Oscillating and static dietary crude protein supply. I. Impacts on intake, digestibility, performance, and nitrogen balance in young Nellore bulls[1]

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ABSTRACT: Effects of dietary crude protein (CP) supply on intake, digestibility, performance, and N balance were evaluated in young Nellore bulls consuming static or oscillating CP concentrations. Forty-two young bulls (initial BW of 260 ± 8.1 kg; age of 7 ± 1.0 mo) were fed ad libitum and were randomly assigned to receive one of six diets with different CP concentrations for 140 d: 105 (LO), 125 (MD), or 145 g CP /kg DM (HI), and LO to HI (LH), LO to MD (LM), or MD to HI (MH) oscillating CP at a 48-h interval for each feed. At the end of the experiment, bulls were slaughtered to evaluate carcass characteristics. Linear and quadratic effects were used to compare LO, MD, and HI, and specific contrasts were applied to compare oscillating dietary CP treatments vs. MD (125 g CP/kg DM) static treatment. Dry matter intake (DMI) was not affected ($P > 0.26$) by increasing or oscillating dietary CP. As dietary N concentration increased, there was a subsequent increase in apparent N compounds digestibility ($P = 0.02$), and no significant difference ($P = 0.38$) was observed between oscillating LH and MD. Daily total urinary and fecal N increased ($P < 0.01$) in response to increasing dietary CP. Significant differences were observed between oscillating LM and MH vs. MD, where bulls receiving the LM diet excreted less ($P < 0.01$; 71.21 g/d) and bulls fed MH excreted more ($P < 0.01$) urinary N (90.70 g/d) than those fed MD (85.52 g/d). A quadratic effect was observed ($P < 0.01$) for retained N as a percentage of N intake, where the bulls fed LO had greater N retention than those fed HI, 16.20% and 13.78%, respectively. Both LH and LM had greater ($P < 0.01$) daily retained N when compared with MD. Performance and carcass characteristics were not affected ($P > 0.05$) by increasing or oscillating dietary CP. Therefore, these data indicate that although there is no alteration in the performance of growing Nellore bulls fed with oscillating CP diets vs. a static level of 125 g CP/kg DM, nor static low (105 g CP/kg DM) and high (145 g CP/kg DM) levels; there may be undesirable increases in environmental N excretion when the average dietary CP content is increased. The results suggest that dietary CP concentrations of 105, 125 g/kg DM, or within this range can be indicated for finishing young Nellore bulls, since it reaches the requirements, reduces the environmental footprint related to N excretion, and may save on costs of high-priced protein feeds.

Key words: bulls, crude protein, Nellore, nitrogen, performance

INTRODUCTION

There are growing concerns about the effects of feedlot operations on air and water quality. Ammonia (NH_3) is the main gas emitted into the atmosphere from manure decomposition that affects environmental ecosystems and represents an unproductive loss of dietary nutrients (Liu et al., 2017). Several factors can affect the excretion of nitrogen such as feed intake, chemical composition of the diet, and efficiency of nutrient utilization (Muñoz et al., 2015). Strategies that increase production efficiency can conserve resources, improve environmental stewardship, and represent a great opportunity for mitigating N emissions per unit of livestock product.

Beef cattle may convert 20% to 30% of their dietary N into animal protein, consequently, about 70% to 80% is excreted in the urine and feces. The excess dietary N that is excreted accumulates in the atmosphere, soil, and groundwater and is detrimental to the ecosystem (NASEM, 2016). Promising strategies to alleviate N excretion and improve N retention involve manipulating the dietary crude protein (CP) content. Reducing the dietary CP content in finishing diets can decrease N excretion, mainly via urine, without a negative impact on performance (Amaral et al., 2014; Menezes et al., 2016). Additionally, oscillating CP concentration can enhance N retention in growing sheep (Cole, 1999; Kiran and Mutsvangwa, 2009; Doranalli et al., 2011) and finishing cattle (Cole et al., 2003; Archibeque et al., 2007a).

Dietary nutrient oscillation seems to affect the homeostatic and homeorhetic processes of host animal and ruminal microbial population in a manner that promotes a period of accelerated microbial growth due to an increase in N assimilation by ruminal microorganisms (Amaral et al., 2016). Therefore, the reduction in N excretion by meeting the ruminally degradable protein and metabolizable protein requirements of animals, without decreasing performance, has great potential to reduce the environmental impact of beef cattle production and increase economic returns for producers. However, to the best of our knowledge, no systematic empirical research exists addressing the question of how oscillating CP dietary content affects N excretion and productive performance of growing and finishing *Bos indicus* animals in tropical conditions.

We hypothesized that 1) oscillation of the dietary CP concentration would enhance growth performance, reduce N excretion, and improve N retention; and 2) it is possible to reduce CP during feedlot stages, without adversely affecting animal performance and efficiency. These hypotheses were tested by evaluating three static dietary CP concentrations; and three oscillating CP concentrations vs. a static level of 125 g CP/kg DM, by determining intake, apparent digestibility, performance, feed efficiency, and carcass characteristics of young Nellore bulls.

MATERIALS AND METHODS

Dietary Treatments and Animals

The experiment was conducted at the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil. Animal care and handling followed guidelines set by the UFV (process 59/2016). Dietary CP levels were determined according to the protein requirements for Nellore bulls suggested by the BR-CORTE system (Valadares Filho et al., 2016), where 125 g CP/kg DM was established as the adequate CP concentration for bulls in this age and weight category. Therefore, we used 125 g CP/kg DM as our medium, or average, treatment, and the oscillating treatments were compared with this static treatment.

Forty-two weaned Nellore bulls (initial BW of 260 ± 8.1 kg; age of 7 ± 1.0 mo) were fed ad libitum and were randomly assigned to receive one of six dietary treatments (*n* = 7 bulls per treatment) with different CP concentrations for 140 d, either: 1) Low (LO; 105 g CP/kg DM), 2) Medium (MD; 125 g CP/kg DM), 3) High (HI; 145 g CP/kg DM), 4) Low to High (LH; Oscillating dietary CP concentration of 105 to 145 g CP/kg DM at a 48-h interval), 5) Low

to Medium (LM; oscillating dietary CP concentration of 105 to 125 g CP/kg DM at a 48-h interval), and 6) Medium to High (MH; Oscillating dietary CP concentration of 125 to 145 g CP/kg DM at a 48-h interval). The chemical composition of the three diets used in this experiment is presented in Table 1. Briefly, the Low diet (105 g CP/ kg DM) provided 673.2 g RDP /kg CP and 326.8 g RUP /kg CP; the Medium diet (125 g CP/ kg DM) provided 696.7 g RDP/kg CP and 303.3 g RUP/kg CP; and the High diet (145 g CP / kg DM) provided 713.7 g RDP/kg CP and 286.3 g RUP/kg CP.

Each treatment was group-housed in a feedlot pen (48.0 m²) with one electronic feeder (model AF-1000 Master; Intergado Ltd., Contagem, Minas Gerais, Brazil) and one electronic waterer per pen (model WD-1000 Master; Intergado Ltd.). Before the experiment, each bull was fitted with an ear tag (left ear) containing a unique radio frequency transponder (FDX-ISO 11784/11785; Allflex, Joinville, Santa Catarina, Brazil). The bulls were allowed a 21-d adaptation period to the experimental conditions and treated against internal and external parasites by administration of injectable ivermectin

Table 1. Proportion of ingredients and nutrient composition of the experimental diets

Item	Experimental diets[1]		
	Low	Medium	High
Proportion			
Corn Silage	50.0	50.0	50.0
Ground corn	39.4	39.4	39.4
Soybean meal	2.38	4.92	7.46
Wheat bran	6.09	3.05	0.00
Urea	0.47	0.98	1.49
Salt	0.30	0.30	0.30
Limestone	0.06	0.06	0.06
Mineral mix[2]	0.29	0.29	0.29
Sodium bicarbonate	0.75	0.75	0.75
Magnesium oxide	0.25	0.25	0.25
Total	100	100	100
Chemical composition			
Dry matter, g/kg as-fed	406.0	406.1	406.2
Organic matter, g/kg DM	944.1	943.8	943.5
Crude protein, g/kg DM	102.7	122.3	141.9
Rumen degradable protein, g/kg CP	673.2	696.7	713.7
Rumen undegradable protein, g/kg CP	326.8	303.3	286.3
Ether extract, g/kg DM	42.8	42.1	41.4
Neutral detergent fiber, g/kg DM	321.3	314.2	307.1
Indigestible neutral detergent fiber, g/kg DM	98.09	95.7	93.4
Nonfiber carbohydrates, g/kg DM	480.6	476.2	471.8

[1]Low = 105 g CP/kg DM; Medium = 125 g CP/kg DM; High =145 g CP/kg DM.

[2]Mineral mix = 7.83 g S/kg; 5,950 mg Co/kg; 10,790 mg Cu/kg; 1,000 mg Mn/kg; 1,940 mg Se/kg; 1,767.4 mg Zn/kg.

(Ivomec; Merial, Paulinia, Brazil). The experiment was divided into five 28-d experimental periods, where the bulls were weighed at the beginning and end of the experiment after undergoing 16 h of fasting to measure initial and final BW, and weighed every 28 d to evaluate and monitor average daily gain (ADG) and BW. Diets were formulated according to the BR-CORTE system (Valadares Filho et al., 2016) to achieve an ADG of 1.1 kg. The diets (50:50 forage to concentrate ratio) consisted of corn silage and a concentrate that was formulated with ground corn, wheat bran, soybean meal, urea, ammonium sulfate, sodium bicarbonate, salt, and mineral mix. Chemical composition and amount of feed in diets are shown in Table 1. The RDP was calculated according to Brazilian Tables of Chemical Composition of Feeds described by Valadares Filho et al. (2016), and the RUP was estimated by difference.

The total mixed rations were provided twice a day, at 0700 and 1600 h. Feed delivery was adjusted daily to maintain minimum refusals the next day and ad libitum intake. The appropriate feed delivery for each group was based on refusal weight each morning. Electronic feeders were evaluated at 0600 h daily to quantify orts and adjust daily feed delivery to a maximum of 2.5% orts. According to the amount of refusals, the total mixed ration 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; Intergado Ltda., Contagem, Minas Gerais, Brazil; Chizzotti et al., 2015).

Sample Processing and Chemical Analysis

Feeds offered and refused were weighed daily, sampled, and frozen. Weekly, corn silage and refused feeds were pooled, oven-dried at 55°C for 72 h and ground at 2 mm to determinate the indigestible neutral detergent fiber (iNDF) concentration and 1 mm for other analyses, with a Wiley mill (TECNAL, SP, Brazil). The total DM was evaluated using a drying oven at 105°C for 16 h. Based on the amount of DM from each animal refusal, pooled samples were made for each 28-d period. Samples of each one of the concentrate ingredients were collected directly at the feed mill, and corn silage samples were collected daily and stored in a freezer at −20°C.

To evaluate apparent total-tract nutrient digestibility, grab samples of feces were obtained from each bull over two 5-d periods, from days 36 to 40 and 98 to 102. Within each period, collections were conducted at 1800 h on day 1, at 1200 h on day 2, at 0600 and 1800 h on day 3, at 1200 h on day 4, and at 0600 h on day 5. These collection times were used in order to obtain proportional and representative samples to the oscillating and fixed treatments. A composite sample from each animal was created per period and processed as described for silage and orts. Indigestible NDF was used as a marker to estimate fecal DM excretion.

Pooled samples of corn silage, concentrate ingredients, refusals, and feces were quantified in terms of dry matter (DM), organic matter (OM), N, and ether extract (EE) according to the AOAC (2012, method numbers 934.01, 930.05, and 981.10; 2006, method number 945.16, respectively). Neutral detergent fiber (NDF) was analyzed according to the technique described by Mertens et al. (2002) without the addition of sodium sulfite, 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 fiber analyzer (Ankom200, Ankon Technology, Macedon, NY). The NDF content corrected to ash (Mertens 2002) and protein (Licitra et al. 1996) content was estimated. The fecal DM excretion was obtained by dividing the iNDF intake by the fecal iNDF concentration. To quantify iNDF, the fecal samples, concentrate, refusals, and corn silage were placed in filter bags (model F57, Ankon) and incubated in the rumen of a rumen-cannulated animal for 288 h (Valente et al. 2011). Nonfiber carbohydrates (NFC) were calculated according to Detmann and Valadares Filho (2010), where NFC (% DM) = 100 − [CP − (CP derived from urea + urea) + NDF + EE + ash].

Blood Sampling

Jugular blood samples were obtained on days 56 and 112 prior to morning feed delivery, placed into evacuated tubes (Labor Import, Osasco, São Paulo, Brazil), and immediately cooled in ice. Blood samples were transported on ice to the laboratory and centrifuged to separate plasma ($1,200 \times g$ for 10 min at 4°C). Once separated, plasma was removed by pipetting, aliquoted into 2-mL tubes, and immediately frozen at −40°C until analysis could be performed. Plasma was analyzed for plasma urea N using an automated biochemistry analyzer (ModelBS200E; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China).

Slaughter and Sampling

Prior to slaughter, bulls were fasted from feed for 16-h to estimate shrunk body weight (SBW). Bulls were slaughtered via captive bolt stunning followed by exsanguination. After slaughter, the carcass of each bull was separated into two halves, weighed to quantify hot carcass weight and dressing percentage, and then chilled at 4°C for 18 h. Next, half-carcasses were removed from the cold chamber, weighed, and cold carcass yields were calculated. Subcutaneous fat thickness was then measured using a digital caliper in the region between 11th and 12th rib cut.

Statistical Analyses

The experiment was carried out under a completely randomized design, where the bulls were the experimental units. Constant CP concentration treatment comparisons followed the decomposition of orthogonal polynomials in linear and quadratic effects to compare 105, 125, and 145 g CP/kg DM. Moreover, specific contrasts were applied to compare oscillating dietary CP treatments vs. MD (125 g CP/kg DM) static treatment. Hartley's Fmax test was used to account for treatments homogeneity of variances; and the residual normality was investigated by Shapiro–Wilk test. Both ANOVA assumptions were verified for all variables. The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) software was used to perform all statistical analyses assuming the significance level of 0.05.

RESULTS AND DISCUSSION

Voluntary Intake and Digestibility

Voluntary intake and digestibility data are presented in Table 2. Dry matter, OM, NDF, and NFC intake were not affected ($P > 0.05$) by increasing dietary CP, nor by oscillating dietary CP compared with MD. Mean intake of DM and other constituents, except for the planned difference in N (Table 3), were not different between dietary treatments, suggesting that neither the dietary CP content nor the way CP is delivered in diet restricts or stimulates intake parameters. As such, the absence of the effects of dietary CP content in voluntary intake suggests that the dietary inclusion of 105 g CP/kg DM, even though considered the lowest CP content relative to other treatments, fulfilled minimum requirements for microbial growth and feed degradation in the rumen (Kidane et al., 2018). It is suggested (Marini and Van Amburgh, 2003; Brake

Table 2. Voluntary intake and apparent digestibility by bulls fed with differing crude protein concentrations

Item	Treatment[1]						SEM[2]	Contrasts[3]				
	LO	MD	HI	LH	LM	MH		Linear	Quadratic	M vs. LH	M vs. LM	M vs. MH
Intake, kg/d				*Dry matter*								
Apparent digestibility, g/kg DM	7.71	7.62	7.29	7.54	7.51	7.62	0.21	0.27	0.33	0.51	0.77	0.77
	732.4	730.1	734.5	737.5	731.9	716.4	4.37	0.54	0.99	0.94	0.72	0.01
Intake, kg/d				*Organic matter*								
	7.28	7.19	6.88	7.11	7.08	7.19	0.20	0.27	0.33	0.51	0.77	0.77
Apparent digestibility, g/kg DM	754.1	751.8	759.6	764.5	750.8	739.1	4.38	0.27	0.79	0.59	0.71	0.02
Intake, kg/d				*Neutral detergent fiber*								
	2.52	2.43	2.27	2.41	2.42	2.40	0.07	0.10	0.05	0.34	0.37	0.24
Apparent digestibility, g/kg DM	631.3	619.1	601.7	629.6	625.0	575.8	11.1	0.10	0.23	0.65	0.40	< 0.01
Intake, kg/d				*Nonfiber carbohydrates*								
	3.67	3.60	3.41	3.56	3.56	3.59	0.10	0.19	0.18	0.44	0.61	0.55
Apparent digestibility, g/kg DM	849.8	867.0	870.5	870.5	853.2	857.0	5.35	0.59	< 0.01	0.66	0.03	0.35

[1]LO, low (105 g CP/kg DM); MD, medium (125 g CP/kg DM); HI, high (145 g CP/kg DM), LH, oscillating low (LO; 105 g CP/kg DM) to high (HI; 145g CP/kg DM) each 48 h; LM, oscillating low (105 g CP/kg DM) to medium (MD; 125 g CP/kg DM) each 48 h; MH, oscillating medium (125g CP/kg DM) to high (145g CP/kg DM) each 48 h.

[2]SEM = standard error of the mean.

[3]Linear and quadratic contrasts compared 105, 125, and 145 g CP/kg DM; M vs. LH compared 125 vs. oscillating 105 to 145 g CP/kg DM each 48 h; M vs. LM compared 125 vs. oscillating 105 to 125 g CP/kg DM each 48 h; M vs. MH compared 125 vs. oscillating 125 to 145 g CP/kg DM each 48 h.

et al., 2010) that under such low CP diets, it is expected that the higher turnover rate of urea N with reduced clearance in the kidneys and increased clearance from the digestive tract would compensate for the low level of dietary CP for rumen microbes. Additionally, the oscillation frequency of 48 h is probably in synchrony with retention time of digesta in the rumen, which would ensure that greater rates of urea-N recycling with the low CP diet, as in the treatments LM and LH, would occur when ruminal NH_3-N concentration is sub-optimal in terms of supporting microbial growth. Similar findings are reported in finishing *Bos taurus* steers (Archibeque et al., 2007a; Westover, 2011), ram lambs (Kiran and Mutsvangwa, 2009), and dairy cows (Brown, 2014) fed oscillating CP diets.

In addition to the above intake parameters, no significant differences for DM and OM digestibility ($P > 0.05$) were observed with increasing dietary CP content. However, significant effects for DM ($P = 0.01$) and OM digestibility ($P = 0.02$) were observed between oscillating MH and MD static CP concentration, where the DM and OM digestibilities were reduced when oscillating 125 to 145g CP/kg DM at a 48-h interval. According to Westover (2011), digestibility accounts for a large portion of variation in nutrient utilization in feedlot cattle and increased dietary CP concentration can be utilized to improve

DM digestibility in roughage and mixed rations. However, the results from this experiment showed that increasing dietary CP had no significant effect on DM or OM digestibilities, probably because of similar DM and OM intake among treatments.

Previous studies with sheep reported that DM digestibility was not altered by CP concentration and oscillation (Ludden et al., 2002; Kiran and Mutsvangwa, 2009; Doranalli et al., 2011). In contrast, Archibeque et al. (2007a) observed that DM digestibility increased from low (91 g CP/kg DM) to medium (118 g CP/kg DM), high (139 g CP/kg DM), and oscillating CP diets (91 to 139 g CP/kg DM) in steers. Cole (1999) reported that apparent DM digestibility tended to decrease with increasing dietary CP and oscillating dietary CP concentration at 24 and 48 h basis for lambs. These results likely differ due to different protein concentrations and sources, timing of CP oscillations, animal species, and other confounding components of the ration, such as the inclusion level of concentrate and forage.

No significant difference was observed ($P > 0.05$) for NDF digestibility when dietary CP increased; however, there was a significant difference between MD vs. MH ($P < 0.01$), where the NDF digestibility was lower when oscillating MH compared with MD. These differences can be explained

Table 3. Nitrogen balance of bulls fed with different crude protein levels

Item	Treatment[1]						SEM[2]	Contrasts[3]				
	LO	MD	HI	LH	LM	MH		Linear	Quadratic	M x LH	Mx LM	M x MH
N intake, g/d	135.2	149.7	197.6	171.9	143.8	165.9	2.38	<0.01	<0.01	0.02	<0.01	<0.01
N digested, % of intake	67.9	70.5	73.0	71.1	67.2	69.91	0.60	0.02	<0.01	0.38	0.01	0.03
Fecal N, g/d	43.9	44.3	52.1	49.4	47.0	50.0	1.21	<0.01	0.01	0.07	0.81	<0.01
Urine N, g/d	67.7	85.5	116.1	92.8	71.2	90.7	2.39	<0.01	<0.01	0.33	<0.01	<0.01
Urine N, % of excreted N	61.1	64.0	67.7	64.9	59.4	64.2	0.71	<0.01	<0.01	0.09	<0.01	<0.01
Retained N, g/d	23.8	29.0	29.1	29.9	26.4	27.3	1.16	0.96	<0.01	0.13	<0.01	0.04
Retained N, % of N intake	16.2	17.9	13.8	18.8	19.1	15.4	0.39	<0.01	0.58	<0.01	0.01	0.23
Blood urea N, mg/dL	11.2	21.5	23.8	11.8	10.7	20.9	1.72	0.49	<0.01	0.84	<0.01	<0.01

[1]LO, low (105 g CP/kg DM); MD, medium (125 g CP/kg DM); HI, high (145 g CP/kg DM); LH, oscillating low (LO; 105 g CP/kg DM) to medium (MD; 125 g CP/kg DM) each 48 h; MH, oscillating medium (125g CP/kg DM) to high (145g CP/kg DM) each 48 h.

[2]SEM = standard error of the mean.

[3]Linear and quadratic contrasts compared 105, 125, and 145 g CP/kg DM; M vs. LH compared 125 vs. oscillating 105 to 145 g CP/kg DM each 48 h; M vs. LM compared 125 vs. oscillating 105 to 125 g CP/kg DM each 48 h; M vs. MH compared 125 vs. oscillating 125 to 145 g CP/kg DM each 48 h.

by the proportion of wheat bran in the diets (122.4, 61.4, and 0 g/kg DM for LO, MD, and HI CP diets, respectively) and the NDF content of wheat bran that consequently resulted in a reduction of NDF content from LO to HI CP diets. Kiran and Mutsvangwa (2009) reported a linear increase in NDF digestibility from low, medium, high, and oscillating CP-pelleted diets fed to ram lambs. Amaral et al. (2016) observed in an in vitro assay that apparent ruminal digestibility of NDF was not affected by increasing dietary CP, nor by oscillating dietary CP (100 to 140 g CP/kg of DM) compared with a static supply of 120 g CP/kg DM.

A quadratic effect ($P < 0.01$) was observed in NFC digestibility when dietary CP increased. This performance apparently contradicts the patterns observed on NFC intake estimates, even though no significant difference was observed, the NFC intake decreased as the CP content in the diet increased. The NFC intake pattern seemed to be caused by decreasing NFC levels in the diet as nitrogen supplementation increased. A significant difference was observed between MD vs. LM ($P = 0.03$), where the NFC digestibility was reduced when oscillating LO to MD CP content compared with static MD. The biological responses observed in this study are not normally observed, as an illustration, Menezes et al. (2016) and Cavalcante et al. (2005) did not observe any influence of dietary CP level on NFC digestibility, on the other hand, Lazzarini et al. (2009) reported a quadratic pattern on the digestibility coefficient of NFC, with a decrease in the NFC digestibility according to increase in CP diet levels, associated with a linear reduction in NFC intake. A possible explanation for the variation in NFC digestibility between studies are differences in feed processing, dietary CP concentrations and source, feed additives, interactions among feedstuffs, and levels of feed intake (Westover, 2011).

Nitrogen Balance

This study was designed to provide a linear increase in dietary N from sub-adequate (low) to adequate (medium) and excessive (high) concentration, based on protein requirements for young Nellore bulls estimated according to the BR-CORTE system (Valadares Filho et al., 2016). The nitrogen balance is reported in Table 3 and was calculated according to Cole et al. (2006) and Cole and Todd (2009), where urine N was obtained based on the difference of N intake, fecal N, and retained N. A quadratic effect was observed ($P < 0.05$) for N intake with increasing dietary CP levels. As

would be expected, steers fed the HI CP diet had greater N intakes (197.6 g/d) than steers fed the MD (149.7 g/d) or LO CP diets (135.2 g/d). A significant difference was observed between oscillating dietary CP treatments and the MD static treatment, where N intake was greater ($P \leq 0.02$) for bulls fed LH and MH in comparison to those receiving the MD treatment, whereas bulls fed LM consumed less ($P < 0.01$) CP than those fed MD.

As dietary N concentration increased, there was a subsequent increase in apparent nitrogenous compounds digestibility, as stated by the quadratic effect ($P < 0.01$) yielding a greater apparent digestibility in the bulls fed HI (730.2 g/kg of DM) than those fed MD (704.9 g/kg of DM) or LO diets (679.1 g/kg of DM). No significant difference ($P > 0.37$) was observed between oscillating LH and MD. A significant difference was observed between oscillating LM and MD ($P < 0.01$) and between oscillating MH and MD ($P = 0.03$), and in both situations, the greater digestibility value was obtained by the static MD treatment. A similar pattern was observed by Archibeque et al. (2007a) where the steers fed high (139 g CP/kg DM) or oscillating (139 to 91 g CP/kg DM) diets had greatest apparent CP digestibility than steers fed medium (118 g CP/kg DM) or low (91 g CP/kg DM). According to Rufino et al. (2016), the differences in CP digestibility could be because the CP apparent digestibility coefficient is proportional to CP intake and can be considered a direct consequence of the dilution of the metabolic fecal fraction.

There was a significant quadratic effect ($P < 0.01$) on fecal N when dietary CP increased, and a significant difference ($P < 0.01$) between oscillating MH and MD was observed; however, no significant difference ($P > 0.06$) was observed between oscillating LH, oscillating LM, and the MD static treatment. Significant effects of CP concentration on fecal N were reported by Vasconcelos et al. (2009) and Hales et al. (2013) due to increasing CP intake. However, some authors (Menezes et al., 2016; Jennings et al., 2018) reported a lack of a dietary effect on fecal N excretion for finishing bulls. In a study involving static and oscillating CP concentration for finishing steers, Archibeque et al. (2007a) observed that daily fecal N did not differ between steers fed high (139 g CP/kg DM) or medium (118 g CP/kg DM), but was reduced when steers were fed oscillating (139 to 91 g CP/kg DM each 48 h) or low (91 g CP/kg DM). The variation in fecal N excretion can be associated with increased microbial protein synthesis (Prates et al., 2017), as according to the NASEM (2016), 20% of microbial N is indigestible and can be excreted in feces. As

observed by Cole et al. (2005), 30% to 50% of N intake is excreted in feces by beef cattle fed "typical" finishing diets; thus, the appropriate formulation of diets to meet the nutritional requirements of cattle to reduce the excretion of polluting compounds without decreasing animal performance is of fundamental importance.

The rate of environmental emission of N, such as losses as ammonia volatilization to the atmosphere, nitrate diffusion in soil and groundwater, and denitrification and nitrous oxide emission in the atmosphere, is influenced by the source (fecal or urinary N). Fecal N (mainly undigested dietary, microbial, and endogenous proteins) differs substantially from N in the urine (mainly urea, allantoin, hippuric acid, creatinine, ammonia, and uric acid); the latter is more soluble and rapidly metabolized by microorganisms, affecting the severity of the environmental impact (Chizzotti et al., 2016). Daily total urinary N was greatest in the bulls fed HI (116.1 g/d) compared with those fed MD (85.5 g/d) and LO diets (67.7 g/d) as evidenced by quadratic effects ($P < 0.01$). There was no significant difference ($P = 0.33$) in urinary N between bulls fed oscillating LH and those fed MD static diets. Significant differences ($P < 0.01$) were observed between oscillating LM and MH vs. MD, where bulls receiving the LM diet excreted less (71.2 g/d) and bulls fed MH excreted more urinary N (90.7 g/d) than those fed MD (85.5 g/d), suggesting that the apparent effect of oscillating dietary CP content is more associated with dietary CP than with the way CP is delivery in diet.

The route of N excretion, such as fecal N and urinary N, was dependent on diet composition and greater than 75% of N excretion that was found in urine when high protein and high concentrate-based diets were used (Swensson, 2003; Cole et al., 2005; Hristov et al., 2011). Cole et al. (2003) evaluated three CP concentrations in diets where steers were fed as follows: constant 120 g CP/kg DM, constant 140 g CP/kg DM, and oscillating 100 or 140 g CP/kg DM at 2-d intervals. Greater N excretion was reported for steers fed constant 140 g CP/kg DM compared with all other steers (Cole et al., 2003). Archibeque et al. (2007a) reported that daily total urinary N was greatest for the steers fed high (139 g CP/kg DM), intermediate for steers fed medium (118 g CP/kg DM) or oscillating (139 to 91 g CP/kg DM), and least for steers fed low CP diets (91 g CP/kg DM). In this study, considering the results described above, the low and oscillating LM diets resulted in an average 22.52% and 40.15% less urinary N losses, respectively, than the medium, oscillating MH, oscillating LH, and high CP-based

diets resulting in a smaller environmental impact, which is explained due to the average CP content of the diets as stated before. According to Menezes et al. (2016), the efficiency of N utilization is affected by dietary CP content, and urinary and fecal N excretion increases linearly with protein intake. If protein contents in the diet are higher than the animal nutrient requirements, then increased N excretion results, mainly via urine. The reduction in N excretion by meeting the nutritional requirements of animals, without decreasing performance, has great potential to reduce the environmental impact of beef cattle production and increase economic returns to producers (Prados et al., 2016).

There was a quadratic effect ($P < 0.01$) on N retained (g/d) with increasing dietary CP levels where the bulls fed the HI and MD CP diet had greater N retained (29.1 and 29.0 g/d, respectively) than those fed low (23.8 g/d). A significant difference for N retention (g/d; $P < 0.04$) was observed between oscillating LM and MH vs. MD, where bulls receiving the MD diet retained more N. No difference ($P = 0.13$) was observed on N retention between oscillating LH and MD. When we consider retained N as a percentage of N intake, we observed a quadratic effect ($P < 0.01$, where the bulls fed with LO CP had greater N retention than those fed HI (16.20% and 13.78%, respectively). Significant differences ($P < 0.01$) were observed between LH vs. MD and LM vs. MD, and no difference ($P = 0.23$) was observed between MH and MD.

It is known that the efficiency of nitrogen utilization by animals is low; this results in high amounts of nitrogen excretion (Steinfeld et al., 2006). According to Hutchings et al. (1996), nitrogen use efficiency of beef cattle is approximately 10%, and the nitrogen retention in animal product ranges from 5% to 20% of the total consumed. Some causes of low nitrogen retention can be related to a grazing system with low quality of forage (low N supply) or feedlot diets that are excessive in nitrogen due to overestimation of the animal's requirements or use of inconsistent requirement systems to the climate conditions and genetic groups (Detmann et al., 2014). According to Cole et al. (2003), oscillating CP does not seem to affect N retention when supplemental CP was highly degradable (i.e., urea) but do affect N retention when supplemental CP contained appreciable amounts of ruminally undegradable CP (Cole, 1999) suggesting that some ruminally undegradable CP could potentially be fermented in the large intestine and the N recycled to the rumen. Archibeque et al. (2007a) observed that nitrogen retention was greater in steers fed oscillating (139 to 91 g CP/kg DM with 2-d

interval) and medium (118 g CP/kg DM) diets compared with steers fed low (91 g CP/kg DM) or high (139 g CP/kg DM).

It has been reported that plasma urea N (PUN) concentration is correlated with CP intake (Valadares et al., 1997). In the present study, there was a significant quadratic effect ($P = 0.01$) in PUN levels when dietary CP increased and a difference ($P < 0.01$) between oscillating MH and LM vs. MD. No difference ($P = 0.84$) was observed between oscillating LH and the MD static CP diet. The increase observed for PUN concentrations with increased concentration of dietary CP (11.21, 21.45, and 23.76 mg/dL for LO, MD, and HI treatments) can be explained by the increase in daily N intake, as described by Prates et al. (2017). A linear increase of serum urea-N concentration with an increased supply of dietary CP was described in Nellore heifers and bulls (Prates et al., 2017), British x Continental steers (Gleghorn et al., 2004), and in *Bos grunniens* (yak; Guo et al., 2012).

Animal Performance and Carcass Characteristics

Animal performance and carcass characteristics were not affected ($P > 0.05$) by dietary CP content or oscillating dietary CP (Table 4), suggesting that oscillating CP diets were not detrimental as well as do not bring any evident benefit to bull performance. This response is similar to the results of previous research demonstrating that feeding supplemental protein at 48-h intervals to ruminants has no negative impact on animal performance and carcass characteristics (Ludden et al., 2002; Ludden et al., 2003; Archibeque et al., 2007b; Westover, 2011). In contrast to this study, Cole et al. (2003) observed a greater ADG in steers fed oscillating CP concentrations (10% to 14% CP at 48-h intervals) than in steers fed the same quantity of CP on a continuous basis (12% or 14% CP). As discussed by this author, the variability in results could be caused by several factors including timing of CP oscillations, CP concentrations in diets vs. animal requirements, degradability of CP, or diet composition/fermentation ability.

Data from the literature suggest that for growing animals, dietary CP content influences weight gain (Winchester et al., 1957), and an optimal CP for beef cattle is approximately 13% over the grow out period (Gleghorn et al., 2004) and is greater during early feeding and less during the finishing phase, as cattle approach final weight (Todd et al., 2008). Amaral et al. (2018) developed a study with Nellore and crossbred Nellore x Angus bulls divided into three groups receiving diets with 100, 120, and 140 g CP/

Table 4. Animal performance and carcass characteristics of bulls fed with different crude protein levels

Item[1]	Treatment[2]						SEM[3]	Contrasts[4]				
	LO	MD	HI	LM	LH	MH		Linear	Quadratic	M x LH	M x LM	M x MH
Initial SBW, kg	276.1	274.8	278.9	273.7	275.1	277.2	9.09	0.75	0.95	0.85	0.92	0.93
Final SBW, kg	439.6	447.1	432.9	444.6	455.3	448.3	14.1	0.46	0.98	0.80	0.71	0.67
ADG, kg/d	1.17	1.25	1.13	1.23	1.32	1.25	0.06	0.15	0.79	0.51	0.34	0.35
G:F	0.15	0.16	0.15	0.16	0.17	0.16	0.01	0.11	0.24	0.19	0.10	0.14
Hot carcass weight, kg	266.8	270.4	262.9	267.4	275.9	272.9	8.44	0.53	0.99	0.96	0.77	0.61
Cold carcass weight, kg	261.1	265.7	258.3	262.2	271.0	268.0	8.43	0.53	0.92	0.93	0.69	0.56
Hot carcass dressing, %	60.7	60.5	60.7	60.2	60.6	60.9	0.42	0.67	0.85	0.39	0.68	0.77
Cold carcass dressing, %	59.4	59.4	59.7	59.0	59.5	59.8	0.43	0.73	0.79	0.53	0.96	0.53
Carcass length, cm	127.4	131.4	129.3	129.3	129.7	128.7	1.93	0.28	0.09	0.50	0.15	0.64
Fat thickness, mm	4.9	3.9	3.2	3.8	4.0	5.1	0.50	0.41	0.06	0.11	0.16	0.77

[1]SBW, shrunk body weight; ADG, average daily gain; G:F, gain-to-feed ratio

[2]LO, low (105 g CP/kg DM); MD, medium (125 g CP/kg DM); HI, high (145 g CP/kg DM); LH, oscillating low (LO; 105 g CP/kg DM) to high (HI; 145g CP/kg DM) each 48 h; LM, oscillating low (105 g CP/kg DM) to medium (MD; 125 g CP/kg DM) each 48 h; MH, oscillating medium (125g CP/kg DM) to high (145g CP/kg DM) each 48 h.

[3]SEM = standard error of the mean.

[4]Linear and quadratic contrasts compared 105, 125, and 145 g CP/kg DM; M vs. LH compared 125 vs. oscillating 105 to 145 g CP/kg DM each 48 h; M vs. LM compared 125 vs. oscillating 105 to 125 g CP/kg DM each 48 h; M vs. MH compared 125 vs. oscillating 125 to 145 g CP/kg DM each 48 h.

kg DM. This author observed that calves that were weaned and thereafter finished in feedlot should receive diets with CP content of approximately 120 g CP/kg DM during the initial growing phase (84 d). At the end of this period, or during the finishing phase (56 d), dietary CP content could be reduced to 100 g CP/kg DM without affecting animal performance during this phase. In agreement with Amaral et al. (2018), this study shows that reducing CP from 145 to 105 g CP/kg DM or oscillating CP supply did not affect animal performance and carcass characteristics of growing Nellore bulls.

A possible explanation for the lack of effect of dietary treatments obtained in the present study is that the total dietary CP content of the low CP treatment (105 g CP/kg DM) may be sufficient to supply degradable CP for rumen microbial activity and MP for muscle production, as proposed by Hynes et al. (2016). The present study found that increasing dietary CP concentration had no significant effect on total DMI or ADG; however, it increased N retention (Table 3). It is important to highlight that protein and fat are components of the gain, and protein has a lower energetic efficiency of deposition than fat, likely because it is influenced by the mix of amino acids available and the energy cost associated with body protein turnover (Baldwin, 1995). Additionally, when averaged over the entire feeding period and animals are fed to a constant endpoint, the body composition of gain and the diluting effects water gain on cost of lean weight gain may minimize the effects of protein vs. fat gain (Tedeschi et al., 2010). Data regarding the chemical body composition of the bulls and its pattern of deposition can be found in a complimentary paper (Menezes et al., 2019).

Therefore, these data indicate that although there is no alteration in the performance of growing Nellore bulls fed with oscillating CP diets vs. a static level of 125 g CP/kg DM, nor static low (105 g CP/kg DM) and high (145 g CP/kg DM) levels; there may be undesirable increases in environmental N excretion when the average dietary CP content is increased. The results suggest that dietary CP concentrations of 105, 125 g/kg DM, or within this range can be indicated for finishing young Nellore bulls, since it reaches the requirements, reduces the environmental footprint related to N excretion, and may save on costs of high-priced protein feeds.

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