltrán *et al.* (2012), reports contents of 31.4 g/kg of protein in milk. Mohammed *et al.* (2016), conducted an experiment to evaluate the grazing of *Cynodon nlemfuensis*, in addition to a concentrate based on 60% corn grain, 30% soybean and 10% soybean husk vs *Cynodon nlemfuensis* grazing associated with *Leucaena leucocephala* and supplemented with sorghum grains, not finding an influence on the protein (30 g/kg). Granzin and Mc Dryden (2005), report an increase on protein in milk when supplementing animals with Monensin, who found protein contents of, 30 g/kg, and 32 g/kg.

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