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Influence of crude protein content and flint maize processing methods on the performance of early-weaning Nellore calves

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Abstract

This study aims to determine the effects of dietary crude protein (CP) content of early-weaned calves; and the influence of flint maize processing methods on intake, total tract nutrient digestibilities and performance of Nellore heifer calves. Fifteen early-weaned Nellore female calves (4 ± 0.5 months; 108 ± 13.1 kg) were used. In phase 1, animals were fed one of the following diets for 112 days: 130, 145 or 160 g CP/kg dry matter (DM). In phase 2, animals received one of the two diets for 84 days: 0.60 dry ground maize grain, 0.30 whole-plant maize silage plus 0.10 mineral-protein supplement or 0.90 snaplage plus 0.10 mineral-protein supplement. In phase 1, intake and digestibility of dietary components were not affected ($P > 0.05$) by increasing dietary CP content. Daily total urinary nitrogen (N) and urinary urea N increased ($P < 0.05$) in response to increasing dietary CP content. Animal performance was not affected ($P > 0.05$) by dietary CP content. In phase 2, maize processing methods did not affect ($P > 0.05$) intake and digestibility of dietary components as well as animal performance, carcase characteristics and carcase composition. Therefore, based on the current experimental condition, we conclude that dietary CP concentrations of 130 g/kg DM can be indicated for early-weaned Nellore calves. However, more studies are recommended to validate this result and to evaluate concentrations below 130 g CP/kg DM for early-weaned Nellore calves. Moreover, snaplage could be used as an exclusive fibre and energy source for finishing cattle in feedlot.

Introduction

Early weaning for calves reared in a feedlot production system may be defined as separating calves from their dams before 180 days of age (Rasby, 2007). This management technique has been used to improve body condition score of the cows after calving (Houghton *et al.*, 1990; Arthington and Kalmbacher, 2003; Arthington and Minton, 2004), since they have greater nutritional demands during the lactation phase (Arthington *et al.*, 2005). In this context, the majority of primiparous cows are in a condition that needs a greater amount of nutrients to support lactation and continued growth (Cooke *et al.*, 2020).

Although early weaning of calves has been used to preserve primiparous dams, the allocation of their calves in a feedlot system might be a viable strategy for producers. Some studies (Myers *et al.*, 1999; Arthington *et al.*, 2005; Arthington and Vendramini, 2016) have reported that early-weaned calves have exceptional gain : feed and an improvement in their carcase quality when fed a total mixed ration (TMR) in the feedlot compared to normal weaned animals. Nevertheless, it is worth mentioning that early-weaned beef calves are in the growth phase, and therefore, have different nutritional requirements compared to beef cattle in the finishing phase. Thus, starch-rich feedlot diets could provide a greater early fat deposition and negatively influence the performance of these animals, being important to provide diets that meet their nutritional requirements.

However, the nutrient requirements and performance of early-weaned *Bos indicus* beef calves allocated in a feedlot system during the growing and finishing phases are not well documented in the literature. Energy and protein requirements for calves have been established based on diets containing milk proteins, which have a high digestibility and biological value (NRC, 2001; Valadares Filho *et al.*, 2016) compared to other ingredients. According to Davis and Drackley (1998), calves may not use non-milk proteins at such high efficiencies as the protein from milk, and some adjustments may need to ensure an adequate supply of amino acids for growth when such protein sources are used. Moreover, protein requirements could be lower for beef calves in feedlots compared with those in pasture systems.

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According to Valadares Filho *et al.* (2016), suckling beef calves [140 days of age, 150 kg and 0.6 kg/day average daily gain (ADG)] in pasture feeding systems have been shown to have crude protein (CP) requirements of approximately 145 g CP/kg dry matter (DM). Amaral *et al.* (2018) showed that male beef calves in traditional weaning (240 days of age) allocated in a feedlot should receive diets with a CP content of approximately 140 g CP/kg DM to achieve an ADG of 1.2 kg during the initial growing phase (112 days). However, these authors suggest that the CP content could be reduced to 120 g CP/kg DM during the finishing stage for Nellore and to 100 g CP/kg DM for Crossbred. Similarly, Menezes *et al.* (2019) suggested that the protein requirements for finishing young Nellore bulls can be met through dietary CP concentrations of 105–125 g/kg DM. Therefore, these data suggest that CP requirements decrease with age and as cattle reach final body weight (BW) (Todd *et al.*, 2008; Amaral *et al.*, 2018), it being possible to adjust diets according to the phase of the production system.

Fine grinding is the primary maize processing method adopted by most Brazilian feedlots (Millen *et al.*, 2009; Oliveira and Millen, 2014; Pinto and Millen, 2018). However, feedlots have shown interest in using processing methods based on ensiling flint maize with high moisture (Pinto and Millen, 2018; Bernardes and Castro, 2019) to maximize starch–protein matrix breakdown (Mahanna, 2008; Hoffman *et al.*, 2011; Silva *et al.*, 2020*a*, 2020*b*), and consequently, increase starch digestibility and nutrients' utilization (Owens *et al.*, 1986). In that context, snaplage is a type of maize silage that usually contains maize grain, cobs and husks, which are harvested when the maize grain contains approximately 40% moisture (Mahanna, 2008). The use of a forage harvester fitted with a maize snapper header and an on-board grain processor allows simultaneous harvesting and chopping of snaplage components (Lardy and Anderson, 2016; Ferraretto *et al.*, 2018). Also, snaplage is considered a high-energy feed product (Lardy and Anderson, 2016) that could be used as an exclusive fibre and energy source in feedlot diets or in combination with other feeds (Godoi *et al.*, 2021*b*). Therefore, diet formulation using snaplage will vary according to the harvest methods and desired performance. Furthermore, the use of snaplage in feedlots may improve nutrients' digestibility and facilitate daily operations.

Based on the protein requirements suggested by Valadares Filho *et al.* (2016) and previously mentioned, we hypothesize that: (1) it is possible to feed early-weaned beef calves with lower feedlot dietary CP content without adversely affecting animal growth and performance and (2) lower dietary CP concentration would reduce nitrogen (N) excretion through urine and faeces to the environment. Therefore, this study was conducted aiming to verify the effects of dietary CP contents of early-weaned calves.

Regarding maize processing methods, we hypothesize that snaplage can be used as an exclusive fibre and energy source in feedlot cattle. Thus, this study was conducted aiming to verify the influence of flint maize processing methods on intake, total tract nutrient digestibilities and performance of Nellore heifers.

Materials and methods

The experiment was conducted at the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil. The total experiment lasted 196 days. In phase 1, three CP contents were evaluated in early-weaned Nellore female calves for 112 days. The CP contents were: (1) low (130 g CP/kg DM), (2) medium (145 g CP/kg DM) and (3) high (160 g CP/kg DM). In phase 2, maize processing methods were evaluated in the growth and finishing of Nellore females and lasted 84 days. The results of both phases, phases 1 and 2, were combined in the current study to provide information regarding the performance of early-weaned calves allocated in a feedlot system until the finishing phase.

Animal handling, experimental designs and diets

Fifteen early-weaned Nellore heifer calves (initial BW of 108 ± 13.1 kg) were used in the study. The animals were weaned with approximately 4 months of age in the beef cattle sector, and they were immediately transported to the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa. Nellore female calves were born from primiparous cows. During 40 days before the weaning, calves received *ad libitum* creep-feeding supplementation as a previous acclimation period to the feedlot diet. The supplement was formulated with ground maize, wheat bran, soybean meal, urea, ammonium sulphate, salt and mineral mix. Moreover, each calf was fitted with an ear tag (left ear) containing a unique radio frequency transponder (FDX-ISO 11784/11785; Allflex, Joinville, Santa Catarina, Brazil), and treated for the elimination of internal and external parasites by the administration of injectable ivermectin (Ivomec; Merial, Paulinia, São Paulo, Brazil).

Phase 1

The remaining 15 early-weaned Nellore female calves were randomly assigned to receive one of the three *ad libitum* dietary treatments ($n = 5$ female calves per treatment) with different CP concentrations, as follows: (1) low (130 g CP/kg DM), (2) medium (145 g CP/kg DM) and (3) high (160 g CP/kg DM). Urea + ammonium sulphate (9 : 1) and soybean meal were used as a strategy to increase the CP content of the diets. It is worth mentioning that urea + ammonium sulphate and soybean meal ratio was similar (approximately 1 :7 .5) to avoid a large variation in the proportion of rumen degradable protein (RDP) and rumen undegradable protein (RUP) among the experimental diets. Feedstuffs and chemical composition of experimental diets during phase 1 are detailed in Table 1. Diets were formulated according to the BR-CORTE system (Valadares Filho *et al.*, 2016) to achieve an ADG of 0.6 kg/day. The RDP was calculated according to the Brazilian Tables of Chemical Composition of Feeds described by Valadares Filho *et al.* (2016), and the RUP was estimated by difference.

Phase 2

At the end of phase 1, the animals were randomly redistributed into two groups ($n = 6$ female calves per treatment). Each group received two animals from each previous treatment. So, the remaining three animals were assigned to another study. The animals were fed *ad libitum*, receiving the following treatments: (1) 0.60 dry ground maize grain, 0.30 whole-plant maize silage plus 0.10 mineral-protein supplement (DM basis; DMG), or (2) 0.90 snaplage plus 0.10 mineral-protein supplement (DM basis; SNAP-90). The proportions of snaplage used in this experiment were approximately 0.78 grains, 0.13 cob and 0.9 husk. Diets were formulated according to BR-CORTE recommendations (Valadares Filho *et al.*, 2016) to provide 125 g CP/kg DM and support an ADG of 1.0 kg/day (Table 2).

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Table 1. Feedstuffs and chemical composition of experimental diets during phase 1

Item	Diets		
	Low	Medium	High
Feed, g/kg of DM			
Whole-plant maize silage	600	600	600
Dry ground maize grain	199	199	199
Soybean meal	61.9	81.8	102
Urea + AS[a]	8.22	10.9	13.3
Wheat bran	122	99.5	77.4
Mineral mix[b]	4.49	4.49	4.49
Common salt	4.49	4.49	4.49
DM, g/kg as fed	405	405	405
Chemical composition g/kg of DM			
OM	933	942	942
CP	130	144	157
RDP, g/kg CP[c]	744	748	750
RUP, g/kg CP[d]	256	252	250
EE	29.3	28.8	28.4
NDF[e]	379	373	368
NFC[f]	413	409	406
Starch	328	323	318

[a]Urea + ammonium sulphate in a 9 : 1 ratio.
[b]Mineral mix = 7.83 g S/kg; 5950 mg Co/kg; 10 790 mg Cu/kg; 1000 mg Mn/kg; 1940 mg Se/kg; 1767.4 mg Zn/kg.
[c]Calculated according to Brazilian Tables of Chemical Composition of Feeds described by Valadares Filho *et al*. (2016).
[d]Rumen undegradable protein (g/kg CP) = 1000 – rumen degradable protein (g/kg CP).
[e]Corrected for residual ash and nitrogen compounds.
[f]NFC = 100 – [(CP – CP from urea + urea) + NDF corrected for residual ash and residual nitrogenous compounds + EE + ash].

Table 2. Feedstuffs and chemical composition of experimental diets during phase 2

Item	Diets	
	DMG	SNAP-90
Feed, g/kg of DM		
Whole-plant maize silage	300	–
Dry ground maize grain	600	–
Snaplage	–	900
Soybean meal	60.5	60.5
Mineral premix[a]	30.7	30.7
Urea + AS[b]	8.80	8.80
DM, g/kg as fed	571	567
Chemical composition g/kg of DM		
ME (MJ/kg)[c]	13.9	13.9
OM	942	956
CP	125	125
EE	33.2	30.5
NDF[d]	223	234
Starch	529	538

[a]Premix guarantees (per kg of DM): 200–220 g of Ca, 10 mg of Co (min), 500 mg of Cu (min), 22 g of S (min), 333 mg of Fe (min), 178.41 mg of F (max), 10 g of P (min), 25 mg of I (min), 17 g of Mg (min), 1500 mg of Mn (min), 1100 mg of monensin, 100 × 109 CFU of *Saccharomyces cerevisiae* (min), 6.6 mg of Se (min), 50 g of Na (min), 100 000 IU of vitamin A (min), 13 000 IU of vitamin D_3 (min), 150 IU of vitamin E (min) and 2000 mg of Zn (min).
[b]Urea + ammonium sulphate in a 9 : 1 ratio.
[c]Total digestible nutrients (TDN) was estimated using concentrations of CP, EE, ash, NDF, lignin, acid and neutral insoluble CP as described by the BR-CORTE system (Valadares Filho *et al*., 2016) and then metabolizable energy (ME) was calculated using the following equations: DE = TDN × 4.4; and ME = 0.9455 × ED – 0.3032 (Valadares Filho *et al*., 2016) multiplied by the conversion factor from Mcal/kg to MJ/kg of 4.184.
[d]Corrected for residual ash and nitrogen compounds.

Facilities and experimental procedures

In both phases, the animals were housed in collective pens (48.0 m^2) with electronic feeder (model AF-1000 Master; Intergado Ltd., Contagem, Minas Gerais, Brazil) and waterer (model WD-1000 Master; Intergado Ltd.). Animals were weighed at the beginning and the end of each phase after undergoing a 16-h fasting period to measure the initial and final BW. Also, animals were weighed every 28 days to evaluate and monitor ADG and BW.

The TMRs were provided twice a day at 07.00 h and 15.00 h. Feed delivery was adjusted daily to maintain minimum orts the next day and *ad libitum* intake. The appropriate feed delivery for each group was based on orts weight each morning. Electronic feeders were evaluated at 06.00 h daily to quantify orts and adjust daily feed delivery to a maximum of 2.5% orts. According to the amount of orts, the TMR was reduced (more than 2.5% orts at morning evaluation) or increased (less than 2.5% orts at morning evaluation) to reach *ad libitum* intake. Each treatment was delivered to the electronic feeder and consequently provided unique access to individual animals. Using the electronic identification tags, individual daily feed intake was recorded and measured using electronic equipment (model AF-1000 Master; all Ltda., Contagem, Minas Gerais, Brazil; Chizzotti *et al*., 2015).

All feeds were sampled daily. Feed samples were oven-dried (55°C), ground in a knife mill (R-TE-650/1, Tecnal, Piracicaba, São Paulo, Brazil) using 1-mm screen sequentially, packed in plastic bags, and stored at room temperature (20°C) for further laboratory analyses.

Sample collections, digestibility assays and slaughter procedures

To evaluate apparent total tract digestibility and to estimate nutrient excretion, two digestibility assays were performed in each phase. The spot sampling of faeces was collected through rectal palpation over a 3-day period. In phase 1, spot faecal samples were collected on day 50 to 52 and day 106 to 108. Moreover, in phase 2, spot faecal samples were collected on day 40 to 42 and day 80 to 82. The collections were conducted at 06.00 h on day 1, at 12.00 h on day 2 and at 18.00 h on day 3 of each digestibility assay (Prados *et al*., 2017; Amaral *et al*., 2018). Faeces were packed in aluminium trays, partially dried in a forced ventilation oven at 55°C, and then ground in a knife mill as described above. These collection times were used to obtain proportional and representative samples for each treatment. A composite sample from each animal was created per period. Indigestible neutral detergent fibre (iNDF) was used as a marker to estimate faecal DM excretion.

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Spot urine samples were collected by spontaneous urination simultaneously to faeces collection at 12.00 h on day 2 of each phase (Valadares *et al.*, 1999; Prados *et al.*, 2017; Zanetti *et al.*, 2017; Makizadeh *et al.*, 2020). After each collection day, 10 ml of urine was diluted in 40 ml of 0.036 N sulphuric acid solution to avoid bacterial destruction of purine derivative (Chen and Gomes, 1992; Menezes *et al.*, 2016). These samples were stored at −20°C for further laboratory analyses.

The heifers were slaughtered after 196 days at an average shrunk body weight (SBW) of 285 kg, close to the average for young heifers in the Brazilian system (Costa e Silva *et al.*, 2015; Valadares Filho *et al.*, 2016). Before slaughter, heifers were solid fasted for 16-h to estimate SBW. Heifers were slaughtered via captive bolt stunning followed by exsanguination. After slaughter, the carcase of each heifer was separated into two halves, weighed to quantify hot carcase weight and dressing percentage, and then chilled at 4°C for 18 h. The next day, half-carcases were removed from the cold chamber, weighed again and cold carcase yields were calculated. Subcutaneous fat thickness was then measured using a digital caliper in the region between the 11th and 12th rib cut.

The left half-carcase of each animal was totally dissected into muscle, fat and bone, and each portion was weighed separately. After that, the muscle and fat of each bull were homogenized and ground to obtain a composite sample. The bones were sliced with a band saw (Skymsen, model SFL-315HD, Santa Catarina, Brazil) in subsections of 1.5-cm length to obtain a representative sample of the bones. The composite sample of muscle and fat and the sample of bones were lyophilized and then were ground in a knife mill (Fortinox, Piracicaba, São Paulo, Brazil) with a 1-mm screen sieve to evaluate the DM, organic matter (OM), N and ether extract (EE) contents.

Laboratory analyses and calculations

Individual feed ingredients and faeces were quantified in terms of DM, OM, N and EE according to the AOAC (2012, method numbers 934.01, 930.05 and 981.10; 2006, method number 945.16, respectively). NDF was analysed according to the technique described by Mertens *et al.* (2002) without the addition of sodium sulphite, but with the addition of thermostable alpha-amylase to the detergent (Ankom Tech. Corp., Fairport, NY). The analyses of NDF were performed by using a fibre analyser (Ankom Technology, Macedon, NY, USA). The NDF content was corrected for residual ash and protein (apNDF). Estimations of neutral detergent insoluble nitrogen followed the technique described by Licitra *et al.* (1996).

The faecal DM excretion was obtained by dividing iNDF intake by the faecal iNDF concentration. To quantify iNDF, the faecal samples, concentrate, orts, maize silage and snaplage were placed in filter bags (model F57, Ankom) and incubated in a rumen-cannulated animal for 288 h (Valente *et al.*, 2011). Non-fibre carbohydrates (NFC) were calculated following the recommendations of Detmann and Valadares Filho (2010) regarding the use of urea (or urea : ammonium sulphate mixture) in diets. The faecal content of NFC and its digestibility were calculated based on the equation for feed NFC (NRC, 2001; Makizadeh *et al.*, 2020).

The starch analysis was based on an enzymatic method following the recommendations of Silva *et al.* (2019). Briefly, sequential incubations in a water bath with solutions of thermostable α-amylase and amyloglucosidase were performed in standards of D-glucose anhydrous (0, 50, 100, 150, 200 and 250 mg) and samples (i.e. feed and faeces) previously weighed in screw-cap test tubes. This procedure is used to establish a process of starch hydrolysis into glucose. Then, an *o*-toluidine solution is used to allow glucose quantification. The standard absorbance obtained through reading a spectrophotometer (630 nm) is used to adjust the simple linear regression equation without an intercept according to the Lambert–Beer law (Skoog *et al.*, 2017). Thus, the sample content of glucose released from starch is converted into starch content (% of DM).

Urine samples were analysed for N concentration (method 981.10; AOAC, 2012). Uric acid, creatinine and urea in urine were analysed with kits Bioclin® (K0139, K067 and K056, Belo Horizonte, Brazil) and determined by an automated biochemical analyser (Mindray, BS200E, Shenzhen, China). Allantoin was analysed according to the descriptions in Chen and Gomes (1992). Microbial efficiency was estimated as described by Barbosa *et al.* (2011) in accordance with daily purine derivative excretion and measured by sum of allantoin and uric acid excretion via urine. Microbial efficiency was expressed in grams of microbial protein synthesized by kilograms of TDN intake (g MCP/kg NDT) and by kilogram of digestible OM intake (g MCP/kg DOM). N balance was calculated based on the difference of N intake, faecal N and urine N.

Statistical analyses

Data from both phases were analysed under a completely randomized design by PROC MIXED procedures in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), where the animals were considered as the experimental units and treatments were fixed effects in the model.

In phase 1, the orthogonal contrast was used to identify linear and quadratic effects of CP concentration. Analysis of variance assumptions were verified for all variables. The regression equations were performed using PROC REG procedures in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). In phase 2, the least-squares means were compared using the Fisher's least significant difference test. Results were deemed significant when $P \leq 0.05$ and tendency was considered when $0.05 < P \leq 0.10$.

Results

Phase 1

Voluntary intake and digestibility of DM, OM, NDF and starch were not different ($P > 0.10$) among treatments (Table 3). However, there was a linear tendency ($P = 0.07$) for N intake with increasing dietary CP content (Table 4). The apparent digestibility coefficient of N increased linearly ($P < 0.05$) as dietary N concentration increased (0.75, 0.77 and 0.79 for low, medium and high based diets, respectively). The linear regression to estimate the apparent digestibility coefficient of *N* was as follows: apparent digestibility coefficient of $N = 0.588 + 0.0013 \times \text{CPc}$ ($R^2 = 0.40$), where CPc is the crude protein content of the diet (g/kg).

There was no significant difference ($P > 0.10$) on faecal N when dietary CP increased. On the other hand, urinary N (g/day and g/kg of excreted N) increased linearly ($P < 0.05$) as dietary CP increased. The linear regression to estimate the urinary N was as follows: urinary N (g/day) = $0.302 \times$ CPc ($R^2 = 0.98$). No difference ($P > 0.10$) was observed on N retained (g/day and g/kg

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Table 3. Voluntary intake and apparent digestibility coefficient by calves fed three CP contents during phase 1

	Diets[a]				*P*-value	
Item	Low	Medium	High	S.E.M. ($n = 5$)	Linear	Quadratic
DM intake, g/kg BW	23.8	23.1	22.5	0.80	0.291	0.972
Intake, kg/day						
DM	3.57	3.46	3.32	0.248	0.476	0.951
OM	3.34	3.26	3.12	0.233	0.520	0.904
NDF	1.36	1.30	1.22	0.093	0.315	0.938
NFC	1.49	1.46	1.38	0.103	0.448	0.857
Starch	1.17	1.12	1.06	0.081	0.321	0.944
Apparent digestibility coefficient						
DM	0.70	0.70	0.70	0.009	0.418	0.905
OM	0.73	0.74	0.73	0.008	0.516	0.415
NDF	0.60	0.62	0.63	0.016	0.319	0.597
NFC	0.84	0.83	0.83	0.008	0.551	0.938
Starch	0.95	0.95	0.96	0.006	0.225	0.678

[a]Low = 130 g CP/kg DM; medium = 145 g CP/kg DM; high = 160 g CP/kg DM.

Table 4. Nitrogen (N) balance of calves fed three CP contents during phase 1

	Diets[a]				*P*-value	
Item	Low	Medium	High	S.E.M. ($n = 5$)	Linear	Quadratic
N intake, g/day	74.3	85.7	87.5	4.90	0.072	0.436
Apparent digestibility coefficient of N	0.75	0.77	0.79	0.010	0.014	0.580
Faecal N, g/day	18.2	20.1	18.3	1.06	0.948	0.186
Urine volume[b], litres/day	4.97	5.56	4.95	0.717	0.986	0.507
Urinary N, g/day	36.2	45.2	50.1	3.21	0.009	0.606
Urinary N, g/kg of excreted N	663	692	729	17.8	0.019	0.832
Urinary urea N, g/day	28.9	34.5	45.4	2.71	0.001	0.437
Retained N, g/day	19.9	20.4	19.1	3.74	0.877	0.843
Retained N, g/kg of N intake	264	238	213	36.1	0.313	0.995
TDN intake, kg/day	3.08	3.06	2.87	0.217	0.513	0.752
DOM intake, kg/day	2.56	2.52	2.49	0.184	0.783	0.986
Allantoin (mmol/day)	111	108	103	10.5	0.598	0.921
Uric acid (mmol/day)	12.6	12.7	11.4	1.17	0.454	0.650
Microbial N, g/day	60.7	60.2	59.4	4.21	0.818	0.985
gMCP[c]/kg NDT intake	127	126	124	3.5	0.571	0.869
gMCP/kg DOM intake	152	153	147	4.5	0.475	0.522

[a]Low = 130 g CP/kg DM; medium = 145 g CP/kg DM; high = 160 g CP/kg DM.
[b]Urine volume (litres/day) was estimated based on daily urinary creatinine excretion [UCE (mg/day) = $37.88 \times SBW^{0.9316}$; where SBW is the animal shrunk body weight (kg)] and creatinine concentration in urine spot sample as described by the BR-CORTE system (Valadares Filho *et al.*, 2016).
[c]Microbial CP synthesis.

of N intake) between the diets. A significant linear effect ($P < 0.05$) was observed for urinary urea N (g/day) with increasing dietary CP content. The linear regression to estimate the urinary urea N was as follows: urinary urea N (g/day) = $-42.47 + 0.543 \times$ CPc ($R^2 = 0.62$).

No difference ($P > 0.10$) was observed on microbial N production (g/day) or microbial efficiency (g MCP/kg NDT and g MCP/kg DOM) with increasing dietary CP content.

Animal performance was not affected ($P > 0.10$) by dietary CP content (Table 5).

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Table 5. Performance of calves fed three CP contents during phase 1

Item[a]	Diets[b]			S.E.M. ($n = 5$)	*P*-value	
	Low	Medium	High		Linear	Quadratic
Initial SBW, kg	106	107	109	6.3	0.744	0.910
Final SBW, kg	194	191	188	14.4	0.774	0.982
ADG, kg/day	0.78	0.75	0.70	0.083	0.509	0.908
G : F	0.22	0.21	0.21	0.012	0.735	0.947

[a]SBW, shrunk body weight; ADG, average daily gain; G : F, gain-to-feed ratio.
[b]Low = 130 g CP/kg DM; medium = 145 g CP/kg DM; high = 160 g CP/kg DM.

Phase 2

The different flint maize processing methods on the finishing phase of heifers did not alter ($P > 0.10$) the voluntary intake and digestibility of DM, OM, NDF, starch and N (Tables 6 and 7).

There was no significant difference ($P > 0.10$) in faecal N, urinary N (g/day and g/kg of excreted N), and N retained (g/day and g/kg of N intake) among the diets. Also, no difference ($P > 0.10$) was observed on animal performance, carcase characteristics, and carcase composition of heifers fed different flint maize processing methods (Table 8).

Table 6. Voluntary intake and apparent digestibility coefficient by heifers fed different flint maize processing methods during phase 2

Item	Diets[a]		S.E.M. ($n = 6$)	*P*-value
	DMG	SNAP-90		
DM intake, g/kg BW	23.7	21.8	1.62	0.430
Intake, kg/day				
DM	5.56	5.37	0.302	0.655
OM	5.24	5.13	0.286	0.797
NDF	1.24	1.36	0.072	0.272
Starch	2.94	3.03	0.164	0.701
Apparent digestibility coefficient				
DM	0.76	0.77	0.011	0.861
OM	0.78	0.78	0.011	0.779
NDF	0.59	0.60	0.015	0.629
Starch	0.96	0.97	0.004	0.128

[a]DMG = 0.60 dry ground maize grain, 0.30 whole-plant maize silage and 0.10 mineral–protein supplement; SNAP-90 = 0.90 snaplage and 0.10 mineral–protein supplement.

Discussion

Phase 1

Dietary protein can be divided into RDP and RUP (NRC, 1985). Ruminal microorganisms could degrade the digestible proteins and non-protein compounds of diets into peptides, amino acids and eventually to ammonia (NH_3; Hristov and Jouany, 2005), which is the primary source of protein for ruminal microbial growth (Cotta and Russell, 1982).

Rumen microbial growth, and consequently, the microbial CP synthesis can be compromised when ruminants are fed with inadequate amounts of RDP. Also, the inadequate flow of MCP and RUP to the small intestine can compromise animal performance (Hristov *et al.*, 2011) since the metabolizable protein requirements of ruminants are met by MCP, RUP and endogenous protein secretion (NRC, 2001). From a nutritional point of view, non-protein nitrogen sources, such as urea, have been included in diets to increase the RDP (Rufino *et al.*, 2016), and consequently, the amount of NH_3. Also, the use of non-protein nitrogen sources could reduce costs compared with the use of RUP (Rufino *et al.*, 2016), being economically viable to the production system.

Regarding the requirements of weaned calves, the ruminant phase of calves starts from 8 weeks onwards of age (Gupta *et al.*, 2016). Therefore, calves begin to derive their nutrients from solid feeds, mainly through microbial fermentation in the reticulo-rumen (NRC, 2001), resembling the rumen characteristics of adult animals.

The increase in dietary CP content did not affect the intake and digestibility of DM, OM, NDF and starch, suggesting that intake variable are not restricted or stimulated by dietary CP content (Menezes *et al.*, 2019). However, as would be expected, the planned difference in dietary N content resulted in a tendency of the linear increase in N intake as dietary CP content increased. Also, there was a subsequent increase in apparent N compounds' digestibility. Also, according to Rufino *et al.* (2016), CP apparent digestibility coefficient is proportional to CP intake. A similar pattern was observed by Menezes *et al.* (2019) where young Nellore bulls fed high CP diet (145 g CP/kg DM) had greater apparent CP digestibility than bulls fed diets based on medium (125 g CP/kg DM) or low CP content (105 g CP/kg DM).

Microbial protein synthesis was estimated using purine derivative excretion. Microbial protein synthesis and microbial efficiency was not affected by the dietary CP content. According to Clark *et al.* (1992), the availability of energy and nitrogen are the main determinants of microbial growth. Therefore, it suggests that both diets fulfilled minimum requirements for microbial growth and feed degradation in the rumen (Kidane *et al.*, 2018).

Regarding N excretion, the increasing content of CP in diets did not influence the excretion of faeces N. Studies (Menezes *et al.*, 2016; Jennings *et al.*, 2018) have reported the lack of knowledge of dietary effects on faecal N excretion for finishing bulls. On the other hand, the increase of dietary CP content resulted in a linear pattern on urinary N (g/day and g/kg of excreted N) and urinary urea N (g/day). When the protein contents of diets are greater than animal requirements, the urinary N excretion will increase linearly with protein intake (Menezes *et al.*, 2016).

Ruminal microorganisms usually degrade the digestible proteins and non-protein compounds of diets into ammonia-N

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Table 7. Nitrogen (N) balance and microbial efficiency of heifers fed different flint maize processing methods during phase 2

	Diets[a]			
Item	DMG	SNAP-90	S.E.M. ($n = 6$)	*P*-value
N intake, g/day	112	108	6.1	0.669
Apparent digestibility coefficient of N	0.79	0.81	0.017	0.417
Faecal N, g/day	23.6	20.7	2.08	0.337
Urine volume[b], litres/day	5.80	6.29	0.485	0.488
Urinary N, g/day	63.1	62.9	7.30	0.984
Urinary N, g/kg of excreted N	720	744	29.3	0.575
Retained N, g/day	24.9	24.3	3.10	0.893
Retained N, g/kg of N intake	227	234	37.1	0.897
TDN intake, kg/day	4.80	4.77	0.253	0.920
DOM intake, kg/day	4.07	4.06	0.213	0.984
Allantoin (mmol/day)	166	167	9.7	0.945
Uric acid (mmol/day)	18.3	17.8	1.12	0.760
Microbial N, g/day	92.0	92.6	4.94	0.925
gMCP[c]/kg NDT intake	125	126	3.9	0.933
gMCP/kg DOM intake	147	148	4.5	0.873

[a]DMG = 0.60 dry ground maize grain, 0.30 whole-plant maize silage and 0.10 mineral–protein supplement; SNAP-90 = 0.90 snaplage and 0.10 mineral–protein supplement.
[b]Urine volume (litres/day) was estimated based on daily urinary creatinine excretion [UCE (mg/day) = $37.88 \times SBW^{0.9316}$; where SBW is the animal shrunk body weight (kg)] and creatinine concentration in urine spot sample as described by the BR-CORTE system (Valadares Filho *et al.*, 2016).
[c]Microbial CP synthesis.

Table 8. Performance, carcase characteristics and carcase composition of heifers fed with different flint maize processing methods during phase 2

	Diets[a]			
Item[b]	DMG	SNAP-90	S.E.M. ($n = 6$)	*P*-value
Initial SBW, kg	198	204	9.7	0.574
Final SBW, kg	281	289	14.6	0.627
ADG, kg/day	1.05	1.04	0.092	0.738
G : F	0.19	0.19	0.019	0.999
Hot carcase weight, kg	165.3	172.4	8.48	0.540
Cold carcase weight, kg	162.3	169.6	8.47	0.562
Hot carcase dressing, %	58.4	58.5	0.71	0.892
Cold carcase dressing, %	57.3	57.4	0.71	0.958
Carcase length, cm	111	111	2.2	0.834
Fat thickness, mm	3.2	4.0	0.89	0.531
Rib eye area, cm^2	56.9	57.0	2.57	0.979
Physical composition of carcase, %				
Meat	58.2	60.3	1.14	0.224
Fat	26.5	24.6	1.37	0.345
Bones	15.3	15.1	0.43	0.822
Chemical composition of carcase, %				
Water	55.6	57.5	1.06	0.225
Ash	4.7	4.5	0.17	0.322
EE	23.0	21.4	1.41	0.460
CP	16.7	16.6	0.40	0.779

[a]DMG = 0.60 dry ground maize grain, 0.30 whole-plant maize silage and 0.10 mineral–protein supplement; SNAP-90 = 0.90 snaplage and 0.10 mineral–protein supplement.
[b]SBW, shrunk body weight; ADG, average daily gain; G : F, gain-to-feed ratio.

to be used in microbial protein synthesis (Hristov and Jouany, 2005). However, the inefficient use of N by ruminal microorganisms may promote an excess of NH_3 in the rumen. Thus, this excess is absorbed by the rumen wall and transferred through portal blood to the liver, where it is converted to urea and subsequently released into the blood (Hristov *et al.*, 2011). The blood urea is partially filtered in the kidney and excreted in urine or recycled back to the gastrointestinal tract (Lapierre and Lobley, 2001). Therefore, it may explain why N losses are much more variable in urine than in faeces (Dijkstra *et al.*, 2013) since faecal N is made up of undigested dietary proteins, indigestible microbial CP and endogenous N (Prados *et al.*, 2016).

The N retained (g/day or g/kg of N intake), ADG (kg/day) and final SBW (kg) were not affected by dietary CP content, suggesting that the increase in CP in diets does not bring any evident benefit to the performance of early-weaned calves. Commercial dairy calves are usually weaned under 90 days of age since transitioning calves from milk to solid feed is a way to decrease feed costs in commercial dairy production systems (Kertz *et al.*, 2017). Thus, NRC (2001) recommended 180 g CP/kg DM for conventionally reared dairy calves, which generally weigh less than 100 kg. On the other hand, according to Valadares Filho *et al.* (2016), suckling beef calves (140 days of age, 150 kg and 0.6 kg/day ADG) in pasture systems have been shown to have CP requirements of approximately 145 g CP/kg DM. Also, Amaral *et al.* (2018) showed that beef calves in traditional weaning (240 days of age) allocated in a feedlot should receive diets with a CP content of approximately 140 g CP/kg DM to achieve an ADG of 1.2 kg/day during the initial growing phase (112 days), being reduced to 120 g CP/kg DM in the finishing phase. Therefore, these data suggest that it is possible to adjust diets according to each phase and targeted animal ADG.

The results suggest that dietary CP concentrations of 130 g/kg DM can be indicated for early-weaned Nellore calves since it meets their nutritional requirements. Moreover, there is the potential to reduce the environmental impacts of beef cattle production and increase economic returns to producers by the reduction of N losses. Nevertheless, it is also important to highlight that the small sample size was one of the limitations of this study.

Phase 2

Greater amounts of grain in beef cattle diets associated with the use of feeds rich in readily available carbohydrates may increase the propensity for acidosis, which is a common ruminal digestive disorder in feedlots (Elam, 1976; Owens *et al.*, 1998; Nagaraja and Titgemeyer, 2007). Animals with subacute acidosis decrease (Elam, 1976; Allen, 1997) or oscillate (Devant *et al.*, 2015) their

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DM intake in an attempt to stabilize the rumen environment. Also, ruminal pH below 6.0 may decrease fibre digestibility as well as the microbial yield (Hoover, 1986). Therefore, acidosis can lead to marked reductions in cattle performance (Owens *et al.*, 1998; Nagaraja and Titgemeyer, 2007). According to Owens *et al.* (1998), acidosis can also be diagnostic when animals show symptoms such as anorexia, diarrhoea and lethargy. None of these factors was observed during the feedlot period, suggesting the non-occurrence of acidosis. Animals fed diets based on SNAP-90 or DMG showed similar intake and digestibility of DM, OM, CP, NDF and starch.

Some studies (Akins and Shaver, 2014; Ferraretto *et al.*, 2018; Bernardes and Castro, 2019; Godoi *et al.*, 2021*a*, 2021*b*) have reported an improvement in starch digestibility when maize is harvested, stored and fed as snaplage. However, many factors may interfere with the digestibility of starch in diets based on grain silages, such as the moisture content at harvest time and ensiling (Owens *et al.*, 1997; Lardy and Anderson, 2016), particle size (Rémond *et al.*, 2004; Lardy and Anderson, 2016) as well as the length of storage time (Hoffman *et al.*, 2011).

Microbial protein synthesis and microbial efficiency were not affected by flint maize processing methods. Similarly, animal performance, carcase characteristics and carcase composition did not differ when heifers were fed diets based on SNAP-90 or DMG. Hence, corroborating with our hypothesis, snaplage can be used as an exclusive fibre and energy source in feedlot cattle. However, it is worthy to note that a small sample size was one of the limitations of this study.

Although no differences were observed for animals fed diets based on SNAP-90 or DMG, harvest maize as snaplage can increase productivity per hectare compared to the harvest of dry grains (Mahanna, 2008). Also, the ensiling process of grains with high moisture may promote an improvement in starch availability, and consequently, in feed efficiency (Hoffman *et al.*, 2011; Junges *et al.*, 2017; Silva *et al.*, 2020*a*, 2020*b*).

In conclusion, based on the current experimental condition, the dietary CP concentrations of 130 g/kg DM can be adequate for early-weaned Nellore calves since this content can cover the N requirement of animals at this age. However, more studies are recommended to validate this result and to evaluate content below 130 g CP/kg DM in early-weaned Nellore calves. Moreover, this diet reduces the environmental footprint related to N excretion. Regarding flint maize processing methods, snaplage could be used as an exclusive fibre and energy source for cattle finished in feedlot.

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Conflict of interest. The authors declare that they have no competing interests.

Ethical standards. The experiment was conducted following the approval of the animal handling and procedures described herein by the Ethics Committee for Animal Use (protocol CEUAP/DZO/UFV 05/2020).

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