



# Sugarcane and cactus cladodes plus urea: a new option for Girolando dairy heifers

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**ABSTRACT** - This study was performed to identify the ideal amount of concentrate required for a diet based on cactus cladodes, sugarcane, and urea used to feed heifers. Twenty Girolando heifers ( $160\pm8.39$  kg) were randomly distributed into four experimental treatments with 0, 0.40, 0.80, or 1.20 kg day<sup>-1</sup> of concentrate. The basal diet contained [on dry matter (DM) basis] 38.1% sugarcane, 56.5% cactus cladodes [*Opuntia stricta* (Haw)], 0.5% common salt, 1.1% mineral mixture, and 3.8% urea plus ammonium sulfate. The concentrate contained [on DM basis] 87% corn meal and 13% soybean meal. The basal diet and the concentrate were formulated to provide 13% crude protein (CP). The intake of DM (4.34-4.96 kg day<sup>-1</sup>), organic matter (3.96-4.98 kg day<sup>-1</sup>), CP (0.57-0.64 kg day<sup>-1</sup>), non-fibrous carbohydrates (2.20-2.61 kg day<sup>-1</sup>), and metabolizable energy (10.3-12.4 Mcal day<sup>-1</sup>) increased linearly with concentrate amount. Final body weight (196-224 kg), total body weight gain (36.9-61.2 kg), and average daily body weight gain (0.51-0.85 kg day<sup>-1</sup>) increased linearly with concentrate amount. The nitrogen balance was positive and increased linearly (54.8-62.3 g day<sup>-1</sup>) with concentrate amount. Based on the data, we recommend supplying six-month-old Girolando heifers (160 kg body weight) 1.20 kg concentrate day<sup>-1</sup> added to a diet based on sugarcane and cactus cladodes plus urea for better productive and economic performance.

**Keywords:** cactaceous, energy supplementation, non-protein nitrogen, reproduction, tropical forage

## 1. Introduction

Heifer rearing provides high-quality genetic material to replace older cows from the herd. The age at first calving depends on the heifers' feeding and rearing program. Ideally, the age at first calving should occur at 24 months of age with 85% of adult body weight (BW). Thus, the key point for reducing the age at first calving is weight gain from birth to mating. This factor is related to management, feeding, and sanitary conditions. On average, the age at first calving in Brazil is 34 months (Facó et al., 2005; Canazacayo et al., 2018). Analyses of economic indicators of milk production systems in tropical conditions

with crossbred Holstein × Gyr animals showed that reducing the age at first calving increases the profitability of the system as a whole and provides a return on capital invested in replacing heifers (Moreira, 2012).

However, forage availability and quality decrease during the dry season and worsen during prolonged droughts. Raising heifers in this environment is not an easy task. In semiarid regions, the situation is more critical due to the low availability of forage during most of the year, justifying feedlot management (Ferreira et al., 2011). Barros et al. (2018) and Pessoa et al. (2017) showed the feasibility of using cactus cladodes with Tifton hay and sugarcane bagasse as fiber source in diets of crossbred heifers from two to six and 12 to 24 months of age. Cactus cladodes should not be provided to ruminants as an exclusive roughage source due to the low fiber content (Conceição et al., 2016) and the great agronomic and nutritional characteristics sugarcane presents as a potential fiber source in combination with cactus cladodes.

However, sugarcane as the basis for ruminant feed has some nutritional limitations, notably the high amount of ruminal low-degradation fiber that decreases dry matter (DM) intake and, thus, requires increased concentrate feeding. Because of this, Rangel et al. (2010) recommended a 45:55 sugarcane:concentrate ratio.

It was hypothesized that the addition of concentrate to diets based on sugarcane, cactus cladodes, and urea guarantees performance, to allow earlier age at first calving for Girolando dairy heifers. Thus, the objective was to identify the appropriate concentrate amount to add to a base diet composed of sugarcane, cactus cladodes, and urea for Girolando heifers.

## 2. Material and Methods

All procedures were conducted in accordance with the guidelines set by the Brazilian College of Animal Experimentation and approved by the Ethics Committee on Use of Animal for Research (CEUA) (case no. 069/2016). The experiment was performed in Arcoverde, Pernambuco, Brazil ( $08^{\circ}6'03.3''$  S and  $37^{\circ}03'27.7''$  W).

Twenty Girolando heifers, averaging six months of age and  $160 \pm 8.39$  kg of body weight (BW) were housed in covered individual stalls provided with randomly distributed feeders and drinkers in a completely randomized design. The adaptation period was 30 days, in addition to three 28-day experimental periods for data registration of animal performance and intake and sampling.

The basal diet (offered *ad libitum*) for all animals was composed of sugarcane (38.1%), cactus cladodes [*Opuntia stricta* (Haw)] (56.5%), common salt (0.5%), mineral mixture (1.1%), and a 9:1 the urea:ammonium sulfate ratio (3.8%), and the experimental treatments consisted in increasing the concentrate amount of 0, 0.40, 0.80, or 1.20 kg as fed day<sup>-1</sup> (offered separately from basal diet to guarantee the total intake). The concentrate was composed of corn meal (87%) and soybean meal (13%). The basal diet and the concentrate were formulated to meet 13% of crude protein (CP; NRC, 2001; Table 1). The animals were fed at 8:00 and 16:00 h, andorts relative to basal diet were weighted daily before the morning feeding to estimate the intake.

**Table 1 - Chemical composition of diet ingredients, basal diet, and concentrate**

Item	Cactus	Sugarcane	Corn meal	Soybean meal	Basal diet	Concentrate
DM (g kg <sup>-1</sup> as fed)	212	337	876	886	219	878
OM (g kg <sup>-1</sup> DM)	925	986	985	937	900	978
CP (g kg <sup>-1</sup> DM)	36.8	17.6	8.40	47.3	128	131
EE (g kg <sup>-1</sup> DM)	19.3	15.5	40.7	17.1	16.8	37.7
NDFap (g kg <sup>-1</sup> DM)	321	577	140	146	402	141
NFC (g kg <sup>-1</sup> DM)	549	37.6	713	251	454	653

DM - dry matter; OM - organic matter; CP - crude protein; NDFap - neutral detergent fiber corrected for ash and protein; NFC - non-fiber carbohydrates.

To assess the apparent digestibility of the nutrients – dry matter (DMD), organic matter (OMD), crude protein (CPD), neutral detergent fiber corrected for ash and protein (NDFDap), and non-fiber carbohydrates (NFCD) –, samples of feces were collected daily directly from the rectum of the animals from the 16th to the 20th day of the second experimental period, following the collection time: 16th day – 16:00 h, 17th day – 14:00 h, 18th day – 12:00 h, 19th day – 10:00 h, and 20th day – 08:00 h. The samples were stored in plastic bags and frozen (-20 °C; Cochran et al., 2007).

Feeds, orts, and feces samples were dried at 60 °C in a forced-ventilation oven (TE-394/3; Tecnal®, Brazil) for 72 h and processed in a Willey mill (TE-631/14; Tecnal®, Brazil). Subsequently, the samples were compiled per animal and experimental period.

Dry matter (method 934.01), ash (method 942.05), CP (method 968.06), and ether extract (EE; method 920.39) were analyzed according to AOAC (2000). Subsequently, neutral detergent fiber (NDF) was analyzed through heat-stable  $\alpha$ -amylase as described by Mertens (2002), corrected for residual ash and corrected for the nitrogenous compounds contents as described by (Licitra et al., 1996), and acid detergent fiber (ADF) as described by Van Soest (1973). Neutral detergent insoluble nitrogen (NDIP) was analyzed by the Kjeldahl method (Licitra et al., 1996).

The content of organic matter (OM) was calculated as the difference between DM and ash contents. Non-fiber carbohydrates (NFC) were calculated according to Detmann and Valadares Filho (2010), i.e.,  $NFC = 100\% - (\%ash + \%EE + \%NDFcp + (\%CP - \%CPU + \%U))$ . To estimate total digestible nutrients (TDN), we used the equation described by Weiss (1999):  $TDN = dCP + dEE*2.25 + dNFC + dNDF$ , in which  $d$  = digestible. Fecal excretion was estimated by using the indigestible NDF (iNDF) as an internal marker. Samples of feeds, orts, and feces were sieved through a 2-mm sieve and evaluated for their iNDF content by using non-woven fabric bags ( $100 \text{ g/m}^2$ ) and *in situ* incubation over a period of 288 h (INCT-CA N. F-009/1 method (Detmann et al., 2012).

Urine spot samples were obtained from all heifers on the 18th day of the second experimental period during spontaneous urination, approximately 4 h after the morning feeding (Chizzotti et al., 2008). For plasma ureic nitrogen (N) determination, blood samples were collected on the 18th day of the second experimental period, 4 h after the 8:00 h feeding, by coccygeal venipuncture with 21 G  $\times$  1 needle (BD Vacutainer®, EUA) and Vacutainer® tubes, without any additive. The samples were immediately centrifuged (at 3000 rpm, or  $1308 \times g$ , for 20 min), and the serum obtained was stored at -20 °C.

The nitrogen compound balance was estimated by subtracting the nitrogen excretion in the urine and feces from the total nitrogen intake. An aliquot was collected for the urine ureic-N from the urine collected for nitrogen balance estimation. Plasma and urine ureic-N were determined colorimetrically, using a commercial kit (LABTEST®).

Immediately before the analysis, the urine samples were thawed and centrifuged (at  $2700 \times g$  for 15 min). Creatinine concentrations were estimated by using the endpoint reaction method with enzymatic Trinder (Uric Acid Liqueform Ref. 73, Labtest®). According to Valadares Filho and Valadares (2001), the total urinary volume was estimated by dividing the urinary creatinine excretion by the value observed for urine creatinine concentration. Creatinine excretion (CE) was estimated by using a mathematical model proposed by Chizzotti et al. (2008):  $CE (\text{mg kg}^{-1} \text{ of BW}) = 32.27 - 0.01093 \times BW$ . Every 28 days, at the beginning and at the end of the trial period, the animals were weighed after a 16-h solid fasting.

The data were analyzed by the PROC MIXED function of SAS Software (Statistical Analysis System, version 9.2), after being tested for residual normality and variance homogeneity; a value of  $\alpha = 0.05$  for type I error was adopted.

Initial body weight was included in the analysis as a covariate, as described in the model:

$$Y_{ij} = \mu + T_i + \beta(X_{ij} - X) + e_{ij},$$

in which  $Y_{ij}$  = value observed of the dependent variable,  $\mu$  = overall mean,  $T_i$  = Treatment,  $\beta(X_{ij} - X)$  = the covariate effect (initial body weight), and  $e_{ij}$  = the experimental error.

### 3. Results

The basal diet and concentrate had very similar CP contents (128 and 131 g kg<sup>-1</sup> of DM, respectively; Table 1), as expected. Intakes of DM, OM, CP, NFC, and ME increased linearly as the concentrate amount increased (Table 2). Concentrate addition did not influence the digestibility of any nutrients (Table 3). Final body weight, total body weight gain, and average body weight daily gain increased linearly with the concentrate amount (Table 4).

Nitrogen intake, fecal excretion, and N balance increased linearly with concentrate supplementation (Table 5). Urine and plasma urea concentrations were not influenced by supplementation. The urea intake was 0.900, 0.870, 0.810, and 0.800 g kg<sup>-1</sup> BW for 0, 0.40, 0.80, and 1.20 kg day<sup>-1</sup> of concentrate, respectively.

**Table 2 - Nutrient intake of dairy heifers fed different amounts of concentrate in diets based on cactus cladodes, sugarcane, and urea**

Item	Concentrate (kg day <sup>-1</sup> )				SEM	P-value <sup>1</sup>		
	0	0.40	0.80	1.20		L	Q	COV
Dry matter (kg day <sup>-1</sup> )	4.34	4.62	4.69	4.96	0.19	0.037	0.975	<.001
Dry matter (g kg <sup>-1</sup> BW)	24.4	24.9	25.2	26.2	0.10	0.213	0.848	0.784
Organic matter (kg day <sup>-1</sup> )	3.96	4.20	4.25	4.48	0.17	0.005	0.988	<.001
Crude protein (kg day <sup>-1</sup> )	0.57	0.60	0.61	0.64	0.02	0.050	0.952	<.001
NDFap (kg day <sup>-1</sup> )	1.65	1.67	1.58	1.59	0.08	0.422	0.935	<.001
Non-fiber carbohydrates (kg day <sup>-1</sup> )	2.20	2.35	2.45	2.61	0.09	0.004	0.931	<0.01
Metabolizable energy (Mcal day <sup>-1</sup> )	10.3	11.6	11.2	12.4	0.46	0.011	0.915	<0.01

BW - body weight; NDFap - neutral detergent fiber corrected for ash and protein; SEM - standard error of the means.

<sup>1</sup>Linear, quadratic, and covariable.

**Table 3 - Apparent digestibility of nutrients and metabolizable energy of diets**

Item (g kg <sup>-1</sup> )	Concentrate (kg day <sup>-1</sup> )				SEM	P-value <sup>1</sup>		
	0	0.40	0.80	1.20		L	Q	COV
Dry matter	669	702	661	678	0.81	0.825	0.624	0.153
Organic matter	704	737	690	707	0.84	0.571	0.629	0.092
Crude protein (CP)	723	688	699	711	1.93	0.142	0.810	0.704
Neutral detergent fiber	378	407	403	409	1.12	0.101	0.245	0.102
Non-fiber carbohydrates	796	871	799	870	1.37	0.118	0.930	0.018
ME (Mcal kg <sup>-1</sup> of DM)	2.37	2.52	2.39	2.50	-	-	-	-
CP:ME (g CP Mcal <sup>-1</sup> of ME)	54.8	51.6	54.4	52.0	-	-	-	-

ME - metabolizable energy; SEM - standard error of the means.

<sup>1</sup>Linear, quadratic, and covariable.

**Table 4 - Body weight gain of dairy heifers fed different amounts of concentrate in diets based on cactus cladodes, sugarcane, and urea**

Item	Concentrate (kg day <sup>-1</sup> )				SEM	P-value <sup>1</sup>		
	0	0.40	0.80	1.20		L	Q	COV
Initial body weight (kg)	160	158	158	163	8.39	-	-	-
Final body weight (kg)	196	207	211	224	9.25	<.001	0.584	<.001
Total weight gain (kg)	36.9	48.8	52.4	61.2	2.38	<.001	0.575	0.115
Average daily gain (kg day <sup>-1</sup> )	0.51	0.70	0.73	0.85	0.03	<.001	0.539	0.111

SEM - standard error of the means.

<sup>1</sup>Linear, quadratic, and covariable.

**Table 5 - Urea in urine and plasma and nitrogen balance of dairy heifers fed different amounts of concentrate in diets based on cactus cladodes, sugarcane, and urea**

Item	Concentrate (kg day <sup>-1</sup> )				SEM	P-value <sup>1</sup>		
	0	0.40	0.80	1.20		L	Q	COV
Urinary urea (mg dL <sup>-1</sup> )	448	398	406	406	9.34	0.111	0.169	0.054
Plasma urea (mg dL <sup>-1</sup> )	24.9	21.5	17.2	20.5	1.54	0.242	0.295	0.639
Nitrogen intake (g day <sup>-1</sup> )	91.2	96.0	97.6	102	5.13	0.006	0.253	0.002
Urinary nitrogen (g day <sup>-1</sup> )	11.2	10.5	9.38	9.56	2.22	0.434	0.382	0.609
Fecal nitrogen (g day <sup>-1</sup> )	25.2	30.0	29.4	29.6	1.84	0.017	0.735	0.415
Nitrogen balance (g day <sup>-1</sup> )	54.8	55.5	58.8	62.3	6.24	0.034	0.848	0.017

SEM - standard error of the means.

<sup>1</sup>Linear, quadratic, and covariable.

#### 4. Discussion

Although the concentrate was supplied separately and the animals consumed all of it, the DM intake increase was not proportional to the elevation in the amount of the supplied concentrate. The intake differences between the basal diet and the other diets was 0.28, 0.35, and 0.62 kg day<sup>-1</sup> of DM. The DM amount supplied via concentrate was 0.35, 0.70, and 1.05 kg day<sup>-1</sup>; these data indicate a low substitutive effect. The intake of other nutrients followed the behavior observed for DM (Table 2).

Concentrate addition did not influence the digestibility of any nutrient (Table 3), despite the addition of grain, which positively impacts ruminal degradation (Beigh et al., 2017). However, the highest concentrate amount offered to the heifers was 1.20 kg day<sup>-1</sup>, which is less than 25% of the total DM intake. Additionally, the basal diet ingredients (cactus cladodes and sugarcane) is usually reported as highly digestible. For example, in a performance trial with sheep to test the ability of cactus cladodes to replace wheat bran in a sugarcane-based diet, nutrient digestibility increased when the cactus was added (Lins et al., 2016).

The increase in performance can be explained by the increases in DM, OM, CP, and ME intake. Supplementation with only 0.40 kg day<sup>-1</sup> of concentrate allowed adequate weight gain (0.70 kg day<sup>-1</sup>) for this category (6 to 12 months of age), according to Campos and Lzieire (2007), who established strategies for first calving at 24 months of age.

Nitrogen intake followed the same pattern as that observed for DM intake, because the diets were isonitrogenous. The N balance increased as a function of the higher digestible N intake; there was a linear increase in N intake without digestibility changes.

Urine and plasma urea concentrations were not altered by supplementation. The urea intake was 0.900, 0.870, 0.810, and 0.800 g kg<sup>-1</sup> BW for 0, 0.40, 0.80, and 1.20 kg day<sup>-1</sup> of concentrate, respectively. Recommendations for urea use are not well established, but whether the necessary urea amount can cause acute intoxication depends on several factors, including ingestion rate, rumen pH, and adaptation periods. Thus, levels above 0.45-0.50 g urea per kg of BW, ingested within a short time, may lead to intoxication in non-adapted animals (Bartley et al., 1976). Further, adapted animals fed a urea-supplemented diet based on rapidly fermentable carbohydrates as total mix ration (TMR) can tolerate two to three times more urea added to the diet without intoxication symptoms (Huber and Kung Jr., 1981). The same phenomenon occurred in the current experiment, in which urea was used with cactus cladodes in combination with sugarcane; both ingredients present high concentrations of rapidly fermentable carbohydrates (sucrose in sugar cane and pectin and organic acids in cactus). Additionally, the urea amount was supplied in two daily portions as TMR; the mean urea concentration 4 h after the morning feeding ranged from 17.2 to 24.9 mg dL<sup>-1</sup> (Table 5).

Facó et al. (2005) noted that advanced age at first calving causes a negative impact for dairy farms. The current results and previous studies regarding Girolando heifers fed diets composed of cactus

cladodes, urea, and tropical roughage as fiber and energy source and supplied with concentrate feed (Pessoa et al., 2017; Barros et al., 2018) suggest that diet is a feasible strategy to increase the performance of heifers during the growth stage. Interestingly, studies conducted under tropical conditions with different roughage, including corn silage, sugarcane, and sugarcane bagasse, showed that there is a need for higher concentrate amounts (2.00, 1.30, and 2.90 kg day<sup>-1</sup>, respectively; Rangel et al., 2010; Inácio et al., 2017) to reach gains similar to or lower than (0.70 kg day<sup>-1</sup>) achieved in this study using cactus cladodes as the base for the heifers' diet.

A relevant point that should be discussed is the high daily gain (0.85 kg day<sup>-1</sup>) obtained with 1.20 kg of supplementation due to possibilities of fatty deposition in the mammary gland (Sejrsen and Purup, 1997). However, according to Whitlock et al. (2002), higher weight gains may be achieved without damage for fat accumulation when the diets present an adequate balance between the gram CP and Mcal ME, with a ratio between 50 and 70. In the present study, this average balance is 53.2 g CP Mcal<sup>-1</sup> of ME.

The performance of heifers fed only the basal diet and with 0.40 kg day<sup>-1</sup> of concentrate apparently showed an advantage because there is no or a small amount of concentrate implying a falsely low production cost. A simulation was performed (Table 6); considering the average initial weight of heifers (160 kg) and the recommended weight at mating (330 kg), it is clear that, to reach this mating weight, 333, 246, 236, and 197 days of feeding are necessary for animals fed the basal ration combined with 0, 0.40, 0.80, and 1.20 kg day<sup>-1</sup> of concentrate, respectively. Considering only the feeding costs, in this period they would be \$190.28, \$160.53, \$166.87, and \$155.37, respectively. In addition, other factors must be considered, mainly labor.

The results obtained in the present trial showed the great nutritional value of a base diet composed of sugarcane, spineless cactus, and urea once the concentrate treatment level reaches a ratio of 78:22 forage:concentrate. This is proven by the fact that Rangel et al. (2010) showed similar performance results using forage:concentrate ratios of 75:25 and 45:55 for corn silage and sugarcane, respectively, as forage source.

**Table 6 - Feeding costs according to concentrate supplementation**

Item	Concentrate (kg day <sup>-1</sup> )			
	0	0.40	0.80	1.20
US\$ day <sup>-1</sup> of basal diet	0.57	0.56	0.52	0.51
US\$ day <sup>-1</sup> of concentrate	-	0.09	0.18	0.28
Total US\$ day <sup>-1</sup>	0.57	0.65	0.71	0.79
US\$ kg <sup>-1</sup> gain	1.12	0.93	0.97	0.93
US\$	190.28	160.53	166.87	155.37

US\$1 = R\$ 4.17 US dollar to Brazilian real rate at 01/24/2020.

## 5. Conclusions

We recommend supplying six-month-old Girolando heifers (160 kg body weight) with 1.20 kg of concentrate per day<sup>-1</sup> added to a diet based on sugarcane and cactus cladodes plus urea for a better productive and economic performance.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Formal analysis: M.L.M.W. Neves. Investigation: A.A.C. Cruz. Project administration: M.A. Ferreira. Resources: J.C.V. Oliveira and D.C. Santos. Supervision: A.S.C. Véras. Writing-review & editing: J.C.C. Chagas, C.C.F. Monteiro and M.A. Ferreira.

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